**Deadline for Proposals for SOT 2016 Annual Meeting Sessions: April 30, 2015**

**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

**Continuing Medical Education**—Emphasis on state-of-the-art knowledge to assist medical doctors, health professionals, and researchers in lifelong learning for providing high-quality health care

*Note: Any session type may be considered for CME.*

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

**Informational Sessions**—Scientific planning or membership development

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

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

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**Why Submit a Proposal?**

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

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Submit your proposal online at [www.toxicology.org](http://www.toxicology.org)
Preface

This issue is devoted to the abstracts of the presentations for the Continuing Education courses and scientific sessions of the 54th Annual Meeting of the Society of Toxicology, held at the San Diego Convention Center, March 22–26, 2015.

An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 529.

The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 553.

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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that have implications in cancer pathogenesis from chemical exposure. Understanding of chemical carcinogenesis as well as discussing some new frontiers in metabolism and differentiation has been known, new studies indicate these proteins are central in cancer pathogenesis either via their canonical or non-canonical actions driven by chemical exposure. This course will review our current level of understanding of chemical carcinogenesis as well as discussing some new frontiers that have implications in cancer pathogenesis from chemical exposure.

The ability of chemicals to cause cancer is an end point with a deep impact on public health. Understanding the mode of action of chemical carcinogens is critical for risk assessment of the chemicals. The mechanisms by which chemicals can cause cell transformation and neoplastic growth have been central to the discipline of toxicology. It is now apparent that the previous simplistic view that chemicals interact with DNA, induce a mutation which results in the formation of a neoplasm is incomplete. Chemical modulation of metabolism, nuclear receptors, gene expression, DNA repair processes, immune surveillance, inflammation, cell to cell communication and changes in target cell function and structure, and their ability to activate stem/progenitor cells, contribute to the formation of preneoplastic cells and their progression to the malignant state. The multitude of changes in the target cell and its microenvironment must be considered in applying mode of action analysis to potential carcinogenic human risk. Besides the estrogen, CAR, PPAR, and AHR receptors, other nuclear receptors, including HNF-4α, TR, Nur77, and LXR previously not associated with cancer pathogenesis, appear to play a critical role in the formation and progression of cancer. While the role of these receptors in metabolic processes and differentiation has been known, new studies indicate these proteins are central in cancer pathogenesis either via their canonical or non- canonical actions driven by chemical exposure. This course will review our current level of understanding of chemical carcinogenesis as well as discussing some new frontiers that have implications in cancer pathogenesis from chemical exposure.

The exposome has been defined as the totality of our exposures throughout our lifetime. Such a definition deems measurement making it less than useful as a scientific construct. More recently, the concept of the exposome has evolved to represent a measurable entity that encompasses our complex exposures and how our bodies respond to such exposures. The addition of the biological response component to the definition of the exposome positions the field of toxicology to make major contributions to the field. By providing an intellectual foil to the genome-centric framework in biomedical research, the exposome has the potential to elevate the importance of the environment in scientific circles and promote a framework that faithfully integrates the importance of the environment in health and disease. This course will introduce the attendees to the concept of the exposome, explain how it can be used to advance toxicological research by providing a clear translational output, and explain some of the innovative approaches being used to measure the exposome. In order for the exposome to become a useful concept it will be necessary to: (1) capture and quantify the complex exposures, (2) identify and quantify the diverse biological responses, and (3) to integrate these disparate datasets with advanced computing and information and mathematical approaches. This course was designed to address these three objectives in an informative and interactive setting.

Assessing chemical mixture toxicity is often considered an intractable problem. Difficulty increases for complex mixtures as much of their composition is typically unknown. Although mixtures toxicology and risk assessment (RA) are more complex than for single chemicals due to potential interactions, significant advances have been made in recent years. As a number of experiments are designed poorly from a mixtures perspective, this course will provide coherent strategies for design, analysis of mixtures experiments for robust conclusions, and data that are useful in mixtures RA. Key principles and concepts underlying modern mixtures toxicology, RA, legislation, policy and guidance in the United States and other nations will be reviewed. Guidance based on data quality will be provided for application of either whole mixture or component-based RA approaches. Whole mixture RA discussions will include recent research on methods to determine whether mixtures are sufficiently similar such that toxicity information for one mixture can be used to estimate the toxicity of another. Most mixtures RAs are component-based and a number of approaches will be illustrated—highlighting key differences. Those include the hazard index (HI), target organ HI, interaction weighted HI, and index-chemical based (relative potency factor and toxic equivalency factor) approaches. This course emphasizes recent advances and will be of value to experimentalists wanting to conduct mixture studies meaningful for evaluation of risk or safety, and risk assessors who evaluate mixtures data and apply mixtures RA methods.

The ability of chemicals to cause cancer is an end point with a deep impact on public health. Understanding the mode of action of chemical carcinogens is critical for risk assessment of the chemicals. The mechanisms by which chemicals can cause cell transformation and neoplastic growth have been central to the discipline of toxicology. It is now apparent that the previous simplistic view that chemicals interact with DNA, induce a mutation which results in the formation of a neoplasm is incomplete. Chemical modulation of metabolism, nuclear receptors, gene expression, DNA repair processes, immune surveillance, inflammation, cell to cell communication and changes in target cell function and structure, and their ability to activate stem/progenitor cells, contribute to the formation of preneoplastic cells and their progression to the malignant state. The multitude of changes in the target cell and its microenvironment must be considered in applying mode of action analysis to potential carcinogenic human risk. Besides the estrogen, CAR, PPAR, and AHR receptors, other nuclear receptors, including HNF-4α, TR, Nur77, and LXR previously not associated with cancer pathogenesis, appear to play a critical role in the formation and progression of cancer. While the role of these receptors in metabolic processes and differentiation has been known, new studies indicate these proteins are central in cancer pathogenesis either via their canonical or non- canonical actions driven by chemical exposure. This course will review our current level of understanding of chemical carcinogenesis as well as discussing some new frontiers that have implications in cancer pathogenesis from chemical exposure.
6 The New World of Cancer Immunotherapy: Challenges in Bench to Bedside Translation

The concept of harnessing the immune system to eradicate cancer has been a long-term goal in immunology and oncology. After years of disappointment, the field of cancer immunotherapy (CIT) has gained a strong foothold with the recent approval of two immunotherapies (ipilimumab and sipuleucel-T); and encouraging data emerging from clinical trials testing checkpoint inhibitors, chimeric antigen T cells, oncolytic vaccines, and other modalities. This intensive effort to identify new CIT targets and/or develop new modalities to harness the immune system presents new challenges for assessing nonclinical safety to support clinical development. For nonclinical safety assessment, understanding the threshold between desired immunological activity (desired pharmacology), and the potential for exaggerated immunologic stimulation is paramount in the clinical dose selection and identifying biomarkers for patient monitoring. Therefore CIT drug development may require creative models and study designs that incorporate extensive immune monitoring that has not been routinely included when testing conventional cancer therapeutics. The course objective is to provide a general overview of the field of cancer immunotherapy, highlight the unique challenges for CIT drug development, and generate discussion with regards to assessing the unique challenges of developing this new oncology therapeutics to ensure patient safety. Presenters will introduce the concept of cancer immunotherapy and summarize the current landscape of the field of CIT drug development, including the various pathways and modalities currently under development, and discuss the scientific limitations of conventional models for evaluating the pharmacology of novel immunotherapeutics, as well as the promise of novel models that may improve translatability of results to the clinical setting. The course will look into the challenges for nonclinical safety assessment of cancer immunotherapy molecules. Case studies from different CIT modalities will illustrate the challenges. Unique clinical challenges of developing CIT molecules, and the regulatory perspective on the need for nonclinical, clinical and regulatory scientists to partner to ensure patient safety when developing CIT molecules, will also be presented.

7 Toxicology and Regulatory Considerations for Combination Products
J. N. Cammack. AstraZeneca Biologics, Gaithersburg, MD.

Therapeutic and diagnostic products that combine drugs, devices, and/or biological elements are termed, and regulated by the US FDA, as combination products. Technological advances continue to merge product types and blur the historical lines of separation between traditional drugs, biologics, and medical devices. Further, the increasing use of absorbable platforms adds another level of complexity to the development and regulation of certain combination products. US FDA’s medical product centers, the Center for Biologics Evaluation and Research (CBER), the Center for Drug Evaluation and Research (CDER), and the Center for Devices and Radiological Health (CDRH), are utilizing evolving collaboratively efforts in order to address the regulatory challenges of combination products. Because combination products involve components that would normally be developed and regulated under different types of processes and policies, and frequently submitted to different US FDA Centers, these products raise challenging development, regulatory, and review management questions. Differences in these pathways for each combination product type can impact the processes for all aspects of product development and management, especially preclinical testing, but also, clinical investigation, marketing applications, manufacturing and quality control, adverse event reporting, promotion and advertising, and post-approval modifications. The 2014 Sunrise Combination Products CE course introduced the emerging topic; the 2015 CE course will provide in-depth detail on the evolving regulatory processes in developing a successful preclinical evaluation program. In addition to examples of product development scenarios (including drug/biologic-device and antibody drug conjugate combination products), US FDA will provide a reviewer perspective on key program considerations.

8 Advances in Safety Assessment of Medical Devices
N. S. Goud. Boston Scientific Corporation, Spencer, IN.

Medical devices used in the diagnosis and treatment of various diseases are manufactured from polymeric materials and metal alloys, each of which may be associated with safety concerns for the patient. The aim of this course is to provide an outline of the various in vitro, in vivo, and in silico methodologies for the safety assessment of medical devices and to discuss how risk assessment approaches can be used in the biological evaluation process for medical devices. Presentations will provide an overview of the biocompatibility test methods recommended by ISO 10993, US Pharmacopeia, and ASTM and will include examples of test failures and how to resolve them without compromising patient safety. The course will begin with a broad overview of the approaches used to evaluate the biological safety of medical devices. Following the introductory talk, there will be presentations on high profile and toxicologically important topics, the potential health risks associated with the use of metallic hip implants and approaches to evaluate the biological safety of plastic dental materials. One challenge in conducting toxicological risk assessments of compounds released from medical device materials is when there are no adequate toxicity data for these compounds. The course will conclude with a presentation that provides practical guidance on the derivation of exposure limits for leachable chemicals released from medical devices when only limited toxicity data are available. This course should be of broad interest to toxicologists and health care professionals involved in evaluating patient risks to new treatment modalities, and in particular to toxicologists involved in evaluating the safety of medical devices and combination products containing drugs or biologics.

9 Interpretation of Cardiovascular Safety Data in Toxicology Studies
J. L. Kremer. Covance Laboratories Inc., Madison, WI.

The value of integrating cardiovascular (CV) safety evaluation into general toxicology studies has been increasingly recognized in drug development for both pharmaceutical and biotechnology-derived products. These combined study approaches offer a unique opportunity to gain a holistic understanding of drug-related functional, biochemical, and morphological changes in the context of proper pharmacokinetic/pharmacodynamic (PK/PD) data. Past courses have focused on the best practices including study design and execution for integrating CV endpoints into toxicology studies. This course will provide a comprehensive and detailed discussion on the interpretation of CV findings as part of a toxicology study. For example, how are CV functional data interpreted (e.g. heart rate, blood pressure, electrocardiogram, contractility) as compared to traditional endpoints (e.g. pathology). Is a physiological finding (e.g. a decrease in contractility) a primary effect or a compensatory effect to other changes? Is it due to a direct CV effect or secondary to drug-related toxicity? How when should a CV finding be interpreted as a “hazard” versus “adverse”? What are the potential mechanisms for toxicity? How do I utilize the holistic data to design the next steps? How does surgically implanted instrumentation affect or potentially confound a pathology evaluation? The course will start with outlining the questions and focus on how these assessments are used and interpreted in this emerging paradigm of combined CV/toxicology studies. The target audience consists of toxicologists who may have limited exposure to CV or safety pharmacology data or are looking to expand their knowledge in this area. By the end of this symposium, the audience should better understand the considerations and strategies in integrating CV/toxicology studies as well as real-world case-studies (or best practices) for interpretation of these data for drug safety assessment.

10 Is Synthetic Biology the Future of Toxicology?
S. M. Hussain. US Air Force, Wright-Patterson AFB, OH.

One frequent critique of traditional in vitro study design is lack of functional correlation between a submerged cellular monolayer and a full organ or tissue system. However, scientists do agree that during preliminary toxicological screening, when little is known regarding the behavior of a new molecule, simple in vitro models coupled with basic toxicological endpoints are critical for generating a baseline response and determining future actions. Currently, there is a significant discrepancy exists between in vitro and in vivo correlations. One approach to bridge this gap is through the development of enhanced in vitro systems to more closely mimic an accurate physiological environment. When examining a physiological system, two key components need to be addressed are: (1) the three dimensional aspect of an organ or tissue and the cell to cell communication that occurs within this structure; and (2) the dynamic environment that flows in and around the tissue, arising from the cardiovascular system. Early improvements in traditional in vitro designs explored co-cultures that included immune cells, the addition of dynamic media flow, and three dimensional matrices (though limited studies have combined multiple of these variables). The focus of the course is to evaluate the current trends in synthetic biology that are advantageous to enhanced in vitro design. One major focus will be on current organ-on-a-chip research, which incorporates cell to cell communication coupled with dynamic flow of media or air, depending on cell type. In addition, since inhalation is a predominant route of toxicological exposure, this course will explore the design of an artificial nose that represents inhalation and the ability of a compound to cross the olfactory bulb in an effort to predict a neurotoxicity risk.
11 Skeleton System Endocrinology and Toxicology
A. Hoberman. Charles River Laboratories, Horsham, PA.

The skeleton has traditionally been considered within the framework of two tenets: A hard structure for protection of the organism, and a major reservoir for the maintenance of serum calcium. Bone remodeling, the process of remaking our skeleton every decade, reinforces that structure/function correlate. However emerging evidence suggests the skeleton is intimately related to other organ systems including but not limited to organs involved in energy metabolism, reproductive system, immune system, central nervous system and muscle, through paracrine, endocrine, and neural networks. The goal of this course is to explore these interactions further and highlight the importance of including skeletal evaluations in juvenile and standard toxicology studies and their relevance to humans and clinical trials. In addition, an overview of bone biology and the appropriate techniques for assessment of changes in bone will be provided. The presentations will focus on bone biology, its growth during infancy and childhood and the regulatory systems involved in the maintenance of bone quality during adulthood; the techniques available for bone evaluations in toxicology studies; why bone has recently been accepted as an endocrine system and what the functions of hormones secreted from bone are; and explore the complex relationships unfolding between bone and the different biological systems and the implications in drug development.

12 Strategies in Investigative Toxicology in a Pharmaceutical Setting
D. Simic. Jansen R&D (Johnson & Johnson), Spring House, PA.

Investigative toxicology is a broad discipline encompassing multiple tools and strategies to help generate and test hypotheses as part of target safety assessments and derisking efforts in support of discovery and development programs. In most pharmaceutical and biotechnology companies, investigative toxicology exists as either a stand-alone lab, or the function is embedded within various support groups. Discovery and development programs that call upon investigative toxicology to manage safety liabilities and facilitate understanding of toxicity issues face a number of challenges. These include adequate communication across stakeholders, steep learning curves, identification of clear deliverables that require resource prioritization and constantly shifting interests. This "best practices" session will highlight steps on how to overcome such challenges by focusing on three key functions: (1) designing testable hypothesis, (2) communication of the meaningful experimental findings, and (3) proposing rationales and decision processes for the timely resolution of the issue(s). Specifically, best practices will be highlighted in relation to the stage of the program within the R&D pipeline. The presenters will focus on optimal investigative toxicology strategies applicable during target safety evaluation, lead optimization, pre-IND, IND, late stages of the compound development and life cycle management, with case examples. The utility of tools such as genomics, RNAi, metabonomics, in vitro assays and informatics for the integration of supportive data (e.g. clinical chemistry, histopathology, TK and biomarkers), and application of communication tools such as MindMaps will be discussed. Finally, a regulatory perspective on the utility, impact, and practical considerations of submitting investigative toxicology studies to regulatory authorities to assess clinical risk will be presented.

L. Buyn. Texas A&M University, College Station, TX.

Toxicogenomics is a mature field which provides invaluable information on the molecular events preceding or accompanying toxicity; however, most traditional use of gene expression and other ‘omic data in toxicology is largely the same as it was ten years ago: The mode-of-action analysis, classification/prediction, and biomarker discovery. As the technological advances keep driving costs down, new challenges are facing toxicologists to manage safety liabilities and facilitate understanding of toxicity issues face a number of challenges. These include adequate communication across stakeholders, steep learning curves, identification of clear deliverables that require resource prioritization and constantly shifting interests. This “best practices” session will highlight steps on how to overcome such challenges by focusing on three key functions: (1) designing testable hypothesis, (2) communication of the meaningful experimental findings, and (3) proposing rationales and decision processes for the timely resolution of the issue(s). Specifically, best practices will be highlighted in relation to the stage of the program within the R&D pipeline. The presenters will focus on optimal investigative toxicology strategies applicable during target safety evaluation, lead optimization, pre-IND, IND, late stages of the compound development and life cycle management, with case examples. The utility of tools such as genomics, RNAi, metabonomics, in vitro assays and informatics for the integration of supportive data (e.g. clinical chemistry, histopathology, TK and biomarkers), and application of communication tools such as MindMaps will be discussed. Finally, a regulatory perspective on the utility, impact, and practical considerations of submitting investigative toxicology studies to regulatory authorities to assess clinical risk will be presented.

14 New and Emerging Tobacco Products—Biomarkers of Exposure and Injury
D. J. Conklin* and I. T. Zelikoff. 1Department of Cardiovascular Medicine, University of Louisville, Louisville, KY and 2Department of Environmental Medicine, New York University, Tuxedo, NY.

On June 22, 2009, the Family Smoking Prevention and Tobacco Control Act was signed into law, giving the US FDA the authority to regulate new and emerging tobacco-derived products. Subsequently, there is an obvious need to provide scientific data in order to inform US FDA’s decision-making regarding these products. Thus, biomarkers of exposure, biomarkers of injury, and controlled, acute, and chronic, as well as longitudinal exposure studies, are being conducted to better define what exactly new and emerging tobacco-derived products do in a variety of preclinical and clinical settings. New and emerging tobacco- and nicotine-derived products come in a dizzying array of products including electronic cigarettes, smokeless tobacco (including snus, snuff, and gutka), shisha for hookah/water pipes, disposable lozenges, and nicotine gels that contain, deliver, and/or generate a varied number of harmful or potentially harmful constituents (HPHCs), making it challenging to predict the biological effects of exposures based solely on traditional cigarette exposure studies. This symposium will provide a broad overview of ongoing studies attempting to identify biomarkers of exposure, biomarkers of injury, and acute and chronic effects in the cardiovascular, pulmonary, and reproductive organ systems resulting from exposures to new and emerging tobacco products and/or their HPHCs.

15 Biomarkers of Exposure to Tobacco Smoke and Emerging Tobacco Products
N. Benowitz. Department of Medicine, University of California San Francisco, San Francisco, CA. Sponsor: D. Conklin.

Biomarkers of exposure include measures of nicotine and alkaloid intake, gaseous and particulate phases of tobacco smoke. Nicotine exposure can be estimated using plasma, saliva, or urinary cotinine and urine total nicotine equivalents. Other alkaloids with potential activity including nornicotine, anabasine and anatabine can be measured in urine. Gaseous phase biomarkers include blood carboxyhemoglobin, expired carbon monoxide, and urine mercapturic metabolites of volatile organic chemicals (VOCs), including acrolein, acrylamide, acrylonitrile, butadiene, benzene and others. VOCs are believed to contribute to cardiovascular, pulmonary and cardiovascular disease caused by tobacco smoke exposure. Tar phase biomarkers include tobacco-specific nitrosoamines, polycyclic aromatic hydrocarbons (PAHs) and the nicotine. Patterns of toxicant exposure vary by type of tobacco product and conditions of exposure. For example water pipe use exposes users to higher level of carbon monoxide and benzene, compare to cigarette smoking. Ratios of anabasine/nicotine exposure differ in smokeless tobacco users compared to cigarette smokers. The pattern and time course of biomarkers after exposure to secondhand smoke will be discussed.

16 Pulmonary Effects of Exposure to Tobacco Smoke and New Tobacco Products
I. Jaspers. Department of Pediatrics, University of North Carolina at Chapel Hill, Chapel Hill, NC.

Tobacco smoke has significant effects on mucosal immune responses including airway surface liquid/mucus dehydration and increased incidence of viral infections, suggesting that the lung’s innate host defense system has been impaired. While traditional cigarettes have been studied for their adverse respiratory immune effects, whether and how new tobacco alternatives affect lung mucosal immune responses is largely unknown. Usage of “little cigars” which are often flavored and are thus attractive to younger smokers, has risen 240%, while in NC, up to 50% of college students claim to have tried Hookah, with >10% being regular Hookah smokers. Similarly, the use of e-cigarettes is on the rise and the US. Collaborative research efforts are now underway measuring the potential adverse impact of tobacco alternatives on the lung’s innate defense system. These include determining the impact of tobacco alternatives on specific aspects of the airway surface liquid homeostasis and mucin/mucus biology using innovative in vitro smoke exposure systems, as well as airway samples from smokers of alternate tobacco. These studies will be integrated with clinical studies aimed at determining genomic and epigenomic biomarkers as...
associated with tobacco alternatives using samples obtained from human volunteers, and focusing on changes in antiviral host defense in these subjects. The overarching goal of these collaborative studies is to identify novel biomarkers associated with tobacco-induced changes in lung innate defense, which can be applied to understand potential toxicity of any new and emerging tobacco products.

17 Reproductive/Developmental Effects of Exposure to New and Emerging Tobacco Products and to Nicotine Delivery Devices in a Mouse Model

J. T. Zelikoff. Department of Environmental Medicine, New York University, Tuxedo, NY.

Exposure of adult males to toxic chemicals is a major contributor to decreased fertility via alterations in gametogenesis, loss of Sertoli or Leydig cell function, or through effects from endocrine disruption that in turn results in reduced sperm numbers, loss of motility, or increased numbers of sperm with morphological defects. Studies examining how toxicant exposure in utero may also contribute to diminished male fertility in the offspring are extremely limited. To these ends, we will present data from rodent studies that examined the effects of maternal exposure during pregnancy to vapor from electronic cigarettes or smoke generated from shisha (a Middle Eastern tobacco product heated in hookahs). Findings from these studies demonstrated that prenatal exposure of mice to E-cigarette vapor resulted in an ~50% decrease in offspring total sperm numbers (compared with control). Moreover, in utero exposure to both E-cigarette vapor and hookah smoke decreased the number of individual offspring with motile sperm (~80% and 70% for E-cigarette and shisha, respectively). These data show that toxicant-induced alterations in male fertility may occur not only by direct chemical exposures after puberty, but can also be adversely impacted by early life exposures during in utero development adding another adverse health outcome for the prenatally exposed offspring. Studies will also be presented demonstrating similar effects on sperm parameters in adult male mice associated with exposure to a globally-relevant smokeless tobacco.

18 Cardiovascular Effects of Exposure to Harmful and Potentially Harmful Constituents (HPHCs) of Tobacco Products

D. J. Conklin1, 2, L. Chen3, and S. Srivastava4, 5. 1Department of Cardiovascular Medicine, University of Louisville, Louisville, KY, 2Department of Environmental Medicine, New York University, Tuxedo, NY, and 3Tobacco Regulation and Addiction Center, American Heart Association, Dallas, TX.

Smoking is the leading cause of preventable deaths, and although cigarette smoking increases the risk of several chronic diseases, nearly half of smoking related mortality is linked to cardiovascular deaths. Moreover, 80-85% of the non-cancer health risk (i.e., cardiovascular disease risk) is attributed to acrolein, an unsaturated aldehyde. Acrolein and other aldehydes amongst the greater than 8,000 chemicals in tobacco smoke represent the most harmful and potentially hazardous constituents (HPHCs) of tobacco products. As carbonyls have been identified in new and emerging tobacco products including electronic cigarettes, hookah and smokeless tobacco (ST), we investigated the direct effects of these HPHCs on cardiovascular disease using both acute and chronic exposures. To accomplish this in a preclinical setting, animals were exposed to varying intensities of HPHCs, tobacco smoke and smokeless tobacco, and urinary metabolites of exposure-derived aldehydes were measured. Accordingly, we have tested the acute and chronic effects of selected HPHCs including acrolein, crotonaldehyde and benzene on platelet activation, platelet-leukocyte aggregate formation, insulin resistance and atherogenesis (using apoe-null mice and rats) as markers of CVD risk. Moreover, effects of these HPHCs are compared with the effects of nicotine exposure alone. Thus, our overall goal is to identify biomarkers of exposure to HPHCs in tobacco products and to relate these to atherosclerosis progression — the major underlying cause of CVD. Finally, elucidation of the relationship between biomarker of exposure and biomarkers of cardiovascular injury will enable comparisons between the toxicity of different and new tobacco products and inform the design of future human studies for evaluating the CVD risk of tobacco product use.

19 Cardiovascular Effects of Tobacco Products, Nicotine Delivery Products, and Secondhand Smoke: Exponential Effects in Humans

S. Schick. Department of Medicine, University of California San Francisco, San Francisco, CA. Sponsor: D. J. Conklin.

Cigarette smoking is a major cause of cardiovascular disease (CVD). However, the mechanisms by which smoking causes cardiovascular disease and the cardiovascular risks of other popular tobacco products (smokeless tobacco), new tobacco products (e-cigarettes) and proposed products (reduced nicotine cigarettes) are not adequately understood. Modern tobacco and nicotine delivery products deliver a wide range of nicotine, particles and other cardiotoxins. Many of the toxins that are in cigarette smoke are also found in secondhand cigarette smoke. Disturbances in the function of vascular endothelium (the lining of arteries, which plays an important role in regulating vascular function) and the activation of the autonomic nervous system, as well as increased inflammation, oxidative stress and propensity to thrombosis (clotting), are key mechanisms in the progression of CVD and validated biomarkers of CVD risk. To assess the cardiovascular effects of tobacco products, we are conducting controlled, short-term exposures of human subjects and measuring endothelial function, autonomic nervous activation, inflammation, oxidative stress and thrombogenesis. By comparing products with a wide range of toxicant deliveries, we hope to identify the compounds and mechanisms by which tobacco product use causes cardiovascular disease.

20 The Role of Connexin-Based Channels in Toxicity

M. Vinken1 and B. L. Upham1, 2. 1Toxicology, Vrije Universiteit Brussel, Belgium, Belgium and 2Department of Pediatrics & Human Development, and Center for Integrative Toxicology, Michigan State University, East Lansing, MI.

Connexins and their channels control tissue homeostasis at three levels, all which involve separate mechanisms. First, they form gap junctions, composed of two hemichannels of neighboring cells, of which each hemichannel is composed of six connexin proteins. As such, these gap junctions mediate the traffic of small, hydrophilic molecules between cells, a flux called gap junctional intercellular communication (GJIC) that controls gene expression and physiological functions. Secondly, hemichannels can form a separate pathway for communication, between the intracellular compartment and the extracellular environment. Thirdly, connexin proteins can affect the homeostatic balance independent of their channel-forming activities by directly interfering with gene expression. Dysfunction of connexin channels have been implicated in many diseases including cancer, reproductive dysfunction, peripheral neuropathies, liver disease, cataracts, deafness, teratogenesis, cardiac arrhythmias, and skin diseases. In the first presentation, general features of connexin-based channels will be discussed, as well as their mechanistic involvement in liver disease and toxicity. In the second presentation, the critical role of gap junction in redox signalling will be addressed. In the third presentation, toxicant-induced disruption of gap junctional intercellular communication in uterine muscle, with implications for parturition will be outlined. The fourth presentation will focus on the use of gap junction function as a biomarker for elucidating toxicant-induced mechanisms of tissue dysfunction using advanced genomic strategies. Overall, this symposium will address significant advances and recent novel concepts in connexin biology and their application to toxicology.

21 Integrative Role of Gap Junctions in Redox Signaling

B. L. Upham1 and P. Babica1. 1Pediatrics & Human Development, Michigan State University, East Lansing, MI and 2RECETOX - Research Centre for Toxic Compounds in the Environment, Masaryk University, Brno, Czech Republic.

Although oxidative toxicants can induce high levels of reactive oxygen species (ROS), which can induce cellular damage to DNA, protein and lipid; low levels of ROS are now known to control gene expression. Gene expression is highly regulated by the coordination of cell signaling systems that maintain tissue homeostasis. Most research has focused on redox regulation of signal transduction within a cell, but we introduce a more comprehensive-systems biology approach to understanding oxidative signaling that includes gap junctional intercellular communication, which plays a role in coordinating gene expression between cells needed to maintain tissue homeostasis. This talk will introduce data supporting the hypothesis that gap junctions are critical in modulating the levels of second messengers, such as low molecular weight reactive oxygen, needed in the transduction of an external signal to the nucleus in the expression of genes. Thus, any comprehensive-systems biology approach to understanding toxic-induced oxidative, as well as non-oxidative, signaling should include gap junctions, in which aberrant gap junctions have been clearly implicated in many human diseases.
22 Connexin Signaling in Liver Toxicity and Disease
M. Vinken, Toxicology, Free University Brussels, Brussels, Belgium.

This presentation will start with a general overview of connexin channel biology and physiology, including their structure, distribution, and mechanisms driving their function and regulation. Inherent to their pivotal role in maintaining tissue homeostasis, it is not surprising that connexins and their channels are frequently involved in the impairment of this critical balance, as occurs during toxicity and disease. Being the major organ involved in the biotransformation of xenobiotics, liver connexins are primary targets in these processes. This actually defines the main focus of the current presentation, whereby particular attention will be paid to the mechanisms underlying connexin channel dysfunction. Indeed, a wide variety of chemical and biological toxic compounds are known to negatively influence connexins in the liver, including environmental pollutants, biological toxins, organic solvents, pesticides, pharmaceuticals, peroxides, metals and phthalates. Interestingly, gap junctional intercellular communication seems to be specifically targeted by tumor promoters and epigenetic carcinogens, both in vivo and in vitro. Hence, inhibition of gap junction functionality could be considered as a suitable indicator for the detection of non-genotoxic carcinogenicity.

23 Uterine Muscle Gap Junctions As Toxicant Targets
R. Loch-Caruso, Environmental Health Sciences, University of Michigan, Ann Arbor, MI.

Mechanisms by which environmental contaminants may contribute to disorders of parturition, such as preterm labor or arrested labor, remain elusive. Connexin channels, or gap junctions, play a key role in parturition by allowing coordination of uterine contractions necessary for successful labor. Mechanisms by which environmental toxicants may alter uterine contractility through modulation of uterine muscle gap junctions will be illustrated in this talk with findings from experiments with the insecticide lindane. Lindane inhibits uterine contractions and inhibits function of gap junctions in myometrial cell cultures without significant change to expression or phosphorylation (at serine 368) of connexin43. Data will show how reactive oxygen species play key roles in lindane-induced inhibition of uterine contractions and myometrial gap junctions. These findings will be interpreted in the context of cell regulatory mechanisms of gap junctions with an emphasis on potential implications for parturition.

24 Gap Junction Function: A Biomarker for Elucidating Toxin-Induced Mechanisms of Tissue Dysfunction
P. Babica1,2 and B. L. Upham2. 1RECETOX - Research Centre for Toxic Compounds in the Environment, Masaryk University, Brno, Czech Republic and 2Pediatrics & Human Development, Michigan State University, East Lansing, MI.

Gap junctional intercellular communication (GJIC) plays a very central role in regulating signal transduction systems that maintain tissue homeostasis, thus GJIC can serve as an excellent biological anchor for genomic approaches to determine changes on the transcriptome, proteome and lipoproteome that can lead to adverse health effects. This talk will present proteomic approaches in determining the early molecular signaling events occurring in response to toxicants, and how these pathways are integrated with GJIC using a rat liver epithelial cell line with oval cell characteristics. Some of the early molecular events discovered by proteomic approaches include lipid signaling pathways, arachidonic acid release, protein kinase A, CREB, annexins, and mitogen activated protein kinases. At least four independent signaling pathways were determined to directly regulate GJIC by toxicants. These results have been integrated into a cybernetic cell signaling model of controlling gene expression, and offers potential new biomarkers of toxicity and more accurate risk assessment of adverse health effects of pharmacological agents, and environmental toxicants and toxins.

25 Environmental Exposures and Alzheimer’s Disease: Epidemiology, Mechanisms, and Future Strategies
J. R. Richardson1 and A. Kanthasamy. 1Environmental and Occupational Medicine, Robert Wood Johnson Medical School, Piscataway, NJ and 2Iowa State University, Ames, IA.

Alzheimer’s disease (AD) is the most common neurodegenerative disease worldwide and is expected to increase three-fold over the next 40 years. To date, a massive amount of effort has focused on identifying genetic contributors to AD. Although there is a growing list of susceptibility genes that collectively contribute to AD, the largest GWAS study published on AD (>74,000 individuals) identified only 1 out of 19 loci as an individual strong contributor to AD. This finding has led to calls for studies to examine the potential influence of environmental and lifestyle factors on risk for AD. Given the wide-spread prevalence of AD and an ever-aging population, the role of environmental exposures in AD is a grossly understudied arena. This workshop brings together experts in the field of toxicology, neuroscience, and epidemiology to highlight the potential mechanisms by which environmental exposures contribute to AD. Experimental design and cutting-edge technologies relevant to discerning environmental influences on AD will also be discussed. The workshop contains presentations and a roundtable discussion that will address five primary questions: (1) What epidemiological strategies are likely to provide the most robust information on the association between AD and environmental exposures? (2) What information can we apply to AD from experiences studying the role of environmental exposures in other neurodegenerative diseases? (3) What is the role of environmental exposures in the etiology of AD? (4) Do epigenetic alterations represent a mechanism by which environmental exposures contribute to AD? (5) Does regulation of protein aggregation and transport of pathogenic proteins by environmental exposures contribute to the progression of AD?
The interaction of senescent-related, genetic, and environmental factors likely contributes to the etiology of late-onset sporadic Alzheimer’s disease (AD). We recently reported that serum levels of a long-lasting residue of the organochlorine pesticide DDT (p,p’DDE) were significantly higher in patients with AD. Further, those carrying a polymorphism in APOE and having high serum levels of DDE performed significantly worse on the mini-mental state exam, suggesting the potential for gene-environment interactions. Mechanistically, DDT/DDE appears to increase expression of amyloid precursor protein and secretion of Aβ in cells and animals. To address the role of gene-environment interactions, APOE 3/3 or 4/4 expressing N2A cells and human neural stem cells expressing these polymorphisms are currently being used to determine the mechanistic basis of these interactions. This presentation will provide a summary of our findings to date and discuss strategies for selecting models to pursue mechanistic questions regarding gene-environment interactions in AD.

Despite several lines of evidence suggesting the involvement of metals in Alzheimer’s disease, the epidemiological research in this area is limited. We and others have explored the question of metals—in particular lead—and cognitive aging in different settings. Our work has focused on large cohorts such as the Normative Aging Study (NAS) and Nurses’ Health Study (NHS). Taking advantage of X-Ray Fluorescence techniques to analyze lead in bone—a marker of cumulative lead exposure—we have found associations between cumulative lead exposure and worse cognitive performance among both men and women, as well as modifications of these associations by genetic polymorphisms such as those in the hemochromatosis and glutathione-s-transferase genes. The extension of this kind of epidemiology to metal exposures and Alzheimer’s disease specifically has been very limited, and essentially limited to aluminum (Al) and lead (Pb). These studies, however, while sometimes suggestive, have generally suffered from methodological issues that make drawing strong inferences difficult. Important limitations of these studies include accurate and appropriate exposure assessment, and study design limitations. These are issues that are not unique to this question. Problems like accurate exposure assessment, appropriate timing of exposure assessment, selection biases in study participant selection and follow-up can all influence the strength of conclusions that can be drawn from any epidemiological study. Drawing on examples from other work of ours and others, I will discuss some of the epidemiological pitfalls of concern in the study of environmental risk factors for Alzheimer’s disease, approaches to try and avoid them, and current efforts to address the question of the role of metal exposures in Alzheimer’s disease.

The workshop will explore how these factors can be used to engineer selectivity. Specifically, recent data has shown that various aspects of ADC design play a major role in both the pharmacokinetic and toxicity profile of these molecules. The workshop will seek to explore how these factors can be used to engineer second-generation ADCs with improved therapeutic indices.

Antibody-drug conjugates (ADC) provide an exciting therapeutic opportunity to effectively treat various cancer types by combining the high specificity of an antibody with a highly potent cytotoxic agent. Therapy with highly potent cytotoxic drugs on its own is known to induce serious and often dose-limiting toxicities in multiple tissues. The promise of ADC targeted therapy concept is to improve the therapeutic index significantly by delivering the cytotoxic payload specifically to antigen expressing tumor cells while minimizing systemic exposure/ off-target toxicity. The successful introduction of T-DM1 (Kadcyla®) and brentuximab vedotin (Adcetris®) in clinical practice vitalized the area of ADC research with an increasing number of projects entering clinical development. However, these successes should not obscure the fact that there are still significant gaps in our understanding of the underlying mechanisms and pivotal factors influencing efficacy or tolerability of an ADC. Consequently, numerous setbacks still occur in (non)clinical development due to insufficient efficacy or unacceptable toxicity. The workshop will discuss recent progress and challenges in the design and development of more efficacious ADC with improved tolerability. Specifically, the workshop will discuss and provide case examples for the chemical aspects of payload design, the complex bio-analytical strategies involved in exposure characterization of the ADC and its components, the challenges of nonclinical safety assessment of an ADC with properties of large and small molecules and a regulatory perspective and lessons learned from submitted INDs.

The structural complexity of antibody-drug conjugates (ADCs) presents unique analytical challenges. In addition to any synthetic heterogeneity present, biotransformation products are also known to occur in vivo. Due to the complex and heterogeneous structures, a number of bioanalytical assay methodologies are typically used for the quantitative and qualitative analysis of the fate of these biotherapeutics in vivo. These methods incorporate strategies for both large and small molecules. Unique to ADCs, these molecules can be catabolized in a variety of ways, depending on their antibody, linker and drug components, thereby releasing distinct species that can contribute to both efficacy and toxicity. These are formed mainly by partial/full deconjugation of the linker-drug from the ADC, modification and/or release of the cytotoxic molecule payload and even its subsequent metabolism. Novel technologies beyond immunoassays are increasingly being applied to better characterize the catabolic fate of the ADC. This presentation will review bioanalytical strategies used to qualitatively assess the biotransformations undergone by the ADCs in vivo, followed by the development of novel methods for quantitative analysis of total antibody, antibody-conjugated drug and unconjugated drug levels. Specific challenges will be highlighted in the context of case examples including that applied to the drug development for the approved ADC, Kadcyla® and other ADCs with new cytotoxic payloads that are still in preclinical clinical development.

The development of antibody-drug conjugates (ADC) with new cytotoxic payloads that are still in preclinical/clinical development.
ADICs represent a real opportunity for truly targeted therapy. Arming antibodies with highly potent warheads is achieved either through random / stochastic conjugation at available cysteine or lysine residues or through site-specific modification of the antibody to engineer site(s) for conjugation. Stochastic conjugation results in an ADC with a variable drug load (DAR, drug antibody ratio), and an average DAR, with site-specific approaches generating ADICs with a specific DAR. Typically non-clinical safety studies for ADICs, are conducted in species in which binding to the target is negligible, either the ADC doesn’t bind the target with equivalent affinity to man, or the target is so tumour specific that it is not expressed in sufficient quantities or the relevant location. As such these studies often only inform about non-target related toxicity and exposure, providing limited data on target engagement. In many ways this can be considered as the worst-case scenario in terms of normal tissue exposure to ADIC. An area that will be discussed is the skin toxicity associated with many ADIC’s. Skin is one of the major sites of antibody catabolism and in the absence of target-mediated distribution, will likely represent a major target organ for ADIC’s. Ultimately these studies are designed to provide a safe starting dose in the clinic, in which the desired target is typically preferentially expressed on the tumour target. As such nonclinical safety studies for ADIC’s are really designed to provide the safest and most pragmatic / quickest route to clinical entry, after which the ultimate safety profile / therapeutic index can be determined. DAR is well known to impact on exposure and safety, with lower DAR species tending to have a more favorable profile, with the nature of the linker (cleavable vs non-cleavable) also having a significant role. Less well described is the impact of conjugation site and the impact this can have on safety, exposure and bio-distribution. Case studies will be discussed to highlight the toxicity and exposure profile of both stochastic and site-specific ADIC’s, highlighting opportunities and challenges on the route to clinical entry.

### 35 Regulatory Perspective for Nonclinical Development and Safety Assessment of Antibody-Drug Conjugates

S. Ricci, Division of Hematology Products, Office of Hematology and Oncology Products, US FDA, CDER, Silver Spring, MD. Sponsor: M. Hinrichs.

This talk will provide a regulatory perspective for nonclinical development of antibody-drug conjugates (ADC) that contain small molecule cytotoxics. The preclinical safety evaluation of ADICs present unique challenges, some of which are addressed in existing FDA or ICH Guidance but also, some that are not. For example, how can existing safety data regarding the toxicity profile of the small molecule or the naked antibody be leveraged to streamline nonclinical development of a novel ADC? How closely should the nonclinical development of an ADC follow guidance provided in ICH S6? How is the first-in-human starting dose calculated? The presentation will review regulatory strategies and key elements of nonclinical development programs for ADCs to support oncology clinical development. In addition, FDA conducted a retrospective review of mature IND applications to support ADCs to evaluate best practices regarding first-in-human starting dose selection and to better understand the translatable of animal findings to clinical experience. Results of this analysis will be presented.

### 36 Linking Early-Life Stages: The First Step toward Lifecourse Risk Assessment

S. P. Darney, Office of Research and Development, US EPA, Research Triangle Park, NC.

Adverse health effects associated with chemical exposures are often greatest during periods of growth, differentiation, and development in embryonic, fetal, infant, and/or childhood life stages. Historically, risk assessment has considered the most critical and sensitive developmental window of exposure for each individual contaminant. However, a pregnant woman experiences exposures to a complex array of environmental contaminants and may transfer them to her fetus across the placenta and/or her newborn through breast milk. Furthermore, mother and child will experience similar environmental exposures and modifying factors in their homes, schools, and communities, all of which may impact subsequent physiology, disease susceptibility, and lifelong health. To begin to address this complexity, we need ways to link exposures as they accrue and health outcomes as they emerge across time and life stages. This workshop will introduce lifecourse theory as applied to early-life stages, specifically to maternal and child health. Then experts in exposure and physiologically-based pharmacokinetic (PBPK) modeling will provide innovative approaches for predicting fetal and neonatal exposures based on the transfer of chemicals from pregnant women to their fetuses across the placenta and to their infants through breast milk, and for predicting biological effects of those exposures across life stages to adulthood. Drawing from a variety of data sources (human biomonitoring, longitudinal children’s health studies, exposure modeling, and biomarker discovery) case studies will demonstrate applications of lifecourse PBPK models to selected chemical classes. The workshop will conclude with an integrative panel discussion on how to apply lifecourse models in risk assessment. (DISCLAIMER: The views expressed in this abstract do not necessarily reflect US EPA policy).

### 37 The Lifecourse Health Development Perspective on Chemical Exposures

N. Halfon, UCLA Center for Healthier Children, Families, and Communities, Los Angeles, CA. Sponsor: S. Darney.

The Lifecourse Health Development model provides an organizing framework for synthesizing existing knowledge about life course health and depicting health as a development process influenced by multiple factors nested in various interacting physical, social and biological environments. It incorporates and builds upon emerging research from various professional fields that attempts to explain health development pathways and trajectories that are influenced by cumulative and time-specific risk, preventive and promoting factors. Some of these influences include epigenetic mechanisms triggered by environmental conditions that may alter gene expression and disease susceptibility within single or across multiple generations; critical and sensitive periods such as the perinatal stage and the effects of behavior and environmental conditions on health development during those periods; the importance of maternal health prior to pregnancy and its implications on the lifelong health of newborns; the role of the placenta in regulating the intrauterine environment; and the critical role of early mother-child interactions in establishing children’s behavioral and mental health. Lifecourse theory stresses the value of longitudinal studies and lifetime perspectives spanning from the prenatal stage to old age, and the importance of including multi-level data in developing health development trajectories and advanced complex models to predict critical pathways from environmental risk to optimal lifelong health.

### 38 PBPK Models for Human Pregnancy and Lactation Life Stages: A Case Study with PFOA and PFOS

H. H. Clewell1 and A. Loccisano1, 2The Hamner Institutes for Health Sciences, Research Triangle Park, NC and 2R.J. Reynolds Tobacco Co, Winston Salem, NC.

Risk assessment and interpretation of available human data during gestation and lactation are hindered by a lack of methods for estimating maternal, fetal, and neonatal pharmacokinetics (PK). Developmental toxicity studies in animals have raised concern about potential reproductive and developmental effects of perfluorocarboxylic acid (PFOA) and perfluorooctane sulfonate (PFOS); however, in humans conflicting results have been reported for associations between maternal plasma concentrations of PFOA and PFOS and these outcomes. We developed physiologically-based pharmacokinetic (PBPK) models for PFOA and PFOS for the gestation and lactation life stages in humans to understand how the physiological changes associated with development affect tissue distributions of these compounds in the mother, fetus, and infant. Model simulations were in good agreement with available data on PFOA and PFOS concentrations in maternal, fetal, and infant plasma, and in maternal milk. The models have been used to estimate maternal, fetal, and neonatal PFOA plasma concentrations in communities with contaminated drinking water, as well as to estimate maternal exposures from biomonitoring data. Additional data on plasma protein binding of perfluoroalkyl acids (PFAAs) and how that is affected by pregnancy, placental transfer kinetics, and renal resorption of PFAAs in pregnant and lactating women, the fetus, and infant would improve model predictions. Also, more data on PFAA concentrations in the fetus and infant would be helpful. These models are useful for comparing pharmacokinetics across life stages and can help address concerns regarding possible adverse health effects due to PFOA/PFOS exposure in the fetus and infant.
Polybrominated diphenyl ethers (PBDEs) are persistent chemicals that were widely used in consumer products for several decades. In animals, PBDEs are endocrine disruptors and neurotoxins, and increasing interest exists over their potential effects in humans. Women in the U.S. have breast milk concentrations of PBDEs that are among the highest in the world leading to concern over the potential health implications to infants during sensitive stages of development. In order to improve infant exposure assessments for PBDEs, lactational exposure models were developed to predict the concentration and distribution of PBDEs in U.S. breast milk. Congener-specific (BDE-28, -47, -85, -99, -100, -153, and -154) linear regression models were developed from existing milk and serum data. Models demonstrated high predictability as determined by internal and external validation procedures. Models were applied to nationally representative 2003–04 NHANES serum data for U.S. women of child bearing age. The highest predicted median U.S. breast milk concentrations were for BDE-47 (30.6 ng/g lipid) and BDE-99 (6.1 ng/g lipid) with the median concentration of 7PBDEs estimated at 54.2 ng/g lipid. These models provide a sustainable method for estimating population-level concentrations are available. Moreover, when applied to NHANES data, measured ABPP from humans and mice to lifestage PBPK modeling for improved predictions of internal dosimetry from pregnancy to adults, since conventional genomic and proteomic methods are poor predictors of enzyme activity.
The Tox21 Program has profiled a diverse collection of >10,000 chemicals (i.e., 10K library) across a set of nuclear receptor and stress response pathway assays at 15 concentrations, with each assay run 3 times to improve the robustness of the data. The activity profiles generated have been made public and are being analyzed in terms of structure-activity relationships and biological relevance to assess their potential to serve as predictive signatures for in-depth toxicological testing priority- itization, toxicity mechanism interpretation, and extrapolation to in vivo toxicity endpoints. The data generated on the 10K library were used to establish a “crowd- sourcing competition” for individual researchers to develop computer models to predict chemical toxicity. The challenge is to use data generated by assays in the Tox21 (Toxicology in the 21st Century) high-throughput screening program to predict how chemicals will interfere with biochemical pathways, using only their structures.

The cross-partner targeted testing working group evaluates prediction models and prioritization schemes developed from Tox21 data. Multiple Tox21 assays related to estrogen signaling were used to develop prediction models of estrogen receptor agonism or antagonism. Results from 1777 test compounds were used to populate the models and a database of highly curated agonism or antagonism. The purpose of Tox21 Phase III, initiated in 2013, is to overcome these limitations by incorporating into the testing strategy more physiologically-relevant cell types (e.g., HepaRG cells, ES and iPSC-differentiated cell populations) and lower organisms (e.g., zebrafish, C. elegans), coupled to high content screening and high throughput transcriptomics platforms to assess chemical toxicity potential. Equally important are continuing efforts to make all data public and to increase stakeholder involvement by establishing formal and informal relationships with investigators and/or organizations interested in contributing to this effort.

As the Tox21 collaboration approaches its decade-long anniversary, its longer term importance are continuing efforts to make all data public and to increase stakeholder involvement by establishing formal and informal relationships with investigators and/or organizations interested in contributing to this effort.
Exposure to environmental pollutants such as smoking and air pollution while pregnant is associated with reproducible epigenetic changes that may be a consequence of DNA damage, or may be part of the non-genotoxic mechanisms of carcinogenesis. Our recent studies provide critical additional insights into linkages between genotoxic and epigenetic mechanisms of carcinogenesis. First, using a multi-strain mouse model of the human population, we showed that important inter-individual (e.g., inter-strain) differences exist in both genotoxic and epigenotoxic effects of the classic genotoxic carcinogen 1,3-butanediol and other chemicals. Second, we confirmed the hypothesis that the chromatin remodeling response is the underlying mechanism for the inter-strain differences in butadiene-induced DNA damage. These novel findings demonstrate the mechanistic linkages between the genome (e.g., DNA sequence variants), epigenome (e.g., chromatin status and non-coding RNAs), and molecular initiating events (e.g., DNA damage) elicited by a classic genotoxic carcinogen butadiene.

Polyaromatic hydrocarbons (PAHs) are highly toxic, small molecular weight compounds that form during incomplete combustion of organic matter, such as diesel fuel, coal, wood or tobacco, and their inhalation is associated with increased severity of atopic diseases. Inhaled PAHs are metabolized and then activated in forming DNA-damaging molecules, which has the potential to impair immune function. However, the precise molecular and cellular mechanisms through which PAHs negatively impact immunity remain enigmatic. Our recent studies demonstrate how ambient PAH exposures mediate changes in systemic immunity through epigenetic modifications of key regulatory and effector immune loci that are involved in atopy. Studying individual allergic children (those with asthma and/or allergic rhinitis) from an area with increased ambient air pollution levels, including PAHs (Fresno, CA), we found that higher than average PAH exposure significantly associated with sustained diminished humoral and cellular immunity and atopy in children. Compellingly, this dysregulated immune function directly associated with increased methylation of Cpg sites in a key locus for regulatory T cell (Treg) function, forkhead box protein 3 (FOXP3), and the locus for the characteristic Th1 cytokine, interferon gamma (IFNG). Moreover, these epigenetic changes were sustained over time and associated with more pronounced cellular functional changes, specifically Treg dysfunction and the promotion of a characteristic Th2-polarizing inflammatory environment, in atopic children. These findings highlight how epigenetic changes mediated by ambient air pollutants can have long-term immunomodulatory effects and exacerbate atopic diseases.

The human placental barrier plays a significant role in modulating fetal exposure to xenobiotics in a gestational age dependent manner. Significant components of this barrier are the influx (e.g. OCT3) and efflux transporters (e.g. P-gp and BCRP) highly expressed in the placenta. Therefore it is important to quantify the gestational age-dependent expression of these transporters and to understand the mechanisms by which their expression is regulated. A number of state-of-the-art methods have been used to assess the expression (proteomics) and function (PET imaging) of placental transporters. For example, recent research has aimed to quantify the expression of transporters in human placenta of varying gestational age using LC/MS/MS. Using this method, we have begun to quantify the expression of transporters in human placentae of different gestational ages. These data together with recombinant cell line data will allow us to predict the placental transfer of drugs. In addition, PET imaging has been used to visualize the activity of P-gp at the nonhuman primate blood-placental barrier. Inhibition of placental P-gp using the inhibitor cyclosporine A increased the AUC(fetal life)/AUC(maternal plasma) of 11(C)-verapamil (a P-gp substrate) from mid- to late gestation in nonhuman primates. Collectively, these data are being used to populate a novel PBPK model in the Unadkat laboratory to predict the fetal exposure to xenobiotics including drugs of abuse. This model will be validated with umbilical vein/maternal plasma concentration ratio obtained at term. Once validated, such a model will significantly advance our ability to predict fetal exposure to drugs and therefore provide a rationale basis for risk assessment. Supported by P01DA035207.
The breast cancer resistance protein (BCRP/ABCG2) is abundantly found on breast tissue and milk should be 1.2- to 16-times greater than the levels of perchlorate in breast milk vs. urine (Kirk et al., 2013; Téllez et al., 2005) to predict the infants ingest perchlorate in the breast milk, inhibit iodide uptake by the infant thyroid. The primary purpose of this study was to compare measured levels of perchlorate in breast milk vs. urine (Kirk et al., 2013; Téllez et al., 2005) to predictions from a published PBPK model (EPA, 2009; McNabhan et al., 2014) as a test of the model’s accuracy. Unlike previous analyses, the measured urinary excretion rates (or concentrations) were used to estimate exposure levels. The published PBPK model was found to under-predict most of the observed breast milk levels. Therefore the secondary purpose of the study was to revise model parameters for transfer of perchlorate into breast milk, to better describe the data. The range of concentrations observed in breast milk indicate that the rate constants for NIS-mediated transport in breast tissue and milk should be 1.2- to 16-times greater than that used in the current PBPK models. For a fixed perchlorate maternal exposure of 20 µg/L in drinking water, this range in transfer constants lead to predicted radio-iodide uptake inhibition (RAIU) ranging from 1.5-7.9% in the 7-day old breast-fed infant, and 1.3-6.0% in the 60-day infant.

Perchlorate is a competitive inhibitor of the sodium-iodide symporter (NIS), which transports iodide into the thyroid and other tissues in animals and humans. Iodide uptake by the thyroid is necessary for the synthesis of thyroid hormones. NIS is expressed in mammary tissue and glands, providing for iodide transfer into breast milk. Sufficient levels of iodide are necessary for the healthy development of infants. Perchlorate also competes with transfer of iodide into breast milk and has been measured in the breast milk of exposed women. Thus, perchlorate exposure can both reduce iodide transfer to suckling infants via breast milk and, because the infants ingest perchlorate in the breast milk, inhibit iodide uptake by the infant thyroid. The primary purpose of this study was to compare measured levels of perchlorate in breast milk vs. urine (Kirk et al., 2013; Téllez et al., 2005) to predictions from a published PBPK model (EPA, 2009; McNabhan et al., 2014) as a test of the model’s accuracy. Unlike previous analyses, the measured urinary excretion rates (or concentrations) were used to estimate exposure levels. The published PBPK model was found to under-predict most of the observed breast milk levels. Therefore the secondary purpose of the study was to revise model parameters for transfer of perchlorate into breast milk, to better describe the data. The range of concentrations observed in breast milk indicate that the rate constants for NIS-mediated transport in breast tissue and milk should be 1.2- to 16-times greater than that used in the current PBPK models. For a fixed perchlorate maternal exposure of 20 µg/L in drinking water, this range in transfer constants lead to predicted radio-iodide uptake inhibition (RAIU) ranging from 1.5-7.9% in the 7-day old breast-fed infant, and 1.3-6.0% in the 60-day infant.
63 Iron-Deficient Toxic Milk Leads to the “Mask” Phenotype in Hephaestin Knockout Mice


This study aimed to determine whether calorie restriction causes alteration in the mRNA levels of Mdr1a. Counterintuitive results showed that the mRNA levels of Mdr1a were increased even in the absence of the liver. This suggests that non-hepatic organs are involved in the regulation of Mdr1a expression. Our findings could have implications for the development of new therapeutic interventions targeting Mdr1a.

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66 High-Throughput Pharmacokinetic Modeling Using Computationally Predicted Parameter Values: Dissociation Constants

C. L. Strope, K. Mansouri, J. Kancherla, C. Stevens and J. F. Wambachts

This poster reports examples where relevant lung metabolism in rats was observed and the use of hepatic in-vitro systems resulted in a poor in vitro in vivo correlation (IVIVC). A common feature in the three cases here reported is the absence of significant metabolism in human lungs compared to rat. Case 1: HDAC inhibitors (compounds analogues of Apicidin, containing octanly ketone chain and imidazole group). In rat, the role of the liver and lungs was determined by comparing steady state plasma concentration after femoral vein versus portal vein or carotid artery infusion. Rat Lungs metabolism accounted for 75% and 90% of total in vivo clearance for the two compounds here reported, respectively. The residual clearance was due to hepatic metabolism. Compounds were cleared in vitro by both liver and lung microsomes in rat but only by liver microsomes in human. Case 2: Poly(ADP-ribose) polymerase 1 (PARP-1) inhibitors. A number of compounds in this program were profiled for liver microsomal stability in vitro and for rat PK in vivo. Poor IVIVC and in vivo plasma clearance in excess of hepatic blood flow was observed. Rat CYP1a1 was then identified as the major enzyme responsible for in vivo clearance in rat. Nirabar, a drug belonging to this series and currently in Phase III, shows 50% of total clearance due to extrahepatic metabolism in rat but no evidence of extrahepatic metabolism in human. Case 3: Metabotrophic glutamate receptor 4 (mGluR4) allosteric modulators. Poor IVIVC was observed in rat, with relatively stable compounds in liver microsomes and hepatocytes. For a few representative compounds, the in vivo hepatic extraction ratio (E%) was directly measured in vivo. Significant extrahepatic disposition was indeed confirmed in some cases (e.g. 77%, 91% of total clearance).

65 Extrahepatic Metabolism May Complicate the IVIVC in Rats

P. Singh and M. Fonsi. Drug Metabolism and Pharmacokinetic, CGToxLAB, Erevex, France.

This poster reports examples where relevant lung metabolism in rats was observed and the use of hepatic in-vitro systems resulted in a poor in vitro in vivo correlation (IVIVC). A common feature in the three cases here reported is the absence of significant metabolism in human lungs compared to rat. Case 1: HDAC inhibitors (compounds analogues of Apicidin, containing octanly ketone chain and imidazole group). In rat, the role of the liver and lungs was determined by comparing steady state plasma concentration after femoral vein versus portal vein or carotid artery infusion. Rat Lungs metabolism accounted for 75% and 90% of total in vivo clearance for the two compounds here reported, respectively. The residual clearance was due to hepatic metabolism. Compounds were cleared in vitro by both liver and lung microsomes in rat but only by liver microsomes in human. Case 2: Poly(ADP-ribose) polymerase 1 (PARP-1) inhibitors. A number of compounds in this program were profiled for liver microsomal stability in vitro and for rat PK in vivo. Poor IVIVC and in vivo plasma clearance in excess of hepatic blood flow was observed. Rat CYP1a1 was then identified as the major enzyme responsible for in vivo clearance in rat. Nirabar, a drug belonging to this series and currently in Phase III, shows 50% of total clearance due to extrahepatic metabolism in rat but no evidence of extrahepatic metabolism in human. Case 3: Metabotrophic glutamate receptor 4 (mGluR4) allosteric modulators. Poor IVIVC was observed in rat, with relatively stable compounds in liver microsomes and hepatocytes. For a few representative compounds, the in vivo hepatic extraction ratio (E%) was directly measured in vivo. Significant extrahepatic disposition was indeed confirmed in some cases (e.g. 77%, 91% of total clearance).

64 Calorie Restriction Significantly Decreases the Intestinal Absorption of Dioxin in Mice

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There is wide variation in how patients respond to therapeutics, both beneficially and adversely. Known factors that significantly contribute to pharmacokinetic variation include genetic variation, age, disease, drugs, and food. Our preliminary microarray studies showed that calorie restriction (CR) may alter the mRNA expression of multidrug resistance protein 1a (Mdr1a; P-glycoprotein) in mice. This study aimed to determine whether calorie restriction causes alteration in the pharmacokinetics of Mdr1a substrate dioxin. Ten week old C57BL/6 male mice were fed either an ad libitum diet or 75% of the diet (CR) for 3 weeks. To determine dioxin pharmacokinetics, mice were administered 200 ng/kg tritium-labeled dioxin by oral gavage. Blood and tissues were collected at 1, 2, 4, and 12 hrs after dioxin administration. Concentrations of dioxin in plasma and tissues were quantified by liquid scintillation counting. CR significantly decreased plasma dioxin concentrations (35-40%) at 1, 2, and 4 hrs after administration. Additionally, dioxin concentrations were 1.2 to 7.5-fold higher in the intestine of calorie restricted mice at 4 and 12 hrs after dioxin administration, respectively. CR increased mRNA expression of Mdr1a in the duodenum (5-fold), jejunum (12- fold) and ileum (5-fold) of 2,3,7,8- tetrachlorodibenzo-p-dioxin. To confirm the role of Mdr1a in the altered pharmacokinetics of dioxin in calorie restricted mice, we quantified plasma and intestine dioxin concentrations in CR or ad libitum fed Mdr1a-null mice. Thus, increased intestinal Mdr1a is responsible for decreased intestinal absorption and lower concentrations of dioxin after calorie restriction in mice. Because Mdr1a transports a wide variety of therapeutics, these results showing that calorie restriction can modulate its expression may be of clinical significance in the absorption of orally administered drugs. (Supported by NIH grant ES009649)
Animal studies of the gasoline oxygenates ethyl tert-butyl ether (ETBE) and tert-butanol (TBA) have shown similar increases in kidney weights following exposure durations of ≥13 weeks but renal tumors were only increased following exposure to TBA. In animals, ETBE is rapidly metabolized to acetaldehyde and TBA; thus, understanding the role of TBA in ETBE toxicity is of critical importance for a dose response analysis. A PBPK model was developed by adapting information from earlier PBPK models of MTBE to calculate internal dosimetrics of TBA following either ETBE or TBA exposure. This model was used to evaluate whether consistent relationships exist between internal dose and kidney effects across routes of exposure and across ETBE and TBA studies. Additionally, we sought to determine if the increased incidences in kidney tumors, which were only observed with TBA treatment, correlate with internal dosimetrics. Relative kidney weight changes were relatively consistent across routes and chemicals when compared on the basis of TBA blood concentration. This is also consistent with chronic studies of TBA and ETBE exposure demonstrating similar histopathological lesions in the kidney. Incidences of renal tubule adenomas or carcinomas across routes and chemicals did not display a consistent relationship for any internal dosimetric, which suggests that other experimental variables may play a role in renal tumor incidence for these two chemicals. These findings demonstrate the utility of this PBPK model to evaluate kidney effects across studies with different chemical treatments and different routes of exposure. The views expressed in this abstract are those of the authors and do not necessarily represent the views or policies of the U.S. Environmental Protection Agency.

This impaired long-term drug incubations required for assessment of low clearance compounds. To overcome these limitations, 3D human liver microtissues (hLiMT) were utilized, since the 3D culture configuration showed preserved cytochrome P450 activity over time and allows for long-term incubations of drugs. In this study, we tested five slowly metabolizing compounds for in vitro hepatic clearance. 3D hLiMT derived from primary hepatocytes of three donors were incubated for seven days with the test compounds Tolbutamide, Theophylline, Risperidone, Prednisolone and Midazolam. The supernatants of hLiMT were assessed for parent compound depletion, indicative for hepatic clearance. The compound elimination differed between hepatocyte donors, reflecting a different CYP activity profile. The CYP3A4 substrate Midazolam, the substrate with reported high in vitro clearance, shows a similar depletion level that that observed for Tolbutamide, the substrate with the lowest in vitro clearance (37.5 % and 38.8%, respectively) at day 7. While risperidone and prednisolone showed lower depletion level than midazolam, although comparable (31.5 % and 26.7%, respectively) that may reflect the known low to moderate expression of CYP3A4 in hepatocytes. The CYP1A2 substrate theophylline, was not metabolized by this in vitro system. In summary, this data demonstrates that 3D hLiMT are a useful system for predicting the intrinsic clearance of slowly metabolized compounds.

The aryl hydrocarbon receptor (AhR) is an important ligand-activated transcription factor. It forms a nuclear heterodimer with the AhR nuclear translocator which binds cis-acting promoter sequences to activate gene transcription. The hepatic clearance rate affects drug half-life, bioavailability, dose and dosing regimes. However, in vitro assays showed so far little predictivity for hepatic clearance, since the activity of drug metabolizing enzymes of hepatocytes in suspension and 2D cultures quickly deteriorates over time.

The significance of exon skipping is the capacity to induce protein diversity from a single selected gene. The cytochrome P-450 (CYP) enzymes metabolize a variety of overlapping substrates including endogenous steroids and fatty acids as well as xenobiotics. Members of the CYP3A family share substrate specificity and small molecule inhibitors are not isoform selective. Several synthetic oligonucleotide chemistries were evaluated following intraportal injections to compare their inhibition of cytochrome P450 3A2 (CYP3A2) in the rat. Efficacy endpoints included midazolam sleep time and erythromycin-O-deethylation, marker substrates for CYP3A4. Studies included evaluation of the area under the plasma concentration versus time (AUC). Saline control rats given 20 mg midazolam (MZ) sleep for 22 ± 1 minute and metabolize erythromycin (ER) at 124 ± 13 pmol/mg/min. Rats treated with two daily 5 mg/kg/day doses of phosphorothioate dioxynucleotides (PSO) increased MZ sleep to 35 ± 2 minutes, decreased ER to 64 ± 8 pmol/mg/min and have AUC of 245 ± 13 µg/mL/min. A PSO with a 3’ ribose sugar administered at 5 mg/kg/day show inhibition of ER to 59 ± 4 µmol/mg/min and AUC of 200 ± 85 µg/min/mL. A PSO containing two C5-propyne modified cytidines administered at 0.075 mg/kg/day show inhibition in ER to 8 ± 2 µmol/mg/min. A phosphorodiamidate morpholino oligomer (PMO) administered at 2.5 mg/ kg/day elevated MZ sleep to 33 ± 2 minutes and inhibited ER to 75 ± 10 µmol/mg/min. A PMO containing two propynyl-uridines administered at 0.5 mg/kg/day inhibited ER to 75 ± 10 µmol/mg/min. The studies reveal similarities in AUC when comparing multiple oligomer chemistries. The optimal approach to enhanced potency utilizes nucleoside base modifications. Synthetic oligonucleotide manipulation of CYP3A gene expression represents a feasible approach to selective isoform inhibition and elucidation of endogenous substrate metabolism.

The prediction of in vivo hepatic clearance by in vitro methods is important for drug discovery and development. The hepatic clearance rate affects drug half-life, bioavailability, dose and dosing regimes. However, in vitro assays showed so far little predictivity for hepatic clearance, since the activity of drug metabolizing enzymes of hepatocytes in suspension and 2D cultures quickly deteriorates over time.
Acyl Glucuronide Formation in Human and Humanized UDP-Glucuronosyltransferase (UGT) 1 Mice

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UDP-glucuronosyltransferases (UGTs) are phase II drug-metabolizing enzymes that catalyze glucuronidation of endogenous and exogenous compounds. Among 19 functional human UGTs, UGT1A family enzymes largely contribute to the metabolism of clinically used drugs. While the UGT1A locus is conserved in mammals such as humans, mice, and rats, species differences in drug glucuronidation have been reported. Recently, humanized UGT1 mice in which the original Ugt1 locus was disrupted and replaced with the human UGT1 locus (hUGT1 mice) have been developed. In this study, acyl-glucuronidation of furosemide, S-naproxen, etodolac, diclofenac, and ibuprofen was determined in human liver microsomes and in liver microsomes from hUGT1 mice. Kinetic parameters of furosemide, etodolac, diclofenac, and ibuprofen acyl-glucuronidation in hUGT1 mice were almost comparable to those in humans, rather than in regular mice. Hepatotoxicity of ibuprofen in hUGT1 mice and regular mice was further investigated by measuring serum ALT levels. ALT levels were increased at 6 hours after dosing in hUGT1 mice and at 24 hours after dosing in regular mice, indicating that the onset pattern of ibuprofen-induced liver toxicity in hUGT1 mice was different from that in regular mice. Our data suggest that hUGT1 mice are promising tools to predict not only in vivo human drug glucuronidation but also drug-induced toxicity in humans.

Role of CYP2B in Phenobarbital-Induced Hepatocyte Proliferation in Mice

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Phenobarbital (PB), a nongenotoxic carcinogen, acts as a promoter for liver tumors in rodents. The mechanism of tumor promotion by PB is not fully understood; but it has been demonstrated that activation of the constitutive androstane receptor (CAR) by PB is a critical event for PB-induced changes in hepatic gene expression and increase in hepatocyte proliferation. A characteristic effect of CAR activation by PB is the prominent induction of hepatic CYP2B2 expression; the latter has been hypothesized to be mechanistically important for PB-induced hepatocyte proliferation. This study was aimed to test this hypothesis, with use of a Cyp2b2/−/+ background, in which all Cyp2b genes are deleted. We speculated that the absence of CYP2B2 would reduce or abolish the ability of PB to induce hepatocyte proliferation. Adult wild-type C57BL/6J and C57BL/6J mice were treated with PB (50 mg/kg, i.p. once daily) for five consecutive days and killed on day 6 for analysis. As expected, the liver-to-body weight ratio, an indicator of hepatocyte proliferation, was increased (by ~45%) in WT male mice, but was unchanged in the null male mice, by the PB treatment. The rate of hepatocyte proliferation, as assessed by the fraction of BrdU-positive hepatocyte nuclei, was significantly lower in PB-treated null male mice, compared to PB-treated WT male mice; however, many proliferating hepatocytes were still detected in PB-treated null male mice, whereas few proliferating hepatocytes were detected in saline treated mice. In contrast, WT female mice were much less sensitive than WT male mice to PB-induced hepatocyte proliferation, and there was no significant difference in hepatocyte proliferation between PB-treated WT female and PB-treated null female mice. These results indicate that CYP2B2 plays a significant, but partial, role in PB-induced hepatocyte proliferation in male mice.
CYP1B1 Enhances Cell Proliferation and Metastasis through Induction of EMT and Activation of Wnt/β-catenin Signaling via Promotion of S1P Expression

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To explore the role of human CYP1B1 in cancer progression, we investigated CYP1B1 action in cells after overexpression or induction with 7,12-dimethylbenz(a)anthracene (DMBA), or inhibition with specific CYP1B1 siRNA or tetra-methoxyxystilbene (TMS), a specific inhibitor and identified that CYP1B1 promotes cell proliferation, migration, and invasion in MCF-7 and MCF-10A cells. To clarify the mechanism induced by CYP1B1, expression level of key proteins such as β-catenin, c-myc, Zeb2, and MMPs were determined subsequent to alteration of CYP1B1 expression levels. The data revealed that CYP1B1 activates β-catenin signaling and induces EMT via up-regulation of transcription for β-catenin and E-cadherin suppressors like Zeb2 and Snail1. S1P transcription factor was showed to be positively regulated by CYP1B1 and suppression of S1P expression and it postulated that S1P acts as a key mediator for CYP1B1 action. To identify whether these events were caused by CYP1B1 activity, cells were treated with 4-hydroxyestradiol (4-OHE2), and similar results were obtained with CYP1B1 overexpression. In conclusion, our data suggest that CYP1B1 promotes cell proliferation and metastasis by induction of EMT and activation of Wnt/β-catenin signaling via S1P induction.

Phase I and II Xenobiotic Biotransformation Responses in Tilapia Species from Lagos Lagoon, Nigeria

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The alarming number of legacy and emerging contaminants that have been released and are currently released through a variety of human activities into the aquatic environment has made them increasingly threatening for the health of the aquatic environment and humans. For developing countries such as Nigeria and in spite of the increasing use/release of these chemicals into the environment, systematic monitoring protocols for these chemicals are either limited or non-existent. An understanding of the presence of these chemicals in biota, such as fish, is important because they serve as food commodity and a potential source of human exposure. In this study, a total of 126 Tilapia species (Sarotherodon melanatheron, Tilapia guineensis and T. zillii) were collected from four sampling points with different loads and sources of domestic and industrial effluents across Lagos lagoon with the aim of investigating the potential modulation of the xenobiotic biotransformation pathways and subsequently establishing their biomarker responses for the Lagos Lagoon ecosystem. Hepatic mRNA expression of phase I- and II xenobiotics were analyzed using validated RT-qPCR. Elevated and significant sex-related differences in mRNA of cyp1a, cyp1b, cyp1c, ugt1, sod, ZnCu-sod, and gst were observed in fish collected at different parts of the lagoon receiving a point source of effluent discharge from a textile factory compared with the other points receiving domestic and sawmill effluents. Overall, our data indicate the suitability of xenobiotic biotransformation responses as biomarkers of exposure and effects on resident organisms from exposure to contaminated effluents discharged into Lagos lagoon.
Measurement of CYP enzyme activity is important in drug metabolism. Acetaminophen is the best example for the concept of chemically reactive metabolites as mediators of adverse drug reactions. It’s bioactivation to electrophilic N-acetyl-p-benzoquinonimine (NAPQI) is catalyzed by CYP3A4 and CYP2E1 in humans. Although there are simple HPLC-based methods for measuring their activities, these methods require high amount of tissue samples. This is a potential problem in human studies, as obtaining tissue sample is difficult. In the present study, we aimed at modifying such methods for smaller amounts of tissues, as well as increasing the analytical assurance. Eleven kidney and nine liver samples were obtained from different individuals submitted to the hospital with various diseases. Whole procedure was approved by the University Ethical Committee. Individual tissue microsomes were incubated with acetaminophen (for determination of NAPQI), nifedipin (for measurement the CYP3A4 activity) and p-nitrophenol (for measurement of CYP2E1 activity) in the presence or absence of GSH, NADPH and NAPQI generating system in 100 μL final volume. Deuteration labelled analogue of the NAPQI-SG conjugate was synthesized and added to the samples for accurate measurement. The minimized amount of microsomes was well sufficient to measure both the amount of conjugate and CYP activities. Considerable variations in the metabolic capacity of individuals were observed for both organs. Amount of NAPQI and CYP2E1 activity was well correlated in liver, while there was no correlation in kidney samples for the same enzyme. No correlation was found between the amount of NAPQI and the activity of CYP3A4 in both organs. The kinetic of formation of NAPQI was found to be dependent to NADPH in liver, while it was independent in kidney samples.
Acetaminophen (APAP) is a widely used antipyretic and analgesic drug that is a leading cause of acute liver failure due to overdose. While the mechanisms of APAP induced liver injury have been extensively studied, a better understanding of the toxicodynamics of overdose could provide the basis for new intervention approaches to improve survival. Recent advances in high-performance metabolomics (HPM) using liquid chromatography-high resolution mass spectrometry enable in-depth study of toxicant effects on metabolism. In this pilot study, HMP analysis of plasma samples from 17 APAP overdose patients was completed to test for metabolites associated with APAP. Results of this metabolome-wide association study (MWAS) showed significant associations with known APAP metabolites and other metabolites belonging to several important metabolic pathways, including cytochrome P450 drug metabolism, N-glycan biosynthesis, and biotin-dependent metabolic pathways. The preliminary findings suggest that interference with metabolism of other xenobiotics may exacerbate hepatic dysfunction and precursors to support mitochondrial function or glycan biosynthesis may prove useful. In summary, the results establish an analytic framework for simultaneous study of toxicokinetic properties involving drug metabolism and associated toxicodynamic changes to the metabolome. This enables a holistic approach to understanding chemical-induced toxicity, providing a useful platform for the study of toxicology in vivo.

Mechanisms of Regulation of Hepatic and Pulmonary Cytochrome P450A1 Enzymes by 3-Methylcholanthrene in Mice In Vivo

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3-Methylcholanthrene (MC) is one of the most potent polycyclic aromatic hydrocarbons (PAHs) present in cigarette smoke, diesel exhausts, and charbroiled meats. Cytochrome P450 (CYP) enzymes play key roles in the activation of PAHs to carcinogenic metabolites. We previously showed persistent induction of CYP1A enzymes by MC in vitro. In this study, we tested the hypothesis that MC elicits persistent induction of CYP1A1 and 1A2 in vivo by sustained transcriptional activation of the corresponding promoters. Thirty two C57B6 (WT) mice were divided into two groups. Group I was treated with vehicle corn oil (CO) (8ml/kg) and group II was treated with a single dose of MC (100mg/kg), i.p. Four animals from each group were sacrificed at 6, 12, 24, and 48 h after MC withdrawal. The mRNA levels, protein content and enzyme activities of CYP1A1 and 1A2 were determined by real-time PCR, Western blotting and fluorimetry, respectively, at different time points. In addition, the binding of MC-AHR nuclear translocator (ARNT) to the AHR responsive element (ARE) promoter region were determined by ChIP assay. The transcription of AHR was also analyzed by immunofluorescence. The ChIP experiments indicated that transcriptional activation of CYP1A1 and 1A2 occurred at 6, 12, and 24 h after MC withdrawal, respectively, but decreased at 48 h. On the other hand, the induction of CYP1A1/1A2 expression at the mRNA, protein and enzyme levels persisted for up to 48 h both in lung and liver tissues. These results suggest that transcriptional activation of CYP1A1 at 6-12 h is sufficient to result in sustained induction of CYP1A mRNA and protein expression for up to 48 h. It is also possible that AHR-independent mechanisms contributed to the sustained induction by MC of CYP1A enzymes, a phenomenon that may be of relevance to PAH-mediated carcinogenesis.

Cytochrome P450 20A1: Diversity and Potential Functions of a Major Orphan P450

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The great majority of CYP enzymes are "orphans," for which substrates and biological roles are unknown and difficult to infer. Function can be inferred for some CYPs with defined functions and with single or few orthologs occurring broadly in metazoan phylogeny. Among conserved P450s, CYP20 is particularly intriguing. CYP20A1 expression in human substantia nigra and hippocampus, and abundant maternal transcript in zebrafish egg suggests roles in brain and early development, but otherwise there is limited knowledge. Studies with zebrafish and literature and database searches are uncovering aspects of CYP20A1 suggesting avenues for de-orphanization. Zebrafish CYP20A1 cDNA was cloned, and sequence alignments show conservation of unusual β-helix and heme-binding motifs. CYP20A1 tissue expression suggests reproduction, immune, hematopoietic and neural involvement in vertebrates. Putative cis-response elements in vertebrate CYP20A1 promoters, and CYP20A1 response in zebrafish embryos exposed to various chemicals suggest regulation by agents involved with steroids, cholesterol and lipid metabolism. Expression in embryos was induced by non-dioxin-like polychlorinated biphenyls that induce steroidogenesis. Results from knockout and transgenic development of CYP20A1 in developing zebrafish delayed development, decreased visual responsiveness, and increased larval hyperactivity. These phenotypes are reminiscent of a human microdeletion syndrome involving a region that contains CYP20A1. Although absent from the Anthropoidea and Nematoda, CYP20A1-like genes occur in most animal taxa including the Ctenophora, a prebilaterian lineage. Zebrafish CYP20A1 has been expressed in E. coli and ferrets, but there are no known orthologs in mammalian species. Studies with zebrafish and literature and database searches are uncovering aspects of CYP20A1 suggesting avenues for de-orphanization. Zebrafish CYP20A1 cDNA was cloned, and sequence alignments show conservation of unusual β-helix and heme-binding motifs. CYP20A1 tissue expression suggests reproduction, immune, hematopoietic and neural involvement in vertebrates. Putative cis-response elements in vertebrate CYP20A1 promoters, and CYP20A1 response in zebrafish embryos exposed to various chemicals suggest regulation by agents involved with steroids, cholesterol and lipid metabolism. Expression in embryos was induced by non-dioxin-like polychlorinated biphenyls that induce steroidogenesis. Results from knockout and transgenic development of CYP20A1 in developing zebrafish delayed development, decreased visual responsiveness, and increased larval hyperactivity. These phenotypes are reminiscent of a human microdeletion syndrome involving a region that contains CYP20A1. Although absent from the Anthropoidea and Nematoda, CYP20A1-like genes occur in most animal taxa including the Ctenophora, a prebilaterian lineage. Zebrafish CYP20A1 has been expressed in E. coli and ferrets, but there are no known orthologs in mammalian species.
were: Acetaminophen: mouse > human, no difference among the mouse variants; cyclophosphamide: WT-Mouse humanized CYP3A4 mouse > OATP1B3 mouse > human; chlorpromazine and disulfiram: Human > humanized CYP3A4 mouse > WT-mouse; and no apparent species/varient differences observed for ethacrynic acid, fluotamide and kethamine, suggesting that human CYP3A4 metabolism may be responsible for mouse-human differences in the hepatotoxicity of cyclophosphamide, chlorpromazine and disulfiram, and OATP1B3 may also be responsible for cyclophosphamide hepatotoxicity. We propose that transgenic mouse hepatocytes may be useful for the elucidation of the role of specific drug metabolizing enzyme pathways in drug toxicity.

**90 Interrogating the Mechanism of microRNA HSA-miR-29A-3P-Mediated Inhibition of CYP2C19 in Human Liver Cells**


Cytochrome P450 2C19 (CYP2C19) is involved in the metabolism of many clinical drugs. Extensive studies have demonstrated that genetic variants and environmental factors affect the expression of CYP2C19. However, the role of microRNAs (miRNAs) in controlling CYP2C19 expression is still unclear. In the present study, we performed in silico analysis to rank putative miRNA/CYP2C19 hybrids with regards to the predicted stabilities of their duplexes and then applied a series of biochemical assays to elucidate the underlying functional mechanisms for the regulation of CYP2C19 by microRNAs. In silico analysis indicated that hsa-miR-23a-3p and hsa-miR-29a-3p could target the coding region of CYP2C19 with hybrid stabilities of ~27.5 kcal/mol and ~23.3 kcal/mol, respectively. An inverse correlation between CYP2C19 mRNA expression level and hsa-miR-23a-3p level, or hsa-miR-29a-3p level was observed in human liver tissue samples. RNA electrophoresis mobility shift assays showed both hsa-miR-23a-3p and hsa-miR-29a-3p miRNAs bind directly to their cognate targets in the coding region of the CYP2C19 transcript. Further, the exogenous CYP2C19 expression in 293T cells and the endogenous CYP2C19 expression in HepaRG cells were suppressed by transfection of hsa-miR-29a-3p, but not by the transfection of hsa-miR-23a-3p. In addition, chemically-induced up-regulation of hsa-miR-29a-3p was significantly inversely correlated with CYP2C19 expression. These results demonstrated the suppressing role of hsa-miR-29a-3p on CYP2C19 expression.

**91 Activity of the MRP2/ABCC2 Efflux Transporter: Comparison of Wild-Type MRP2 and Polymorphic Variants**

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The multidrug resistance-associated protein 2 (MRP2/ABCC2) is a membrane-bound efflux pump that mediates the cellular removal of drugs, chemicals, and environmental contaminants. Single nucleotide polymorphisms (SNPs) in membrane transporters can alter pharmacokinetics, efficacy, and toxicity of substrates. We hypothesized that nonsynonymous SNPs in human MRP2 could influence efflux activity. In the present study, human MRP2 SNPs were generated using site-directed mutagenesis and stably expressed in Flp-In HEK293 cells. The total and cell surface MR2 expression and intracellular accumulation of calcine AM were quantified in cells expressing wild-type (WT) or seven polymorphic MRP2 variants. The transport of CDCF into inverted plasma membrane vesicles of WT MRP2 and its variants was also determined. Compared to WT MRP2, the total protein and mRNA expression of variants C24T, G1249A, G3542T, T3563A, C3972T and G4544A was not significantly changed. However, the C2366T variant, which is located in the ATP-binding domain, had 40-50% lower total and cell surface MR2 expression, which lead to a 3-fold decrease in the efflux of calcine AM. The accumulation of CDCF in the C2366T variant membrane vesicles was 50% lower compared to vesicles containing WT MRP2. Normalization of CDCF transport to protein expression revealed no difference in intrinsic activity suggesting that dysfunction of the MRP2 C2366T variant is a result of overall decreased protein expression. Intracellular glutathione levels were similar between cells expressing WT MRP2 and the variants. Genetic variability in the MRP2 transporter influences expression and function of substrates. The utility of this in vitro technique on prediction of substrate efficacy and toxicity requires additional study. Supported by DK080774, DK093903, ES020522, and ES00502.

**92 RNAi- and Small Molecule-Induced Inhibition of Arylamine N-Acetyltransferase 1 Reduce Anchorage Independent Growth in Breast Cancer Cell Line MDA-MB-231**

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Human arylamine N-acetyltransferase 1 (NAT1) expression is associated with various cancers and affects cell growth rate, morphology, invasiveness, and anchorage independent growth. To investigate these effects further, we stably transfected scrambled shRNA, NAT1 shRNA or NAT1 overexpression plasmids into a single FRT site into the MDA-MB-231 breast cancer cell line. The FRT site allowed insertion of expression constructs into the exact same genetic location in the host cell. We treated the MDA-MB-231 scrambled shRNA cell line with [3H]-diropogenyl] methylidenel-2-sulfanylidene-1, 3-thiazolidin-4-one (rhodamine derivative) to determine whether inhibition of NAT1 using a small molecule inhibitor would result in the same phenotype as inhibition by shRNA. Despite a substantial increase in NAT1 expression, the NAT1 overexpressing cancer cell line showed no significant changes in cell doubling time, colony forming ability, anchorage independent cell growth or relative invasive activity. Both shRNA plasmids and the small molecule inhibitor reduced NAT1 expression about 50%. Partial knockdown of NAT1 using shRNA substantially reduced anchorage independent growth without any significant effect on cell doubling time, colony forming ability or invasion across a basement membrane compared to the scrambled cell line. Inhibition with rhodamine derivative induced significant decreases in both colony forming ability and anchorage independent cell growth without an effect on invasiveness. The data suggest that colony forming assay results reflect cellular toxicity of the small molecule inhibitor and anchorage independent growth assay results reflect a combination of cellular toxicity and ability of rhodamine derivative to reduce anchorage independent growth. Taken together the knockdown of NAT1 by shRNA or rhodamine derivative reduced anchorage independent growth in breast cancer cell line MDA-MB-231.

**93 More Frequent Breast Tumors in Rapid Compared to Slow Rat Nat2 Congenic Fischer 344 Rats Administered Methylnitrosourea**

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Recent investigations suggest possible endogenous role(s) of arylamine N-acetyltransferase 1 (NAT1) in cancer progression. We conducted in vivo studies using F344 rats, congenic at the rat Nat2 locus for high (rapid) and low (slow) Nat2 activity. Rat Nat2 is a homolog for human NAT1. Methylnitrosoureas (MNU), a direct-acting alkylating agent not metabolized by N-acetyltransferase, induces breast tumors in rats. Previous pilot experiments suggested a difference in tumorigenesis in rapid and slow congenic Nat2 rats when injected at 3 weeks of age. In this study, we increased the sample size and added administration at 8 weeks of age. Rapid and slow acetylator female congenic rats were administered a single i.p. dose of MNU (50 mg/kg) and at least 3 rats per strain were given control acidified saline. MNU was administered to 44 rapid and 34 slow acetylator female congenic rats at 3 weeks of age. Rapid acetylator rats developed more palpable breast tumors over time compared to slow acetylator rats (p=0.0085). Tumors were found in 65% of the rapid compared to 56% of slow acetylator congenic rats. Tumor multiplicity and incidence did not differ significantly between rapid and slow acetylator rats. Twenty-four rapid and 33 slow acetylator female congenic rats were administered MNU at 8 weeks of age. Rapid acetylator rats developed more palpable breast tumors over time compared to slow acetylator rats (p=0.050). Tumors were found in 46% of the rapid compared to only 15% of slow acetylator congenic rats. Both tumor multiplicity (p=0.0258) and incidence (p=0.0167) differed significantly between rapid and slow acetylator rats. The results suggest an endogenous role for NAT1 in tumorigenesis that is independent of its role in carcinogen metabolism.

**94 Contribution of Gene Polymorphisms in the Folate Metabolic Pathway to Pancreatic Cancer**

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Pancreatic cancer accounts for 2% of cancer-related deaths in the United States. Several factors have been identified that modify pancreatic cancer risk including low folate intake. Besides poor nutrition and life style determinants, an individual’s folate pathway status may be influenced by genetic variants such as single nucleotide polymorphisms (SNPs) in the genes of the folate pathway. In a cohort
of pancreatic cancer patients and healthy related and unrelated controls, we first determined whether SNPs in selected genes of the folate metabolic pathway were associated with pancreatic cancer. We observed that the LR allele of the L78R SNP in choline dehydrogenase (CHDH) conferred a protective effect on pancreatic cancer development with an odds ratio (OR) of 0.29 and a 95% confidence interval (CI) of 0.12-0.76. In addition, the LR allele in combination with either the MM allele of the V212M SNP in phosphotidylethanolamine methyl transferase (PEMT) (LR/MM) or the RR allele of the Q239R SNP in betaine homocysteine methyltransferase (BHMT) (LR/RR) conferred an additional protective effect (OR=0.15; 95% CI 0.04-0.59; and OR=0.18; 95% CI 0.05-0.06, respectively). We also observed that mean red blood cell folate levels were significantly lower in pancreatic cancer cases compared to their unrelated controls (508.4 ± 215.9 vs 588.3 ± 229.2; p<0.05) while serum folate levels were not significantly different. Lastly, we found that altered serum folate concentrations were associated with the following SNPs: D919G in methylene tetrahydrofolate reductase (MTHFR). The plasma folate levels of individuals having the D919G variant were significantly higher compared to individuals homozygous for the wild type allele (p<0.05). The differences were not statistically significant due to our small sample size, but the trend was consistent. The results suggest that SNPs in the folate pathway affect folate concentrations and may modify pancreatic cancer risk.

95 Identification of ABCB1 Promoter Haplotypes and Their Effects on Placental P-gp Levels

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During gestation, the fetus is exposed to pharmaceuticals, estrogens and environmental toxicants. In utero exposure to these agents can lead to developmental and behavioral problems and diseases including cancer. Placental efflux transporters actively extrude xenobiotics from the placenta back to maternal circulation, thus reducing fetal exposure. P-glycoprotein (P-gp), encoded by the ABCB1 gene, is a major efflux transporter in the placenta. Through multiple mechanisms, single nucleotide polymorphisms (SNPs) in the ABCB1 promoter region can alter P-gp levels, subsequently affecting fetal exposure to xenobiotics. SNPs usually occur in specific combinations or haplotypes, and thus do not exert their effects individually. The transcriptional and translational effects of ABCB1 promoter haplotypes are not fully elucidated. We therefore sequenced 60 placental DNA samples to determine the ABCB1 promoter haplotype structure. We identified 8 SNPs (T-1517A, G-1459A, G-1156Aa, T-1017C, A-414G, G-240A, T-129C, and C153A) in this region. Using PHASE, we inferred 29 haplotypes ranging in frequency from 0.91 to 0.00025. In our study population 7 of these haplotypes were found as paired combinations. We hypothesize that these promoter haplotypes alter ABCB1 transcription regulation and subsequently placental P-gp levels. Comparison of placental P-gp between haplotypes indicate differences in mean protein concentration. However, due to our small sample size, the differences were not statistically significant. We are currently sequencing additional DNA samples to determine the effect of these haplotypes on basal ABCB1 mRNA levels, placental P-gp levels, and on the induction/repression of transcription when exposed to xenobiotics. The long term goal is to define the underlying mechanisms of transcription regulation and to develop biomarkers that can help tailor treatment protocols to the needs of the individual pregnant patient. (Supported by T32ES007254, P30 ES006676 and JSME bridging grant). 96 MGMT P/E Haplotypes Alter Transcription Factors’ Binding and MGMT Promoter Activity

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The O6-methylguanine-DNA methyltransferase (MGMT) removes O6-alkylguanine adducts from DNA. MGMT expression can thus alter sensitivity to chemotherapy and environmental alkylating agents. Previously we identified the haplotype structure encompassing all single nucleotide polymorphisms (SNPs) in the MGMT promoter/enhancer (P/E) region and found that these haplotypes alter MGMT promoter activity. Most SNPs identified were within or in close proximity to putative transcription factors (TFs) binding sites. In this study, we test the hypothesis that MGMT P/E haplotypes affect MGMT promoter activity by altering TF binding to the P/E region. We evaluated the effect of different P/E haplotypes on TF binding using a promoter binding TF profiling array which allows the evaluation of 48 TFs binding profiles. Our data indicate a significant difference in 28 TFs binding profiles that were haplotype dependent. These 28 TFs (which included AP1, NF-1, AP2, p53 and others), consistently showed significant binding alteration (P<0.01) depending on the MGMT P/E haplotype tested and therefore were further evaluated for their role in regulating MGMT expression using siRNA. We co-transfected TF specific siRNAs, which target each TF individually, with MGMT P/E-luciferase constructs representing different MGMT P/E haplotypes. MGMT promoter activities were then measured by the Dual-Luciferase Reporter Assay System. Our data indicate that the siRNAs tested alter MGMT promoter activity and that this regulation is haplotype dependent. These data support our hypothesis that P/E haplotypes can influence MGMT promoter activity by altering TFs binding. The observation that TFs regulatory function may be modulated by siRNAs opens the door for introducing this approach as a potential strategy to regulate MGMT expression in individuals with specific MGMT haplotypes (eg, in cancer patients when alkylation chemotherapy is warranted) (Supported by T32ES007254; P30 ES006676 and JSME bridging grants). 97 Genetic Polymorphisms and Metabolic Activities of CYP1A2, CYP2A6, XO, and NAT2 in Agricultural Communities in Eastern Washington


Diverse ethnic, environmental, and genetic backgrounds may result in differences in enzyme activities. These changes can ultimately lead to increased or decreased susceptibility to various environmental toxins and other chemicals. To better understand genetic polymorphisms and enzymatic activities of cytochrome P450 1A2 (CYP1A2), CYP2A6, xanthine oxidase (XO), and N-acetyltransferase 2 (NAT2), the methyl xanthine and methyl uric acid metabolites of caffeine were measured using an LC-MS/MS method. A total of 252 adult and children urine samples from farmworker and non-farmworker families in Yakima Valley, WA, were obtained and screened. Caffeine and its metabolites were detectable in 82.4% and 70.4% of spot urine samples from adults and children, respectively. On average, adults had lower CYP1A2 activity and higher NAT2 activity compared to that of children. Activities of CYP2A6 and XO were comparable between the two groups. When genetic polymorphisms and the caffeine urinary metabolite ratios for CYP1A2, CYP2A6, and NAT2 were compared, most of the variant alleles had decreased activity in all enzymes and had same directional changes (increasing or decreasing activities) in adults and children. In some cases (e.g. NAT*4/*5), the effect of genotype differed in adults and children. Further research is needed to validate the effects of genetic polymorphisms on the enzymatic activities of CYP1A2, CYP2A6, XO, and NAT2; however, both age and genetic variations may play an important role in understanding the specific risks to populations such as developing children in farmworker families. This work has been supported by contracts HHSN2672007023C and HHSN2752008015C from NICHD, and grants P01-ES009601 and P30-ES007033 from NIEHS, and RD-834514 from EPA. 98 Distinct Metabolic Profiles in Inbred Mouse Strains


Genetic variation may underlie some variation in response to toxicants. Differences in the metabolic response to toxicants may provide an approach to characterize the effects of genetic variation in the phenotypic response to toxicant exposure. Genetic mapping of variation in metabolic response to toxicants may be possible by taking advantage of new approaches in mouse quantitative trait loci (QTL) mapping. In order to assess the potential utility of metabolic profiling in toxicant response, we first characterized the differences in a targeted metabolomics profile between six selected genetically distinct inbred mouse strains. We assessed levels of ~200 metabolites in both serum and liver samples from mice on a defined diet using Triple Quadrupole LC/MS run and identified distinctive metabolomics profiles in each of the different mouse strains. Our finding of reproducible differences in metabolite profiles between inbred strains suggests that mapping of the underlying genetic variation responsible for the differences could be possible using a larger inbred and recombinant inbred mouse QTL mapping panel. We further suggest that metabolic profiling of toxicant response in inbred mice may similarly enable mapping of genetic variation underlying differences in metabolic response to toxicants.
TBBPA is a high-production, flame retardant and industrial chemical. In 2-year bioassay, uterus was a primary site for TBBPA tumors. Here, we performed liver transcript profiling in female Wistar Han rats (Crl:WI(Han)) treated for 15 weeks by oral gavage at 0 and 1000 mg/kg in corn oil to gain insight into TBBPA toxicology while accounting for possible effects of estrous cycling. Rats were staged by vaginal smears (n=5/group) into proestrus, estrus, and diestrus (metestrus excluded due to short duration) before necropsy, liver RNA extraction and microarray analysis. Estrous cycle stage specific hepatic transcript changes were measured with Affymetrix Rat Genome 230 2.0 arrays. No liver histopathology or microarray analysis. Estrous cycle stage specific hepatic transcript changes were measured with Affymetrix Rat Genome 230 2.0 arrays. No liver histopathology was observed as upregulation of multiple glutathione S-transferases, UDP-glucuronosyltransferases, multidrug resistance-associated protein 3, epoxide metabolism enzyme induction. Evidence for activation of an oxidative stress protective response was observed as upregulation of multiple ISGs including MX genes; consequently, uterine IFN secretion during estrous cycling may hormonally affect liver transcription. ISGs regulate endometrial receptivity to implantation as well as survival, growth, and development of conceptus but may also have systemic effects on liver. In conclusion, TBBPA-induced changes on the liver transcriptome were discrete and independent of estrous cycle. Further, potential dysregulation of interferon-mediated processes by TBBPA should be studied.

**100 Whole Genome Transcriptome Profiling of Livers from Rats Treated with the Chemopreventive Agent Oltripraz**

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Oltripraz is a dithiolethione analog that has gathered interest for its chemopreventive and anticancer properties and is currently the subject of multiple nonclinical and clinical investigations. Oltripraz can protect against hepatotoxicity induced by carbon tetrachloride, aflatoxin B1, acetaminophen, alpha-naphthylisothiocyanate and also has beneficial effects in obstructive cholestasis animal models. Here, we investigated the molecular mechanisms that promote liver detoxification using global hepatic transcriptomics profiling with Affymetrix microarrays. Male Sprague-Dawley rats (n=5/group) were dosed orally with 0, 30, 200, and 500 mg/kg/day for 5 days. Mild increases in liver weights at observed x200 mg/kg. Histologically, hepatocellular hypertrophy was apparent and correlated with ultrastructural evidence of proliferation of smooth endoplasmic reticulum. There was a dose dependent increase in global hepatic gene expression changes. Consistent with the ultrastructural changes, robust phase I/II enzyme induction was detected. Oltripraz-induced hepatic gene expression profiles were compared to a toxicogenomics database and showed mild correlation to compounds associated drug metabolism enzyme induction. Evidence for activation of an oxidative stress protective response was observed as upregulation of multiple glutathione S-transferases, UDP-glucuronosyltransferases, multidrug resistance-associated protein 3, epoxide hydrolase, and aldehyde dehydrogenases. This upregulation of oxidative stress protection genes suggests that oltripraz mediates its chemopreventative effects, in part, via a robust detoxification response induced by chemotherapeutic agents. This response should protect against reactive oxidative species and other reactive metabolites, and allow for protection from DNA damage and decreased protein adduct formation.

**101 Withaferin A is a Potent Inducer of the Nrf2-Mediated Environmental Stress Response**


BACKGROUND: Nrf2 is an inducible transcription factor that guards organisms against many forms of toxicity. This is achieved by inducing cytoprotective enzymes such as NAD(P)H:quinone oxidoreductase 1 (NQO1) to facilitate detoxication and elimination of toxins. *Withania somnifera* is a botanical that has been long used in traditional Indian medicine. Though Withaferin A (WA) isolated from *Withania somnifera* shows therapeutic activity in experimental models, it’s potential to induce Nrf2 to protect against chemical stresses is unknown. Here, we showed that WA is a potent inducer of Nrf2 leading protection of cells and organisms against chemical stresses. METHODS: Wild type (WT) and Nrf2-disrupted albino C57BL/6J mice were gavaged DMSO or 7 mg/kg WA. Human mammary epithelial MCF10A cells or WT, Nrf2-disrupted, Keap1-disrupted mouse embryonic fibroblasts (MEF) were treated with graded WA doses. Enzyme expression was quantified by qRT-PCR and western blot. Liver damage provoked by i.p administration of 300 mg/kg acetyaminophen was assessed by serum alanine aminotransferase (ALT) and histology. RESULTS: NQO1 induction observed in liver, small intestines, lung, colon and brain of WA-treated WT mice, but not in Nrf2-disrupted mice. Repeated WA dosing produced increases in cytoprotective gene expression in liver. WA administered WT mice are protected from acetaminophen hepatotoxicity as evidenced by attenuated serum ALT and liver damage. Nrf2 target gene induction observed in MCF10A (CD=80 nM), WT-MEF (CD=200 nM) and Keap1-disrupted MEF compared to MEF with Nrf2 disruption alone and MEF with both Nfr2 and Keap1 disruption. WA is more potent than sulforaphane (CD=1.5 μM) in inducing NQO1 transcripts in MCF10A. CONCLUSIONS: WA is a highly potent inducer of Nrf2 that protects cells and organisms against toxic injury in an Nrf2-dependent, Keap1-independent mechanism and may be a useful chemopreventive agent. Support: NIH R01 CA94076.

**102 Safety Assessment of Pet Food Ingredients Using Cryopreserved Canine Hepatocytes-Based In Vitro Assays**

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This research used canine hepatocytes-based in vitro assays to assess the safety screening of pet food ingredients. Dog hepatocytes were isolated and cryopreserved. Canine hepatocytes in a collagen/gelatin/matteel sandwich configuration were placed in 96-well plates and treated with denatonium benzoate (DB), green tea catechin extract (GTE), hexahydroisohumulone (HX), soybean copper phorphycillin (SCC), tetrahydroisohumulone (TRA) and xylitol (X). Acetaminophen (APAP) was used as a control. LC50 was determined using the alamar blue viability assay after 24hr. The production of reactive oxygen species (ROS) and superoxide (SO) was monitored with two fluorescent dyes for 24hr. LC50 values of individual ingredients were 0.002 (%, wt/wt), 0.004 (%, wt/wt), 0.6 (mg/mL), 3.3 (mg/mL) and 11.3 (mg/mL) for HEX, TRA, SCC, APAP and DB, respectively. However, GTE and X were not toxic in hepatocytes after 24hr. DB showed a significant concentration- and time-dependent increase in ROS production. SO production by DB increased at 4hr onwards, reaching a plateau at 18hr. However, in this study neither ROS nor SO was significantly produced by GTE, X and APAP. In conclusion, canine hepatocytes-based assays can be used to screen the toxicity of dietary food ingredients. This humane in vitro alternative model may provide a baseline for safety assessment of pet food ingredients in dogs. (This research was supported by Mars, Inc.)

**103 Exploring Phenobarbital’s Mechanism of Action in the Rat**

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Phenobarbital (PB) is a model rodent non-genotoxic carcinogen; a tumour promoter in the rat and causes hepatocellular carcinomas in the mouse, both via indirect activation of the constitutive androstane receptor (Car). It has been suggested that PB’s mechanism of action should be considered in the context of Car regulator proteins and their site of response elements and not just the species origin of the receptor (Luister et al., 2014. ToxSci. 139(2):501-511). However, the reason for PB’s species-specific carcinogenicity is still not well understood. Here, we propose that rat hepatocyte-like B13/H cells can be used to explore PB’s mechanism of action in the rat. B13/H cells function in a similar manner to competent hepatocytes (Probert et al., 2014. ToxSci. 137(2):570-70). Treatment of these cells with PB results in the significant induction of Cyp2b1 mRNA (t-test, p<0.05), a marker of PB treatment, as seen in the rat in vivo compared to control, as well as significantly increased Cyp2b activity (t-test, p<0.05) in vitro. Furthermore, B13/H cells do not show significantly altered Cyp2b activity compared to control when treated with the PB inducer, TCPOBOP, demonstrating that B13/H cells are responsive to PB’s species-specific activation mechanisms. Muroh et al., 2013. Sci. Signal. 6: ra31) showed that activation of Car in the mouse by PB is due to inhibition of the Egf receptor (Egfr). We show that treatment of B13/H cells with Egf significantly activates downstream Extracellular signal-regulated kinase (Erk) compared to control, and the Egf inhibitor erlotinib significantly abolishes this induction (one way ANOVA, p<0.05,)
while PB does not. This suggests that, in the rat, PB does not activate Car through inhibiting the Egfr, which is further corroborated by the lack of significant changes in Egrf mRNA and Egrf protein in the rat in vivo comparing PB and control. Our data suggests that the mechanism of PB's indirect activation of Car is different between the rat and the mouse, highlighting a potential factor contributing to species differences in the PB phenotype.

### 104 Differences of Metabolic Functions and Sensitivity to Chemical Compounds between Human Fetal and Adult Hepatocytes

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[Purpose] Liver blood supply is consisted of the portal vein and arteries. Portal vein carries venous blood from gastrointestinal tract which consists 75% of the liver blood supply. Liver is the first organ exposed to chemicals including xenobiotics as well as nutrients absorbed in the small intestine, which may cause liver damage. Function and susceptibility to toxicants of fetal liver is distinct from that of adult liver. In this study, the differences of the metabolic functions between fetal and adult hepatocytes were analyzed to clarify their different sensitivity to chemical compounds. [Methods] Fetal hepatocytes were prepared from six normal human fetal livers (gestation average 16 weeks). Primary adult hepatocytes were obtained from normal liver fragment of three donors. Metabolites were analyzed by CE-TOFMS. Gene expressions were analyzed by Alphymetric gene chip. [Results and Discussion] Principal component analysis of the metabolites and gene expressions clearly classified the fetal and adult hepatocytes. The amounts of most metabolites in the glycolysis/glyconeogenesis pathway, tricarboxylic acid cycle and urea cycle were lower in fetal hepatocytes than in adult hepatocytes. Toxicity tests was showed that tributyltin, acetaminophen and sodium valproate were more toxic to the fetal hepatocytes than to the adult hepatocytes, and perfluorooctanesulfonic acid that tributyltin, acetaminophen and sodium valproate were more toxic to the fetal hepatocytes than to the adult hepatocytes, and perfluorooctanesulfonic acid was vice versa. The results suggested different susceptibility of the fetal and adult liver to toxic insults may be caused by different energy metabolism. [Reference] Comparative metabolome analysis of cultured fetal and adult hepatocytes in humans. Kim, Ishida et al. J Toxicol Sci. 2014.

### 105 Cytotoxic Synergy between Cytokines and NSAIDs Associated with Idiosyncratic Hepatotoxicity Is Driven by Mitogen-Activated Protein Kinases

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Non-steroidal, anti-inflammatory drugs (NSAIDs) are among the most frequent causes of idiosyncratic, drug-induced liver injury (IDILI). Mechanisms of IDILI are unknown; however, in animal models of IDILI the cytokines tumor necrosis factor-alpha (TNF) and interferon-gamma (IFN) are essential to the development of liver injury. Some drugs associated with IDILI interact with cytokines to kill hepatocytes in vitro, and mitogen activated protein kinases (MAPKs) might play a role. Accordingly, we tested the hypothesis that MAPKs are involved in NSAID/cytokine-activated cytotoxicity in HepG2 cells. NSAIDs with high IDILI liability (diclofenac, sulindac sulfide and bromfenac) synergized with TNF to kill HepG2 cells; IFN did not have an effect. Aspirin, an NSAID, did not associated with IFN, did not interact with cytokines to kill HepG2 cells. Diclofenac and ibuprofen treatment induced prolonged activation of each MAPK (JNK, ERK and p38). Inhibition of JNK or ERK reduced cytotoxicity from cytokine interactions with NSAIDs with high IDILI liability. Conversely, an ERK inhibitor potentiated cytotoxicity from cytokine interactions with moderate IDILI liability NSAIDs. An inhibitor of p38 potentiated the cytotoxicity induced by all NSAID/cytokine combinations except naproxen. These findings raise the possibility that some IDILI reactions result from cytotoxic drug-cytokine synergy involving MAPKs. The results also suggest that, even for drugs within the same pharmacologic class, cytotoxic synergy with cytokines occurs by different mechanisms. (Supported by NIH grant DK087886 and the Colgate Palmolive Award for Research in Alternative Methods).

### 106 Berberine Alters Bile-Acid Homeostasis in Mouse Liver and Hepatocytes

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Recent studies suggested that berberine (BBR), a herbal medicine historically used as an antibiotic, may ameliorate or even prevent chemical-induced liver injury. Bile acids (BAs) play an important role in liver function. However, the effects of BBR on BA homeostasis remain largely unknown. The present studies were designed to determine the impact of BBR on BA homeostasis in both in vivo and in vitro systems. C57BL/6 mice were orally administered with either BBR (3, 10, 30, 100, and 300 mg/kg), or vehicle (saline) once daily for two weeks. Then, mouse serum and liver were collected. One portion of the liver sample was freshly fixed in 10% Zinc formalin and processed for H/E staining. We showed that 100mg/kg of BBR caused apparent blood infiltration in mouse liver. In contrast, lower doses and 300mg/kg of BBR had no effect. BAs were extracted from mouse serum and quantified using UPLC-MS/MS. Administration of 10 and 30 mg/kg of BBR increased unconjugated beta-muricholic acid (β-MCA), α-MCA and cholic acid (CA), as well as tauro-conjugated α-MCA and taurine-conjugated α-MCA. In contrast, 300mg/kg of BBR decreased unconjugated and taurine-conjugated α-MCA and DCA. In addition, in mouse liver, BBR dose-dependently increased mRNA expression of the rate-limiting enzyme of BA biosynthesis, cholesterol 7α-hydroxylase (Cyp7a1) and BA-associated transporters, including organic anion-transporting polypeptide (Oatp) 1b3, sodium-taurocholate cotransporting polypeptide (Ntcp), and bile salt export pump (Bsep). However, in cultured mouse Hepcelc7 and human Hep3B hepatoma cells, BBR only increased mRNA expression of Ntcp/NTCP, but not Cyp7a1/CYP7A1, Oatp1b1/OATP1B1 transporters (Oatp1b2 in mouse Hepa1c1c7 cells, Oatp1b1 and OATP1B3 in human Hep3B cells) or Bsep/BSEP. In conclusion, BBR altered BA homeostasis in mouse liver as well as in mouse and human hepatocytes.

### 107 Fibrinogen(ogen) Engagement of αvβ3 Integrin Limits Chronic Liver Fibrosis Induced by a Bile Duct Toxicant in Mice

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Chronic liver disease and fibrosis in humans and animal models is associated with activation of the blood coagulation cascade and deposition of cross-linked fibrin in the liver. Previous studies from our laboratory suggest that fibrin inhibits liver fibrosis caused by chronic experimental biliary injury. However, the mechanisms mediating this protective effect are not fully understood. Here, we tested the hypothesis that fibrin inhibits liver fibrosis through a mechanism requiring its binding to the leukocyte integrin αvβ3. Biliary injury and fibrosis were induced in Fbrm390-396a mice which express a mutant form of fibrinogen that has full clotting function but is incapable of binding integrin αvβ3, thereby down regulating the membrane expression of αvβ3. Clotting injury and fibrosis were assessed at 4 weeks and mice were randomly assigned to treatment groups with fibrinogen or fibrinogen depleted of αvβ3. The data suggest that fibrinogen and αvβ3 integrin are necessary for fibrinogen to inhibit liver fibrosis.

### 108 Hepatotoxic Effects of Lambda-Cyalothrin in Rats

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Lambda-cyalothrin (LCT) is a pyrethroid insecticide widely used in Egypt. The present study was designed to explore the toxic effects of commercial and technical grade LCT on adult male Sprague–Dawley rats. Rats were orally administered various doses of LCT. Animals were observed for any clinical manifestations and
were scarified at the end of the experimental period. Two weeks after daily administration, biochemical responses as well as histopathological changes were studied in rat liver. The sera were collected for estimation of hepatic enzymes (LDH, ALT and AST) activities. Liver tissue samples were obtained and divided into two parts. The first part was used for estimation of liver oxidative stress (reduced GSH and TBARS formation). The other liver tissue part was preserved in formalin and examined histopathologically. The results indicated that commercial LCT grade intoxicated rats showed mild interstitial lymphocytic aggregation and degenerative changes. In conclusion, lambda-chylorothin has hepatotoxic effects and its commercial grade has severe toxic effects than its technical grade.

**109 Kupffer Cell-Mediated Exacerbation of Methimazole-Induced Acute Liver Injury in Rats**


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Methimazole (MTZ), an antithyroid drug, is known to cause liver injury in human. We previously demonstrated that Balb/c mice with MTZ-induced liver injury was accompanied by T helper (Th) 2 cell-mediated immune responses. In the present study, we examined various innate immune responses that may be associated with MTZ-induced acute liver injury in rats as well as to assess their application in highly differentiated 3D HepG2 spheroids. When HepG2 cells cultured in a 3D hydrogel environment they stop proliferating and demonstrate strong differentiation including the expression of liver specific proteins, including albumin and cytokerone P450 enzymes as well as the formation of liver specific bile canaliculi structures which remain stable for several weeks. In this study, HepG2 cells stably expressing GFP tagged stress response proteins were cultured as 3D spheroids. We screened 32 known DILI compounds (including diclofenac, amiodarone, troglitazone) in four different stress responses (DNA damage, oxidative stress, unfolded protein response and NF-κB response) at two different time points (24 and 48 hours). We show for several compounds stress response activation in 3D cultured reporter cells which we were not able to detect in conventional 2D monolayer. Based on this data we conclude that the 3D reporter platform is a powerful tool for early toxicity screening using easy to culture and HepG2 cells with improved metabolic competence measuring early cell state changes in repeated dose exposures.

**111 Alterations of microRNA and Gene Expression in Rats Exposed to the Flame Retardant 1, 2-Dibromo-4-(1, 2-dibromomethyl)-cyclohexane (TBECH)**


TBECH is an additive flame retardant that is detectable in biota and in the environment; however, little is known about its potential health effects in humans. A 28-day subacute rodent study was undertaken to assess potential health hazards associated with exposure. Changes in microRNA (miRNA) and gene expression were analysed in conjunction with toxicological endpoints. Male and female Fischer F344 rats were treated with TBECH (10-5000 mg/kg diet) along with concomitant control. At necropsy, blood and tissues were collected for analysis. Total RNA was isolated from liver and serum and changes in miRNA expression were assessed using microRNA arrays followed by miRNA specific assays. Significant differences in animal weights and food consumption were observed at the highest dose after 7 days and 14 days. Relative liver and kidney weights were significantly affected at the 1250 mg/kg diet dose. Changes in clinical chemistry and hematological endpoints were significantly more altered in male rats. Liver miRNA expression profiles of both males and females showed increases in known regulators of liver (miR-182 and 200b-3p) and kidney (miR-96) cancers. A notable increase was observed of miR-122 in serum, a liver-specific miRNA indicative of liver damage and possible cell proliferation. Gene expression assays also showed changes in xenobiotic metabolism, cell cycle, and toxicity and stress. Our results indicate male rats were more sensitive to TBECH exposure than female rats. Apical endpoint changes related to hepatotoxicity were reflected in altered gene and miRNA expression in serum and the liver.

**110 A 3D HepG2 Spheroid Toxicity Pathway Reporter Platform to Assess DILI Liability**


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Drug-Induced Liver Injury (DILI) is a major problem in drug development and in the clinic. Currently, limited in vitro mechanistic methods are in place for high throughput toxicity testing during early drug development. The assessment of adaptive stress response activation is a likely early measurement for cellular perturbations after chemical exposure. In order to quantitatively assess the adaptive stress response activation and dynamics we developed an in vitro screening platform using HepG2 cell lines where around 6 crucial adaptive stress response proteins are stably tagged with GFP using BAC transgenomics technology. So far the activation of the individual adaptive stress responses was used in 2D HepG2 reporter cultures to classify chemical cell injury liabilities. Here we used our different reporters to assess their application in highly differentiated 3D HepG2 spheroids. When HepG2 cells are cultured in a 3D hydrogel environment they stop proliferating and demonstrate strong differentiation including the expression of liver specific proteins, including albumin and cytokerone P450 enzymes as well as the formation of liver specific bile canaliculi structures which remain stable for several weeks. In this study, HepG2 cells stably expressing GFP tagged stress response proteins were cultured as 3D spheroids. We screened 32 known DILI compounds (including diclofenac, amiodarone, troglitazone) in four different stress responses (DNA damage, oxidative stress, unfolded protein response and NF-κB response)
Emerging evidence supports a role for environmental chemical exposure in the pathogenesis of non-alcoholic fatty liver disease (NAFLD), a disease process tightly linked to increased activity of the blood coagulation cascade. Exposure of C57BL/6 mice to the persistent environmental contaminant 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) yields histopathological features of NAFLD including steatosis, hepatic injury, inflammation, and fibrosis. We examined the ability of TCDD to trigger coagulation cascade activation and the role of the thrombin receptor activated receptor-1 (PAR-1) in TCDD-elevated liver pathology. Wild-type mice were orally gavaged with TCDD (30 μg/kg) or vehicle (sesame oil) every 4 days for 28 days. Exposure was associated with marked coagulation cascade activation indicated by increased plasma thrombin-antithrombin (TAT) levels and widespread hepatic fibrin deposition. Interestingly, TCDD also increased hepatic PAR-1 mRNA expression. To determine the role of PAR-1 in TCDD-induced steatohepatitis, wild-type and PAR-1-/- mice on an equivalent C57B6/J background were treated with TCDD as described above. Of interest, PAR-1 deficiency was associated with a reduction in serum ALT activity, plasma TAT, and interleukin-6 mRNA and protein levels, indicating reduced liver inflammation and injury. Consistent with these changes, preliminary studies suggest TCDD-elevated hepatic collagen deposition was modestly reduced in PAR-1-/- mice compared to wild-type mice. Collectively, these data indicate that TCDD-elevated steatohepatitis is associated with a marked procoagulant response. Moreover, the results suggest that PAR-1 signaling may contribute to TCDD-elicited steatohepatitis.

Role of Fibrin(ogen) in Hepatocyte Proliferation after Acetaminophen Overdose

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Acetaminophen (APAP) overdose is the leading cause of drug-induced acute liver failure in the United States. APAP-induced hepatotoxicity in humans and mice is associated with activation of blood coagulation cascade and deposition of polymerized fibrin clots in the liver. Here, we sought to determine whether fibrin(ogen) contributed to hepatocyte proliferation and liver repair after APAP overdose in mice. Mice with genetically-imposed complete fibrinogen deficiency (Fib−/−) and heterozygous control mice (Fib+/−) were given a hepatotoxic dose of APAP (300 mg/kg, ip) followed by analysis of liver injury and hepatocyte proliferation at multiple time points. Hepatocyte proliferation, was markedly reduced in APAP-challenged Fib−/− mice compared to Fib+/− control mice, which paralleled elevated liver necrosis and hepatocellular damage (i.e., serum alanine aminotransferase activity) at 48 and 72 hours after APAP overdose suggesting a defect in liver repair. Next, we tested the hypothesis that fibrin(ogen) promotes hepatocyte proliferation after APAP overdose through the leukocyte integrin Mac-1/β3, binding motif, as emerging studies suggest macrophages are essential for liver repair. Here, we utilized Fib−/− mice, which express a mutant form of fibrinogen incapable of binding of αMβ2 integrin. Remarkably, molecular markers of hepatocyte proliferation were nearly absent in APAP-treated Fib−/− mice compared to APAP-treated wild-type mice, as early as 24 hours after APAP administration. Overall, the results suggest that hepatic fibrin deposition and engagement of the leukocyte αMβ3 integrin by liver macrophages is an important molecular trigger of hepatocyte proliferation and liver repair after experimental APAP overdose.

Induction of Mitochondrial Biogenesis in Acetaminophen Hepatotoxicity and Possible Role in Liver Regeneration

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Mitochondrial biogenesis (MB) is an adaptive response after mitochondrial dysfunction that helps to meet energy demands and maintain metabolic homeostasis. Numerous liver pathologies, including drug-induced hepatotoxicity, are characterized by mitochondrial dysfunction, and induction of MB has been shown to be protective in some cases. Although mitochondrial dysfunction is known to be critical in the mechanisms of acetaminophen (APAP)-induced liver injury in both mice and humans, induction of MB during APAP hepatotoxicity has not been studied. To investigate this, mice were treated with toxic doses of APAP and euthanized between 0 and 96h later. We found that APAP caused extensive mitochondrial dysfunction, indicated by reduced activity of the electron transport chain (ETC) complex I and IV and massive depletion of hepatic mtDNA from 0 to 12h. Mitochondrial function gradually recovered between 12 and 72h, as determined by the restoration of mtDNA levels and ETC activity, suggesting mitochondrial biogenesis at late time points. In addition, phosphorylation of AMPK and expression of nuclear respiratory factor-1 (Nrf-1) were rapidly induced, indicating activation of the signaling pathways of MB. Consistent with this, several mitochondrial proteins, including ETC complex IV subunit 2 and ATP synthase alpha, increased. The mitochondrial fusion protein Drp-1 also shows massive induction. Intriguingly, the increase in MB markers coincided with liver regeneration, as determined by increased expression of proliferating cell nuclear antigen (PCNA) and Cyclin D-1. The induction of MB was also seen using primary mouse hepatocytes. When we stimulated MB with the Sirtuin 1 activator SRT1720, we observed a significant increase in liver regeneration at 48h. We conclude that MB is induced following mitochondrial dysfunction in APAP hepatotoxicity, and this phenomenon may support recovery and liver regeneration.

RNA Sequencing Analysis of Primary Human Hepatocytes Exposed to PF0A or PFOS

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The environmental contaminants perfluoroanionic acid (PF0A) and perfluorooctane sulfonic acid (PFOS) have significant human health concerns. Rodent studies identify both PF0A and PFOS as possible carcinogens. However, the relevance of these data is contentious because of toxicokinetic and toxicodynamic differences between rodents and humans. We studied the effects of PF0A and PFOS on cultured primary human hepatocytes utilizing RNA sequencing analysis. Cells were exposed to 10 μM of either compound, which is a concentration associated with occupational exposure to these chemicals. Exposure to PF0A for 48 hours resulted in a decrease in 20 genes, as well as an increase in 20 genes. Exposure to PFOS for 48 hours caused significantly more changes in gene expression. PFOS caused a downregulation of 592 genes and an upregulation of 89 genes. Ingenuity Pathway Analysis (IPA) of RNA sequencing data suggested that PF0A altered the
expression of a multitude of genes involved in lipid metabolism, which could result in steatosis. IPA also suggested that the same concentration of PFOS altered the expression of genes involved in liver necrosis and tumorgenesis. The activation of the transcription factors Nanog and Sox11, both of which are associated with stem cell development, also was predicted in response to PFOS. A common factor involved in hepatic steatosis and hepatic tumorgenesis is hepatocyte nuclear factor-4α (HNF4α), which suppresses both steatosis and cancer pathogenesis. IPA analysis identified HNF4α as an upstream regulator of a multitude of transcripts changed in response to either fluorinated compound. Further analysis indicated a substantial decrease in HNF4α protein levels during exposure to either fluorinated compound, especially after PFOS treatment. Together, these data suggest that PFOS might be more hepatotoxic than PF0A, and that these compounds might promote tumorigenesis and steatosis in humans via down regulation of HNF4α.

118 Delayed DNA Repair Induces Cell Cycle Checkpoints and Delays Liver Regeneration after Acetaminophen Overdose

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Acetaminophen (APAP) overdose is a leading cause of Acute Liver Failure (ALF). Liver regeneration followed by APAP induced ALF is a deciding factor in final outcome. A novel incremental dose model involving a regenerating (300 mg/kg, APAP300) and a non-regenerating (600 mg/kg, APAP600) dose of APAP developed in our laboratory has revealed several pathways involved in regeneration after APAP overdose. Here we show that in the non-regenerating dose of APAP, DNA damage followed by inhibited DNA repair results in prolonged activation of cell cycle check point delaying liver regeneration. Liver injury, regeneration and microarray-based global gene expression changes were studied at male C57BL6 mice over time course of 0 to 72 hr following treatment with either APAP300 or APAP600. The ingenuity pathway analysis of microarray data revealed significant differences in DNA damage, replication and checkpoint related pathways between both doses of APAP. Western blot and immunofluorescence staining of PH2AX, Ser139, a hallmark of DNA double strand break, showed prolonged phosphorylation of histone H2AX in mice treated with APAP600 than APAP300. DNA repair mediator proteins 53BP1, BRCA1 were increased in APAP300 treated mice, but not in APAP600 treated mice. Further, expression of XRC4, a NHEJ repair protein was suppressed in APAP600, indicating delay or lack of DNA repair. Western blot analysis still showed that H2AX is phosphorylated at Tyr 142 when it is phosphorylated at Ser139 in APAP300. However, in APAP600 both sites remain phosphorylated at same time. It has been shown that, inability to dephosphorylate H2AX at Tyr 142 prevents DSB repair and induce cell death signal. Further, Real time PCR analysis of p53 target genes showed that p21, GADD45b were significantly induced in APAP600 for longer duration. These data illustrate that lack of prompt DSB repair response occurs after APAP overdose leading to prolonged growth arrest and may be a crucial mechanism involved in inhibition of liver regeneration.

119 Role of Annexin A1 and Calpastatin in Heteroprotection by Thioacetamide against a Lethal Dose of Acetaminophen in Mice

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Compensatory tissue repair (CTR) in thioacetamide (TA)-primed rats protects them against acetaminophen (APAP)-induced lethality. This study was aimed at further investigating the mechanisms of CTR-mediated heteroprotection in mice. Male Swiss Webster mice were primed with a low dose of TA [40 mg/kg bw, in 10 ml normal saline/kg, ip]. TA-induced liver injury, CTR, and expression of annexin A1 (ANX1) and calpastatin (CAST), the endogenous inhibitors for death proteins secretory phospholipase A2 (sPLA2) and calpain, respectively were measured over a time course of 84h after TA-priming. Both centrilobular necrosis and CTR peaked at 36h after a priming dose of TA as indicated by significant increase in alanine transaminase (ALT) and aspartate transaminase (AST) activities, histological findings, and proliferating cell nuclear antigen immunostaining. TA-priming resulted in overexpression of ANX1 and CAST at 36 to 84h and 12-84h, respectively. A lethal dose of APAP (600 mg/kg bw in 10 ml 0.45% NaCl pH 8.2, ip) was given at 36h after TA-priming. TA-priming did not affect the rise in plasma ALT, AST, sPLA2 and calpain levels seen at 2h after the APAP overdose. Neither biochemical markers of liver injury nor histology suggested any escalation of hepatic injury at later time points (12 and 24h after APAP overdose), consistent with 100% survival of the TA-primed mice as compared to non-primed mice which suffered 100% mortality. Depletion of hepatic glutathione (GSH) at 2h after APAP overdose did not affect the survival of TA-primed APAP-overdosed mice. Inhibition of ANX1 and CAST biosynthesis using cycloheximide (40 mg/kg bw in 5 ml distilled water/kg, ip) at 1h before TA-priming led to 100% mortality of APAP-overdosed mice accompanied by abrogation of ANX1 and CAST expression. In conclusion, ANX1 and CAST overexpression abolishes the expansion of liver injury after a lethal APAP overdose in mice.

120 Mechanistic Basis of Altered Morphine Disposition in Nonalcoholic Steatohepatitis

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Opioid-related adverse events occur in up to 13% of post-operative patients and may be attenuated by accounting for inter-individual variability in disposition. Morphine is metabolized to pharmacologically active morphine-6-glucuronide (M6G) and inactive morphine-3-glucuronide (M3G), both of which rely on the influx transporters Mrp2 and Mrp3 for hepatobiliary disposition. Nonalcoholic steatohepatitis (NASH) is the severe stage of nonalcoholic fatty liver disease which, along with its hallmark histopathologic features, alters the expression of biotransformation enzymes and transporters. The purpose of this study was to determine the role of NASH in the inter-individual variability in morphine disposition. Male Sprague-Dawley rats fed a control or methionine and choline deficient diet to induce NASH were administered [3H]-morphine (2.5mg/kg 30Ci/g, iv). Morphine and M3G were measured in plasma and bile over time, cumulative urine, and terminal liver and kidney. To correlate glucuronide disposition differences with pharmacodynamic effect, rats were tested for antinociceptive response over time after administration of M6G (5 mg/kg, ip). NASH decreased morphine concentrations in bile and plasma and increased the M3G/morphine plasma AUC ratio, consistent with upregulation of Ugt2b1. Despite increased systemic M6G exposure, NASH decreased biliary excretion and hepatic accumulation. This pharmacokinetic shift toward systemic retention is consistent with the mislocalization of canalicular Mrp2 and increased expression of sinusoidal Mrp3 in NASH, and may correlate to increased antinociception by the pharmacologically active M6G. This study identifies increased metabolism and altered transporter regulation in NASH as a mechanistic basis for inter-individual variability in morphine disposition that may lead to opioid-related toxicity.

121 Mri21-Facilitated Cyclin D1 Protein Surge Is Crucial for Leptin-Mediated Fibrogenesis in Disinfection Byproduct (DBP)-Induced Hepatotoxicity

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Hepatic fibrogenesis is commonly associated with chronic hepatotoxicity. Studies show that leptin has a crucial role in chronic liver injury like nonalcoholic steatohepatitis (NASH) progression and fibrogenesis. Our laboratory has reported that leptin causes an increase in mIr21, a non-coding RNA. Though the role of miR21 in cyclin D1 translation is known, upstream regulators and their effect in chronic hepatotoxicity is unclear. We hypothesized that leptin mediated mIr21 upregulation suppressed phosphatase and tensin homolog (PTEN) levels in NASH and caused higher NFκB translocation-induced cyclin D1 protein surge. We used a rodent model of toxin induced NASH that is reported by us previously. CYP2E1 ligand, Bromodichloromethane, was administered to provide the necessary “second hit” for NASH progression and to understand the role of leptin and miR21 in causing cyclin D1 surge. Results showed that wild type mice had increased levels of leptin, miR21, NFκB activation and cyclin D1 levels. High cyclin D1 levels correlated well with increased stellate cell activation (high α-SMA levels) and fibrogenesis (increased picrosirius red staining). Mice that were deficient in leptin or miR21 showed significantly decreased levels of cyclin D1 and correlated well with attenuation of NASH symptoms and decreased fibrogenesis. In conclusion, it is shown for the first time that miR21 regulates fibrogenesis at least in part by facilitating cyclin D1 protein surge primarily by NFκB activation through PTEN suppression. Targeting cyclin D1 might be a potential therapeutic option in chronic hepatotoxicity induced liver disease. (Funding: NIH-R00 ES019875 to S.C.)
This research unravels a molecular mechanism connecting water disinfection by-product bromodichloromethane (BDCM) induced nonalcoholic steatohepatitis (NASH) liver fibrogenesis and circulating leptin in mice. We have hypothesized that leptin mediates the upregulation of NADPH oxidase and its subsequent induction of miR21 (micro RNA 21) via NF-kB activation which causes upregulation of TGFβ signaling by inhibition of SMAD7. High fat (60% kCal) diet fed mice were used as a chronic model for inducing fatty liver and subsequent steatohapitic lesions following administration of BDCM. Mice deficient in genes for leptin, p47phox and miR21 were used to prove the role of leptin-NADPH oxidase-miR21 axis in NASH. Techniques like quantitative real-time PCR, dual labeling immunofluorescence microscopy, immunohistochemistry, western blot, ELISA based NF-kB activation assay were used. Results showed that wild type mice livers with NASH had increased oxidative stress, p47phox mRNA expression, NF-kB activation and miR21 levels. These mice livers showed increased TGFβ, SMAD2/3-SMAD4 and gp91phox-p47phox co-localizations, increased immunofluorescence reactivity against Col-Iα and α SMA with a decrease in SMAD7 protein level. Mice deficient in leptin and p47phox showed decreased NF-kB activation and miR21 level that suggest the role of these proteins in inducing NF-kB mediated miR21 expression. miR21 knockout mice showed decreased co-localization events of SMAD2/3-SMAD4 and gp91phox-p47phox, increased SMAD7 protein levels and decreased fibrogenesis. Our study shows a novel role of leptin mediated NADPH oxidase in modulating the actin-myosin couple, BC dynamics and clearance. They should help in the development of screening methods for early prediction of drug-induced cholestatic side-effects.
The interpretation of serum biomarkers such as alanine aminotransferase (ALT) is key to the understanding, treatment, and assessment of risk of drug-induced liver injury (DILI). Caspase-cleaved cytokeratin 18 (cK18), by reflecting apoptosis rather than necrosis, has been proposed as a useful biomarker to help interpret the significance of serum ALT elevations. However, clinical application of cK18 to DILI assessment is in its infancy. While regulatory guidelines indirectly suggest that a 3-fold elevation of ALT signals clinically important DILI, there is no similar guideline for cK18. Furthermore, there is little understanding of how much hepatocyte loss occurs when ALT or cK18 is elevated. We developed a model for cK18 in DILIsym®, a mechanistic model of DILI, and explored the predicted relationship among cK18, ALT, and hepatocellular death for a variety of injury dynamics. DILIsym® predicts that a 3-fold increase in serum ALT and a 1.5-fold increase in cK18 are indicative of similar amounts of hepatocyte death via necrosis and apoptosis respectively. This is true whether the liver injury is acute or prolonged. However, the predicted hepatocyte loss associated with a 3-fold increase in ALT and a 1.5-fold increase in cK18 is dependent on injury dynamics; in a simulated acute injury, these biomarker elevations correspond to the loss of less than 1% of viable hepatocytes, while in more prolonged injury, similar elevations correspond to a hepatocyte loss of 10%. Because cK18 has a higher baseline value than ALT, a 1.5-fold increase in cK18 is larger in absolute terms than a 3-fold increase in ALT. However, more research may be required to assess the sensitivity of cK18 measurements, with emphasis on inter- and intra-individual variability. Our predictions can provide a basis for the design of studies to qualify biomarker relationships. This study also illustrates how a mechanistic model may be applied in the rational development of quantitative guidelines for the use of novel biomarkers.
313 Resveratrol Protects against Acetaminophen Hepatotoxicity by Inducing Stress Genes and Inhibiting Release of Apoptosis-Inducing Factor (AIF) from Mitochondria

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Acetaminophen (APAP) overdose is a major cause of acute liver failure. Toxicity begins with an electrophilic metabolite that reacts with glutathione (GSH) and proteins. Protein binding damages mitochondria and causes oxidative stress. The latter activates signals that converge on the c-Jun N-terminal kinases (JNK) 1/2, which translocate into mitochondria and augment the dysfunction. Bax also translocates to mitochondria and facilitates release of the endonuclease apoptosis-inducing factor (AIF), which cleaves nuclear DNA. Eventually, the mitochondrial membrane permeability transition occurs, with release of additional mitochondria content including endonuclease G and more AIF. The result is necrosis. The natural product resveratrol (RSV) protects against APAP hepatotoxicity. However, the effects of RSV on the basic mechanisms of APAP-induced injury have not been tested. To explore this, we treated mice with 300mg/kg APAP followed by 50mg/kg RSV 1.5h later. Consistent with previous results, RSV protected at 6h (ALT:121±120 vs. 216±42 U/L) and 24h (ALT:1382±300 vs. 430±180 U/L). No differences in protein binding or total GSH degradation were observed. However, there was less glutathione disulfide (GSSG) and lower nitrotyrosine staining after RSV, indicating antioxidant effects are partially responsible. In support of this, we found modest induction of metallocationin in the RSV-treated mice. Surprisingly though, no other stress genes were induced. We also did not see a difference in JNK phosphorylation or translocation into mitochondria. Importantly, however, AIF release from mitochondria into the cytosol was inhibited with RSV, despite no difference in Smac release. These data show AIF release is selectively prevented by RSV. Consistent with this, DNA fragmentation was dramatically reduced in the RSV-treated group. Conclusion: RSV protects against APAP hepatotoxicity by inducing expression of some stress response genes and by preventing AIF release and DNA fragmentation.

314 Role of Integrin-Linked Kinase (ILK) in Liver Injury and Regeneration after Acetaminophen Overdose

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Acetaminophen (APAP) overdose is the major cause of acute liver failure in the US. Compensatory liver regeneration following APAP toxicity is critical determinant in final recovery and stimulating liver regeneration has great potential as a therapeutic strategy for APAP overdose. Extracellular matrix (EM) mediated signaling through integrin-linked kinase (ILK) is known to play important role in hepatic differentiation and in liver regeneration after partial liver resection. However, role of EM signaling via ILK in APAP toxicity and accompanying compensatory regeneration is not known. In this study, we used liver specific ILK knockout (KO) mice to study role of ILK in APAP mediated liver toxicity and compensatory regeneration. ILK KO and wild type (WT) mice were treated with 300 mg/kg APAP. Liver injury and regeneration were studied at 6 and 24hr after APAP dose. ILK KO mice showed remarkable increase in liver regeneration after APAP overdose as studied using PCNA staining and cyclinD1 expression. Studying toxicity parameters showed that improved regeneration in ILK KO mice was secondary to attenuated liver toxicity. Investigation of underlying mechanisms revealed striking decrease in JNK activation in ILK KO mice which is a key mediator of APAP toxicity. Since, effect on APAP toxicity can be mediated through alteration of APAP metabolism, we investigated status of APAP metabolism in ILK KO mice. ILK KO mice exhibited decreased APAP metabolism as seen by reduced glutathione depletion and reduced APAP-adducts formation. Further, decreased APAP metabolism was due to reduced levels of Cyp2e1 gene expression. For further study revealed EM signaling via ILK regulates Cyp2e1 expression in liver. Further, deletion of ILK in liver improved regeneration and attenuated toxicity due to decreased APAP metabolism secondarily to decreased Cyp2e1 expression. These studies indicate that ILK plays a crucial role in regulation of Cyp2e1 and may affect toxicity of several cirrhotic hepatotoxicants including APAP.
Although most antisense oligonucleotides (ASOs) are well tolerated in both mice and humans, mice tend to exhibit a mild but more pronounced innate immune response than patients after ASO treatment. This inflammatory response is typically characterized by a mild but progressive elevation in chemokines such as MIP-1β/CCL4 followed by minimal changes in inflammatory cytokines, multi-organ lymphohistocytic infiltration and splenomegaly. Here, we demonstrate that MIP-1β/CCL4 production in C57BL/6 mice is detected as early as 3 hours post ASO administration in both the plasma (protein) and liver (mRNA) but not in mRNA isolated from the spleen or peripheral blood, with maximal production by 12 hours and return to basal levels by 48 hours. While the magnitude of the MIP-1β/CCL4 production varied markedly from ASO to ASO, circulating levels correlated strongly with the severity of both splenomegaly and increase in Kupffer cell number. Using TLR9+/− mice we demonstrate that that MIP-1β/CCL4 production is dependent on TLR9 signaling, Kupffer cells and thioglycolate-elicited peritoneal exudate cells were evaluated in vitro using a panel of non-CpG ASO’s with a range of proinflammatory potential and TLR9-dependency. Those cells were capable of directly responding to ASO stimulation by producing varying levels of MIP-1β/CCL4 as well as other chemokines and cytokines demonstrating that freshly isolated macrophages can directly respond to ASOs. Together, our findings demonstrate that macrophages, and more particularly Kupffer cells, play an essential role in initiating the inflammatory response to non-CpG ASOs by producing MIP-1β/CCL4 in a TLR9 dependent mechanism.

Rationale: Chronic inflammation drives the development of many debilitating pulmonary pathologies, including fibrosis. Resident lung macrophages secrete IL-1β following exposure to toxic particles, such as silica, which drives the chronic inflammatory response. Two signals are necessary for IL-1β production: activation of the NLRP3 Inflammasome and activation of NF-kB signaling. The regulatory mechanisms of NLRP3 Inflammasome activation and secretion of IL-1β are less understood. This study investigates the contribution of autophagy to NLRP3 Inflammasome activation following silica exposure. Methods: The contribution of autophagy to NLRP3 Inflammasome activation was assessed using inhibitors Ly294002 and 3-MA in Bone Marrow derived Macrophages (BMMφ) and Alveolar Macrophages (AM) exposed to silica challenge with or without endotoxin stimulation. Autophagy induction following particle exposure was assessed using protein markers LC3-II, p62 (sequestome), total ubiquitination of protein, and using a cationic amphiphilic tracer dye. Results: Inhibition of autophagic activation potentiated NLRP3 Inflammasome activity, specifically the release of IL-1β following exposure. Autophagic markers and vesicles were increased in macrophages following silica exposure. Ubiquitinated proteins were also increased following exposure. Conclusions: Autophagy is a primary regulator of NLRP3 Inflammasome activity resulting from exposure to bioactive particles. Accumulation of ubiquitinated protein suggests autophagy is impaired, contributing to the overall inflammatory response. These results have implications in other particle-induced diseases, and re-establishing autophagic function may have therapeutic potential.

We hypothesized that stimulation of BMG with cyanobacterium Oscillatoria LPS (OscLPS) in vitro would result in classical and alternative activation and cytokine and chemokine release in a time-dependent manner. OscLPS was prepared by hot phenol/water extraction and E. coli LPS (ECLPS) 026:B6 (Difco Lab, Detroit, MI) was used as control. Neonatal rat BMG were characterized by confocal microscopy using the surface marker CD11b/c, and treated in vitro with either OscLPS (10^4 ng/mL) or ECLPS (1 ng/mL) for 3, 6, 18, 24, 48 & 72 h at 35.9 °C. Cytokine and chemokine release was quantified by Milliplex® MAP rat cytokine/chemokine multiplex immunoassays. Results: OscLPS stimulated early release (>6 h) of pro-inflammatory cytokines IL-6 and TNF-α and chemokines CINC-1(CXCL-1), MIP-1α(CCL3), MIP-2(CXCL-2); and late release (>6 h) of the anti-inflammatory cytokine IL-10, that was maximal at 72 h. In contrast, ECLPS stimulated early release (>6 h) of pro-inflammatory TNF-α and MIP-1α(CCL3), and late release (>6 h) of IL-6, CINC-1(CXCL-1), and MIP-2(CXCL-2) and (c) anti-inflammatory IL-10, that was maximal at 72 h. Our data support the hypothesis that OscLPS stimulation of BMG results in classical and alternative activation and anti-inflammatory cytokines and chemokines temporal dynamics that differed with ECLPS. Continued investigation of classical and alternative activation of BMG and sequential expression of cytokines and chemokines at the molecular level is ongoing in our laboratory. Support by Midwestern University and the University of Hawai’i at Manoa is gratefully acknowledged.

138 Autophagy Regulates NLRP3 Inflammasome Activity following Silica Exposure

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135 Liver Kupffer Cells Contribute to Early Circulating Chemokine Production after Proinflammatory Antisense Oligonucleotide Administration in Mouse


We have reported that cosmopolitan freshwater and marine Gram negative cyanobacteria Anabaena sp. LPS elicited classical and alternative activation of rat microglia (BMG) after an 18 h in vitro incubation (The Toxicologist CD 138, 2014).

134 Epigenetic Regulation of Proinflammatory Cytokines in Peripheral Blood Mononuclear Cells from Patients with Post-Traumatic Stress Disorder

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Besides the psychiatric disorder experienced by patients with Post-traumatic stress disorder (PTSD), they also suffer from deregulated immune functions. Overexpression of pro-inflammatory cytokines in the peripheral blood has been reported in correlation with chronic inflammation in PTSD patients. However, the underlying mechanism of immune dysfunction in PTSD patients remains elusive. Recent studies suggested that epigenetic regulation may play an important role in the control of gene expression of the pro-inflammatory cytokines. In this study, we first examined genome-wide histone methylation and DNA methylation in the peripheral blood mononuclear cells (PBMCs) using ChIP-seq and MeDIP-seq approaches. Significant differences in histone H3 trimethylation at K4, K9, K27 and K36 sites were identified at the global level. While overall DNA methylation level did not differ significantly between control and PTSD, the promoter of several individual genes were differentially methylated. Among them were pro-inflammatory genes IFN-γ and IL-12. Furthermore, the expression of these cytokines might be regulated by miRNAs. Our miRNA microarray study identified that many miRNAs predicted to directly interact with IFN-γ and IL-12 were down regulated in PBMCs from PTSD patients. Overall, our data suggest that the elevated expression of pro-inflammatory cytokines in PTSD patients may be regulated by multiple
epigenetic mechanisms including histone modification, DNA methylation and miRNA function. This work was supported in part by NIH grants P01AT003961, R01AT006888, R01ES019313, R01MH094755, and P20GM103641 to PN and MN.

140 GLP-1 Mimetics/DPP-4 Inhibitors: A New Role in Obesity-Associated Inflammation and Cholesterol Homeostasis

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Obesity is a major health problem worldwide associated with increased risk of diabetes, cardiovascular diseases and cancer. The mechanisms tying obesity to these health morbidities are still unknown. One hypothesis is that obesity generates a low grade inflammation. Such hypothesis is supported by increased inflammatory markers (e.g., IL-6) in obesity. However, a hallmark of obesity is perturbation of cholesterol homeostasis with high LDL and low HDL levels. There is still a paucity of effective therapeutic agents for obesity. A promising treatment strategy involves approaches to increase the level of the Glucagon-Like Peptide (GLP-1) which reduces appetite and food intake. However, GLP-1 is rapidly metabolized by the dipeptidyl peptidase-4 (DPP-4). As such, GLP-1 mimetics or drugs inhibiting DPP-4 represent a promising treatment. The effect of these approaches on cholesterol homeostasis or obesity-associated inflammation is still controversial. Members of the ATP-binding cassette (ABC) transporters such as ABCA1 and ABCG1 play important roles in modulating cholesterol levels in obesity by acting in a sequential manner to facilitate HDL-cholesterol efflux. We hypothesize that GLP-1 Mimetics/DPP-4 inhibitors will improve reverse cholesterol transport in obesity through an ABCA1/ABCG1 mediated pathway. To test our hypothesis we treated 3T3-L1 adipocytes with the GLP-1 Mimetic Exendin-4 (2 and 50 nM) and with the DPP-4 inhibitor Vildagliptin (5 and 100 nM) and measured the expression of ABCA1, ABCG1 and IL-6. Our preliminary results indicate that such treatment significantly increases ABCA1/ABCG1 expression by 2 fold (p<0.005) and decreases IL-6 expression by 1.8 fold (p<0.005). These data identify a new role for GLP-1 based therapy in modulating cholesterol homeostasis and in reducing inflammation in addition to its role in treatment of obesity (Supported by an Egyptian joint supervision grant and P30 ES006766).

141 Mice Lacking MALT-1 Protease Activity Exhibit Widespread Multiorgan Pathology and Differential Activation of Adaptive and Innate Immune System

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Mucosa-associated lymphoid tissue lymphoma-translocation gene 1 (MALT-1) is a cysteine protease that regulates nuclear factor-kB activation downstream of a diverse set of receptors including TCR, BCR, Dectin, FcεRI and GPCR and acts as a critical regulator of immune functions. To evaluate the toxicological consequences of the disruption of MALT1 function, we developed a genetically altered mouse with a single nucleotide point mutation (C464A) in the protease domain. Male and female mutant mice were smaller in size (adult body weight ~40% less than littermate controls) with multi-organ histopathologic changes including glycosogen depletion and intracellular lipid accumulation in the liver, degeneration and atrophy in the skeletal muscles, mucosal hyperplasia in the stomach and colon, and ovarian hypoplasia. Widespread inflammation observed in multiple organs, including the heart, gastrointestinal tract, skeletal muscles, and lungs (crystalline interstitial pneumonia and bronchoalveolar pneumonia), and the enhanced ability of the macrophages isolated from the mutant mice to produce interleukin-6 in response to Dectin-1 and toll-like receptor agonists suggest that a lack of MALT-1 protease function causes augmentation of the innate immunity. On the other hand, mutant mice had splenic white pulp hyperplasia and absence of germinal centers, diminished antigen dependent antibody response, and were resistant to the induction of experimental autoimmune encephalomyelitis, indicating a suppression of the adaptive immunity. These results suggest that MALT-1 protease function is critical for development and differentially contributes to innate versus adaptive immune responses. The phenotype of MALT-1 mutant mice has striking similarities to the reported disease phenotype of combined immunodeficiency patients with MALT-1 mutations.

142 Upregulated Interleukin-6 Expression Contributes to Erlotinib Resistance in Head and Neck Squamous Cell Carcinoma

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Despite the role of epidermal growth factor receptor (EGFR) signaling in head and neck squamous cell carcinoma (HNSCCC) development and progression, clinical trials involving EGFR tyrosine kinase inhibitors (TKIs) have yielded poor results in HNSCC patients. Mechanisms of acquired resistance to the EGFR TKI erlotinib was investigated by developing four erlotinib-resistant HNSCC cell lines (FaDu, Cal-27, SCC-25 and SQ20B) and comparing their gene expression profiles with their parental erlotinib-sensitive HNSCC cell lines using microarray analyses and subsequent pathway and network analyses. Interleukin-6 (IL-6) was one of thirteen genes that were significantly differentially expressed in all 4 erlotinib-resistant HNSCC cell lines compared to their parental cell lines. Additionally, pathway and network analyses of erlotinib-resistant HNSCC cells displayed a significant upregulation in immune response pathways which are known to lead to IL-6 expression. Finally, an upregulation of IL-6 expression in erlotinib-resistant HNSCC cell lines was validated using RT-PCR and ELISA and blockade of IL-6 signaling using the IL-6 receptor antagonist tocilizumab, was able to overcome erlotinib-resistance in erlotinib-resistant SQ20B tumors in vivo. Overall, erlotinib-resistant HNSCC cells display elevated IL-6 expression levels compared to erlotinib-sensitive HNSCC cells and blockade of the IL-6 signaling pathway may be an effective strategy to overcome resistance to erlotinib and possibly other EGFR TKIs for HNSCC therapy.

143 Activating Transcription Factor 3-Mediated Chemo-Intervention with Proinflammatory Signals in Colon Cancer Cells under Mucosal ER Stress

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The cell-protective features of the endoplasmic reticulum (ER) stress response are chronically activated in vigorously growing malignant tumor cells, which provide cellular growth advantages over the adverse microenvironment including chemotherapeutics. As an intervention with ER stress responses in the intestinal cancer cells, preventive exposure to flavone apigenin potentiated superinduction of a regulatory transcription factor, activating transcription factor 3 (ATF3), which is also known to be an integral player coordinating ER stress response-related gene expression. ATF3 superinduction was due to increased turnover of ATF3 protein via stabilization with HuR protein in the cancer cells under ER stress. Moreover, enhanced ATF3 caused inhibitory action against ER stress-induced cancer chemokines that are potent mediators determining the survival and metastatic potential of epithelial cancer cells. Although enhanced ATF3 was a negative regulator of the proinflammatory chemokine production were repressed by ATF3 through epigenetic regulation. Conclusively, superinduced ATF3 attenuated ER stress-induced cancer chemokine expression by epigenetically interfering with induction of EGR-1, a transcriptional modulator crucial to cancer chemokine production. Thus, these results suggest a potent therapeutic intervention of ER stress response-related cancer-favoring events by ATF3 (This work was supported by the Basic Science Research Program through the National Research Foundation of Korea, funded by Ministry of Education, Science and Technology Grant 2012R1A1A2005837).
Resveratrol (RES) is a naturally-occurring, plant-derived phytoestrogen important in plant defense. In animal models it has been shown to possess antioxidant, anti-carcinogenic and anti-inflammatory functions and is emerging as a potential therapeutic agent for the treatment of inflammatory diseases. In this study, we used a mouse model of multiple sclerosis, experimental autoimmune encephalitis (EAE), to evaluate the effects of RES on T helper cell function. We hypothesized that RES protects against neuroinflammation via regulation of encephalitogenic T cell miRNA expression. We found that RES treatment diminished clinical parameters of EAE, decreasing disease severity scores and circulating cytokine levels. RES treatment also altered immune cell composition and activation in the EAE brain. Microarray analysis of EAE brain-derived CD4+ T cells identified significant up-regulation of several miRNAs with RES treatment. In silico analysis of both predicted and validated miRNA target genes revealed considerable overlap with cell growth and proliferative functions. Focusing on miR-124 and target gene SK1, we identified alteration in the miR-124/SK1 axis specifically in CD4+ T cells from the brains of RES-treated EAE mice, with no significant changes in CD4+ T cells from the periphery. In vitro RES treatment of peripheral-derived mononuclear cells had no effect on miR-124 expression, supporting the brain-specific regulation of CD4+ T cell miRNA expression by RES. Lastly, we evaluated the consequence(s) of RES-mediated alteration of the miR-124/SK1 axis by assessing encephalitogenic CD4+ T cell functionality, including proliferation and cell cycle analysis. The current study demonstrates miR-124 plays a critical role in RES-mediated protection from neuroinflammation and that RES may be an ideal candidate for the treatment of MS and other neuroinflammatory diseases. (Supported in part by P01AT003961, R01AT006888, R01ES019313, R01MH094755, P20GM103641 & BX001357)

Molecular pathways of nonalcoholic steatohepatitis (NASH) are still evolving and there exists no proven treatment regimen in patients. Previous studies have shown that there is an activation of M1 macrophages in the NASH liver following several external or endogenous factors that can include inflammatory stimuli, oxidative stress and cytokines. However, a direct role of oxidative stress in causing M1 polarization in NASH has been unclear. We hypothesized that oxidative stress mediated by CYP2E1 causes M1 polarization in experimental NASH and NO donor administration inhibits CYP2E1 mediated inflammation with concomitant attenuation of M1 polarization. Since CYP2E1 takes center stage in these studies we use a toxin model of NASH which uses a ligand and a substrate of CYP2E1 for inducing NASH. Subsequently we use a methionine-choline deficient diet induced rodent NASH model where CYP2E1 role in its progression has been shown. Results show that CYP2E1 causes M1 polarization bias that includes a significant increase in IL1β and IL12. Nitric oxide donor administration in both models of NASH and CYP2E1 null mice or dialyl sulfide prevented it. Administration of GdC3, a macrophage toxin attenuated both the initial M1 response and subsequent M2 response showing the observed increase in cytokine levels is primarily from macrophages. Based on the evidence of an adaptive NO increase, NO donor administration in vivo, that mechanistically inhibited CYP2E1 catalyzed oxidative stress during the entire study in both models of NASH showed attenuation of M1 polarization bias and NASH progression. These results clearly suggest the role of CYP2E1 in M1 polarization and inhibition of CYP2E1 catalyzed oxidative stress by NO donor (DETA NONOate) can be a promising therapeutic strategy in NASH. (Funding: NIH R00ES019875 to SC)

The ligand-dependent transcription factor aryl hydrocarbon receptor (AhR) was originally characterized because it mediates toxicity of environmental contami-nants, such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Recently, it was demonstrated to be critical for induction of pro-inflammatory TH17 cells, demonstrating its important role in immune homeostasis. We used the mouse model of multiple sclerosis, experimental autoimmune encephalomyelitis (EAE), which is characterized by the stimulation of various T cell subsets, to evaluate other T cells that express AhR. To induce EAE, C57BL/6 mice were immunized with 100 μg myelin oligodendrocyte glycoprotein (MOG) 35-55 with complete Freund’s adjuvant (CFA) in the absence of pertussis toxin (PTx), which is well known to hasten disease, but also inhibits G protein-coupled receptors that might also be important in immune homeostasis. CD4+, CD8+, and γδ T cells exhibited MOG-specific IL-17A+AhR+ and IFN-γ+AhR+ populations in spleenocytes restimulated with 100 μg/ml MOG overnight. These results demonstrated that MOG-mediated AhR expression was increased in the stimulated T cell subsets over time, not only CD4+ TH1/TH17 and γδ T cells but also IFN-γ-producing CD8+ T cells, which results in neuroinflammation and damage of central nervous system, even in the absence of PTx. We also examined T cell populations in vitro and identified that AhR was detected in IFN-γ-producing CD8+ T cells. These results identify various AhR+ T cell populations induced in EAE without PTx, suggesting they could be targeted by alternative nontoxic AhR ligands to inhibit neuroinflammation.

Inflammation has three temporal phases, initiation, amplification and maintenance, and resolution. Inflammation resolution was thought of as a passive process but recent studies show that resolution is a complex process controlled by fatty acid derived specialized pro-resolving mediators. Chemical tissue injuries are often associated with strong and protracted inflammation. These injuries include injuries by exposures to oxidants (inhalation of chlorine gas), electrophiles (smoke inhalation and exposure to tear gas), acids (hydrochloric acid reflux injury, HCl) and skin blistering agents (vesicants, mustard gas). In the past, classical anti-inflammatory strategies were investigated as treatments, interfering with initiation and maintenance of inflammation, with mixed outcomes. We hypothesize that accelerating resolution of inflammation will attenuate the exaggerated inflammatory response following chemical exposures, leading to decreased morbidity and improved recovery. In these studies, pro-resolving agents, Resolv D1, Resolv D2, Lipoxin A4, Protecin DX, and 17(R)Resolv D1 were administered to mice at a dose rate of 2 or 5 μg/kg body weight i.p., following respiratory exposure to chlorine gas or HCl, or cutaneous exposures to a blistering agent (2-Chloroethyl ethyl sulfide) or a tear gas agent (CS). In the cutaneous injury models, pro-resolving agents decreased edema, pro-inflammatory cytokines, and vascular leakage while improving histopathological scores. In the pulmonary injury models, administration of pro-resolving mediators decreased levels of pro-inflammatory cytokines in the lung and score of reduced deterioration in lung function and tissue integrity. These results support our hypothesis and pave the way for more definitive studies in larger sample sizes and in higher mammalian species.

Redox Signaling-Induced TL4 Activation is Crucial for Disinfection Byproduct (DBP)-Mediated NASH Progression

NADPH oxidase and toll like receptor 4 (TLR4) have been implicated in NASH (nonalcoholic steatohepatitis) inflammatory pathogenesis. However, the exact role of NADPH oxidase in induction of TL4 in DBP-mediated NASH has never been shown. We hypothesize that in the liver, NADPH oxidase activation is key to TL4 recruitment to lipid rafts, that in turn upregulates nuclear transloca-tion and DNA-binding of NFκB, leading to NASH progression. A rodent model of bromodichloromethane(BDCM, a DBP)-induced NASH in obesity was used. qRTPCR results showed that livers from BDCM-exposed animals expressed significantly higher mRNA of p47phox. P47phox (cytoplasmic subunit of NADPH oxidase) colocalized with the membrane subunit gp91phox in the diseased liver as shown by immunofluorescence and confocal laser scanning microscopy. Using these techniques, expression and recruitment of TLR4 into lipid rafts were observed to be significantly higher in NASH. The described phenomenon was p47phox dependent since livers from p47phox deficient mice had markedly lower TL4 recruitment. TL4 recruitment was associated with increased NFκB activa-

144 Resveratrol Protects Mice from Experimental Multiple Sclerosis via Regulation of the miR-124/Sphingosine Kinase 1 (SK1) Axis in Encephalitogenic T Cells

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145 Characterization of AhR+ T Cell Responses during Experimental Autoimmune Encephalomyelitis (EAE) Development in the Absence of Pertussis Toxin

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146 Accelerating Inflammation Resolution to Counteract Chemical Cutaneous and Pulmonary Injury

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147 M1 Polarization Bias and Subsequent Toxicity-Induced NASH Progression Is Attenuated by Nitric Oxide Donor DETA NONOate

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The purpose of the present study was to develop a peptide for treatment of multiple sclerosis (MS). We have tested the effect of a novel antiinflammatory peptide (KGGHYAERVG, termed IIIM1) on experimental autoimmune encephalitis (EAE), an animal model of MS. Our findings demonstrate significant reduction in neurological score following oral administration of IIIM1, as compared to the control groups received the vehicle (saline). Structural studies revealed that the entire peptide is required for activity. The peptide caused significant reduction in IL17, MCP-1, MIP1-α production of a new peptide termed RA1 present in Oryza Sativa Japonica group. This Japanese rice peptide ameliorated neurological symptoms in the EAE model. Similar beneficial effect was observed upon oral administration of an extract of Japanese rice. In conclusion, oral treatment with IIIM1 ameliorates EAE symptoms via stimulation of Tregs to proliferate and produce RA1 which reduces EAE symptoms. These findings may explain the relatively low prevalence of MS in Japan and other Japanese rice-eating populations. (This work was supported in part by The Israel Science Foundation grants 747/05, 1563/13).

Obesity is characterized by chronic low-grade, systemic inflammation. CB1 receptors are present in the nervous system, fat cells, and immune cells. Treatment of mice with SR141716A, a cannabinoid receptor 1 (CB1) antagonist, shows significant reduction in food intake. We studied whether treatment with SR141716A can induce a robust anti-inflammatory response in adipose tissue. Diet-Induced Obesity (DIO) model was generated by feeding C57BL/6j mice with high-fat diet (HFD, 60% fat diet) whereas their lean, age-matched controls were fed low-fat (LFD, 10% fat diet). HFD-fed mice were treated with either SR141716A (10mg/kg/day) or vehicle by daily oral gavage for 4 weeks starting at week 12. Pair-feeding was conducted in diet-intake matched controls. Body weight, food intake as well as body composition was recorded. At week 16, mice were sacrificed and their fat pads were separated and analyzed by flow cytometry. SR141716A treatment induced significant weight loss. Body composition records showed a significant reduction in fat mass in SR141716A treated mice. To study the role of microRNA in SR141716A-modulated immune response, we performed high throughput miRNA analysis. In silico analysis showed that SR141716A skewed the adipose tissue macrophage balance to more anti-inflammatory macrophages (M2, Arginases). Induction of myeloid-derived-suppressive cells and Th2 cells followed by suppression of pro-inflammatory macrophages (M1) was observed in SR141716A-treated HFD-fed group. There was a reduction in inflammatory IL-17, MCP-1, IPI-1, and Eotaxin in serum of SR141716A treated group. Our studies demonstrated that blocking of CB1 receptors with an antagonist affects miRNA expression that induces anti-inflammatory properties in adipose tissue in DIO model and associated weight loss. Supported in part by NIH grants P01AT003961, P20GM103641, R01AT006888, R01ES019313, R01MH094755, and VA Merit Award BX001357.

DHA and LPS (p<0.0001). Western blots of nuclear fractions revealed no difference in p47phox and TLR4 null mice. Finally, TLR4 KO and p47phox KO mice or mice treated with apocynin (a NADPH oxide inhibitor) had less fibrosis and NAS scores. In conclusion, the results strongly suggest that redox signaling induced TLR-4 activation is a crucial event in the inflammatory pathogenesis of DBP-mediated NASH.

Inflammation is an important part of the innate immune response and is involved in the healing of many disease processes; however, chronic inflammation is a harmful component of many diseases, which could be exacerbated by toxic chemicals. Not all the regulatory mechanisms of inflammation have been completely understood, but one such possible regulatory mechanism is the endocannabinoid system. Endocannabinoids such as 2-arachidonoylglycerol (2-AG) and anandamide (AEA) are generally anti-inflammatory via engagement of the cannabinoid receptor 2 (CB2) on innate cells; therefore, preventing the degradation of endocannabinoids by specific serine hydrolases such as fatty acid amide hydrolase, monoacylglycerol lipase, and carboxylesterases (CES) might decrease inflammation. We hypothesized that serine hydrolase activity would decrease with a subsequent increase in 2-AG and AEA in a model of inflammation. Mice were injected with lipopolysaccharide (LPS) for 6 or 24 hr, and inflammation in the liver and spleen was confirmed by an increase in interleukin-6 (IL-6) and IL-17 gene expression at 6 hr post LPS. Activity-based protein profiling (ABPP) of serine hydrolases showed no significant difference in various serine hydrolase protein activities in the brain, liver, or spleen after LPS administration. However, 2-AG hydrolase activity in the spleen was decreased at 6 hr post LPS. ABPP-Madpit proteomic analysis suggested that the decreased 2-AG hydrolysis could be due to inhibition of CES2 activity. These studies suggest that the endocannabinoid system could be activated in response to inflammatory stimuli as one mechanism to limit inflammation. These studies also suggest that perturbation of the endocannabinoid system by toxic chemicals could alter the degree of inflammation.
Mechanical ventilation with supraphysiological concentrations of oxygen (hyperoxia) is used for the treatment of critically ill patients with respiratory failure. Unfortunately, prolonged hyperoxia causes significant pulmonary epithelial cell death. Previously, we have demonstrated a critical role for hyperoxia-activated NF-κB in protecting lung epithelial cells against oxidative injury/death in vitro. Here, we further investigated the role of NF-κB in hyperoxia-induced lung injury in vivo, using CC10-1-KoESR transgenic mice, which express a mutant version of the NF-κB inhibitor, IkB, exclusively in airway epithelial cells. Exposure to >99% O2 for 3 days caused significantly more severe lung injury in transgenic mice compared to wildtype mice. The increased severity of hyperoxic lung injury was manifest by elevated protein in the BAL and increased lung wet/dry ratio. This more severe lung injury was accompanied by a marked proinflammatory response, enhanced neutrophil infiltration, and TNF-α secretion in BAL of transgenic mice. Previously, we showed that nuclear protein HMGB1 modulates hyperoxic lung injury. Interestingly, the levels of HMGB1 in the BAL were more elevated in transgenic than wildtype mice. To explore whether airway epithelium contributes to HMGB1 release under hyperoxic conditions, we assessed HMGB1 accumulation in the culture media of normal human bronchial epithelial cells. Prolonged hyperoxic exposure induced HMGB1 translocation and consequent release into the medium. Similar to the observation in vivo, inhibition of NF-κB activation in airway epithelial with Bay 11-7082 worsened hyperoxic cell injury and increased the release of HMGB1 from these cells. These results indicate that the maintenance of airway epithelial integrity through the NF-κB signaling pathways plays a prominent role in the modulation of hyperoxia-induced inflammatory lung injury.
the biochemical differences between each route and show their sensitivities toward proinflammatory ASOs by monitoring indicators such as spleen weight increase, immune cell marker increase, and cytokine or chemokine production. These markers were examined over time in a separate kinetic study performed in C57BL/6 mice and TLR9 KO mice, demonstrating early onset of chemokine production and TLR9-dependency for most oligos tested. A few oligos could still produce substantial proinflammatory responses in the absence of TLR9, indicating that their mechanism of action is TLR9-independent. With the information gathered from in vivo studies, we tested various cell based systems to detect and study these ASOs ex vivo. We determined that established cultured cell lines do not have the sensitivity to easily detect mild proinflammatory ASOs, while primary cell lines show much more potential. More particularly, freshly isolated splenocytes and whole blood responded to these ASOs by secreting both cytokines and chemokines, thus providing a novel tool to detect mild proinflammatory ASOs and study their mechanism of action.

158 Advanced Prediction of Sensitization Potency of Chemicals: THP-1 in Coculture with HaCaT Keratinocytes

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Although several in vitro approaches address key steps of chemical-induced skin sensitization, there is uncertainty how factors such as metabolism and coinciding skin inflammation impact on chemicals’ potential and potency of dendritic cell activation. We integrated this in an in vitro model by coculturing THP-1 cells with HaCaT keratinocytes. In this study, we found high levels of interleukin (IL)-6 and 4,7-fold enhanced IL-8 in coculture supernatants, while no IL-6 was found for THP-1 cells alone. We exposed the cells to 14 sensizers (including 7 prohapten) and 10 non-sensizers for 24h. Compared to THP-1 cells alone, coculture resulted in up to 3,1-fold enhanced maximal CD86 and/or CD54 upregulation on THP-1 cells for 10 of 14 sensizers. All sensizers reached positivity for CD86 (Ameer fluorescence intensity (MFI) ≥ 10 compared to control) and/or CD54 (AMFI ≥ 50). No non-sensitizer gave a positive result regarding CD86, and only 1 non-sensitizer exceeded the threshold for CD54, resulting in an overall accuracy of 96%. Evaluating the concentrations needed to reach positivity, i.e. the potency for CD86 and/or CD54 upregulation, revealed that higher concentrations were needed for 4 of 13 sensizers, mainly hapten, and lower concentrations for 6 of 13, among them 5 prohapten, in coculture compared to THP-1 cells alone. Correlating this potency with human and animal data on sensitization potency revealed a clearly improved correlation with in vivo data compared to THP-1 cells alone. These data indicate that this coculture model has the potential to fill the gap regarding the prediction of sensitization potency.

159 Optimization of the THP-1 Activation Assay to Detect Pharmaceuticals with Potential to Cause Immune-Mediated Drug Reactions

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Despite the important health and economic impact of autoimmune or systemic allergy induced by pharmaceuticals, for such endpoints no reliable preclinical standard approaches are currently available. We have previously established an in vitro test to identify contact and respiratory allergens using the human THP-1 cell line and interleukin-8 (IL-8) production. The present study aimed to challenge this assay for the identification of pharmaceuticals known to be associated with systemic hypersensitivity reactions. THP-1 cells were exposed to drugs associated with systemic hypersensitivity reactions (streptozocin, sulfamethoxazole, neomycin, chlorodine, procainamide, ofloxacin, methyl salicylate), while metformin was used as a non-sensitizer, and D. L. Laskin and D. L. Laskin, Rutgers University, Piscataway, NJ and Robert Wood Johnson Medical School, Piscataway, NJ.

Macrophages play a dual role in the pathogenic response to ozone, contributing to both pro- and anti-inflammatory processes. Galectin-3 (Gal-3) is a lectin known to regulate macrophage activity. Herein we analyzed the role of Gal-3 in macrophage accumulation and activation in the lung in response to ozone. Bronchoalveolar lavage (BAL) and lung tissue were collected 24-72 h after exposure (3 h) of WT and Gal-3-/- mice to air or 0.8 ppm ozone. In WT mice, ozone inhalation resulted in increased numbers of proinflammatory (Gal-3+, iNOS+) and anti-inflammatory (MR-1+) macrophages in the lungs. While accumulation of iNOS+ macrophages was attenuated in Gal-3-/-mice, increased numbers of larger MR-1+ macrophages were noted. This correlated with increased numbers of macrophages in BAL. Flow cytometric analysis showed that these cells were CD11b+ and consisted of Ly6G+ and Ly6G- subpopulations. Greater than 97% of the cells were Ly6G+ macrophages, comprised of proinflammatory Ly6C+ and anti-inflammatory Ly6C- subpopulations. Most of these cells were mature F4/80(CD11c)+ lung macrophages. Treatment of WT mice with ozone resulted in increases in both Ly6G+ and Ly6C- subpopulations; loss of Gal-3 resulted in a decrease in Ly6C- macrophages, with no effects on Ly6G+ cells. Granulocytic (G) and monocytic (M) myeloid derived suppressor cells (MDSC), identified as CD11b+Ly6G+Ly6C-, were also identified in the lung after ozone. In Gal-3-/- mice, the response of GM-CSF to ozone was attenuated, while the response of M-MDSC was heightened. Changes in lung inflammatory cell populations in the lung of ozone-treated Gal-3-/- mice were correlated with reduced tissue injury measured by cytochrome b5 expression. These data demonstrate that both proinflammatory/cytotoxic and anti-inflammatory/ wound-regulating macrophages accumulate in the lung in response to ozone; moreover, Gal-3 plays a role in promoting proinflammatory macrophage activation and inflammation, which contributes to ozone toxicity. (NIH ES004738, AR055073, ES050022).

160 Untargeted Metabolomic Study by LC-QTOF/MS for the Evaluation of Effects of 12-Diindolylmethane on RAW 264.7 Murine Macrophages

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The purpose of this study is to understand how 1,1-bis(3'-indolyl)-1-(p-chlorophenyl) methane (C-DIM12) affects the metabolism of an in-vitro model for inflammation using the murine macrophage cell line RAW264.7. Diindolylmethanes are a class of synthetic compounds based on the naturally occurring 3,3'-diindolymethane, a phytochemical found in cruciferous vegetables. A library of structural analogs of 3,3'-diindolylmethane has been synthesized to optimize and study their anti-carcinogenic properties. Additionally, several studies have examined the naturally occurring 3,3'-diindolymethane for anti-inflammatory properties; however, few studies have evaluated these properties in the synthetic C-DIM compounds. To evaluate C-DIM12 for its potential anti-inflammatory properties, an untargeted metabolomics study was performed. The advantage of an untargeted metabolomics approach is obtaining a global metabolite profile that functions as a diagram of the current state of the cells, which, could, in turn, provide novel insights into the mechanism of action of C-DIM12. A method optimized specifically by our laboratory for this purpose ensures that sample preparation, chromatographic conditions and MS ionization have been refined to maximize the diversity of the detected metabolites. After statistical analysis of the relevant molecular features, a down regulation of inflammatory mediators was found and a Principal Component Analysis shows a unique set of metabolites in the cells treated with C-DIM12. Further analysis of the metabolic pathways implicated could reveal the mechanism of action of C-DIM12 and its potential as an anti-inflammatory therapeutic.

162 TNF Receptor 2 Deficiency Disrupts CD4+ T Cell Differentiation in an Experimental Adoptive Transfer of CD4+ CD45Righ T Cell Model of Colitis

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Tumor necrosis factor alpha (TNF) is key for the induction and perpetuation of inflammation. The effects of TNF are mediated through TNF receptor (TNFR) 1 or 2. Unlike TNFR1, TNFR2 expression is restricted mostly to hematopoietic and endothelial cells, does not contain a death domain, and signals mostly through...
NF-kB signaling to regulate cell survival and function. Given that these two receptors mediate different functions, a better understanding of the mechanisms underlying selective TNFR blockade is needed for improved alternative therapeutic strategies. Adoptive transfer of TNR2-/- CD4 T cells accelerates the onset of colitis, an inflammation of the colon that is controlled by environmental factors including the gut microbiome. These data and our previous observation that TNFR2 signaling augments IL-2 production, a cytokine that is essential for the induction and function of FoxP3+ Tregs, led to the hypothesis that TNFR2 may provide protection in colitis by increasing IL-2 production to control CD4 T cell differentiation in the gut. To test this hypothesis, TNFR2−/−/or interleukin-2R (IL-2R)−/−/− mice deficient in CD45R+B6 CD4 T cells were obtained from B10.A TCR-5.C7 transgenic, Rag-1−/− mice and transferred to H. hepaticus-infected B10.A Rag-1−/− host mice. While these TCR transgenic mice were generated to specifically recognize pigeon cytochrome c (PCC), they also recognize gut commensal bacteria. Our results demonstrate that adoptive transfer of IL-2−/− or TNFR2−/−/− CD4 T cells develops more severe T-cell-mediated colitis, as opposed to the IL-2−/− or IL-2−/−/− CD4 T cell transfer. Genetic deletion of TNFR2 in IL-2−/− mice did not increase disease relative to IL-2 deficiency alone. Together, these results suggest that increased IL-2 production may contribute to TNFR2-mediated protection of inflammatory colitis. 

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163 Membrane-Bound, but Not Soluble, TNF Regulates Interleukin-2 Promoter Activity in a CD4 T Cell Autonomous Manner

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The cytotoxic interleukin-2 (IL-2) is rapidly upregulated by CD4 T cells upon activation and is a critical modulator of autoimmune disease susceptibility and chronic inflammation. We have previously shown that tumor necrosis factor alpha receptor type 2 (TNFR2) signaling augments IL-2 promoter activity in CD4 T cells; however, the underlying mechanisms remain elusive. Given that membrane-bound TNF (mTNF) is superior to soluble TNF (sTNF) in activating TNFR2, the objective of this study was to test the hypothesis that mTNF costimulates IL-2 production. To test this, CD4 T cells from B6 wild-type (WT) and memTNF−/− mice were cultured with or without soluble TNF (sTNF) at concentrations ranging from 0.001 to 100 ng/ml. Following T cell stimulation, CD4+ T cells from memTNF−/− mice were cultured with or without sTNF at concentrations ranging from 0.001 to 100 ng/ml. Overall, these studies suggest a regulatory role for tmTNF, but not sTNF, in the transcription of the IL-2 gene. 

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164 Immunization Study of Keyhole Limpet Hemocyanin or Tetanus Toxoid in Cynomolgus Monkeys for Ex Vivo T-Lymphocyte Stimulation

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Enzyme-linked immunospot (ELISPOT) is a sensitive assay that has historically been used to evaluate antigen-specific T-lymphocyte responses. The ELISPOT assay is unique because it allows for the visualization of individual T-lymphocytes producing cytokine in response to antigenic challenge. With many immunomodulatory drugs in development, there is an increased need for assays that can evaluate the effects of biotherapeutics and their ability to augment or suppress antigen specific T-lymphocyte responses, as well as evaluate their mechanism of action and potential for immunotoxicity. To date, well characterized cell-based assays to evaluate cellular antigen specific responses to KLH (Keyhole limpet hemocyanin), a robust antigen, and TT (Tetanus Toxoid), a less robust antigen, have yet to be fully characterized. The aim of this study was to develop ELISPOT assays against KLH and TT antigens which could then be used to evaluate immunomodulation of antigen specific responses by biotherapeutics in preclinical PK/PD and toxicology studies. To this end, we immunized three female cynomolgus monkeys subcutaneously (SC) with 10 mg KLH and three female cynomolgus monkeys intramuscularly (IM) with 0.5 mL TT. Serum samples were collected for the assessment of traditional T-cell dependent antibody responses (TDAR) and peripheral blood mono- nuclear cell samples were collected on various days and used for the development of ELISPOT methods to assess IL-2 and IFN-γ responses to ex vivo KLH and TT antigen challenge. Various time points and antigen-challenge concentrations were evaluated to determine the time course of response and appropriate ex vivo stimulation conditions for both KLH and TT. IL-2 and IFN-γ responses were detected after ex vivo challenge with KLH and TT with greater responses to KLH than TT.

The major objective of this technique is to use smaller samples for bioanalytical and immunogenicity evaluation in an effort to refine blood collection to reduce stress on animals and reduce the number of animals used for medical research in accordance with the 3Rs initiative. Capillary microsampling provides full pharmacokinetic profiles from single animals avoiding satellite groups thereby reducing the number of animals required on study. Additionally, the technique allows for the correlation of the toxicology data with the exposure and immunogenicity data in the same animals. The aim of this study was to develop bioanalytical assays to determine Humira® concentrations and anti-Humira® antibodies in both microsamples and traditional blood samples. Humira® was administered to Sprague Dawley Rats via subcutaneous injection at 1 mg/kg and macro (0.4 mL K2EDTA) and micro (32 μl hematocrit capillary tube K2EDTA) samples were collected at matched time points post administration. The plasma concentration results of the macro versus micro samples were compared using an ECL based immunoassay qualified on the Meso Scale Discovery® instrument. Although the Cmax varied between 72 hr to 168 hr between animals, the macro and micro sample Cmax times and plasma concentrations were comparable within any single animal. All macro and micro samples collected were within the expected variability of a large molecule ligand binding assay (≤30% variability) demonstrating that capillary microsampling yields comparable pharmacokinetic results to traditional sampling methods. In addition, anti-Humira® antibodies were measured predose, 168 hr and 288 hr post dose. These data demonstrate that capillary microsampling can be an effective technique in obtaining meaningful data from one sample from the same animal.

165 A Comparison of Capillary Microsampling and Traditional Blood Sampling for the Evaluation of Bioanalysis and Immunogenicity of Humira® in Sprague-Dawley Rats

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The T-cell dependent antibody response (TDAR) to antigen (e.g. Keyhole limpet hemocyanin: KLH) is a gold standard for assessing the impact of drug on immune-competence at the preclinical stage of drug discovery. Most preclinical service providers that run TDAR model validate the antigen immunization regimen, including the sampling time course and immunosuppression effects with a known immunosuppressive drug in the strain used in toxicology programs at the provider’s site. Current TDAR models at CRL were initially validated in CD-1 mice and Sprague-Dawley (S-D) rats. However, in some cases, different strains of rodents may need to be used because of their genetic background. The magnitude of the immune responses in these animals may not necessarily be the same as those measured in the more standard strains of animals used in toxicology studies. Here, we present the differences observed in anti-KLH IgG and IgM responses in mice (CD-1 and C57BL/6N) and rats (S-D and Wistar Hannover). In mice, C57BL/6N did not respond as robustly as CD-1 animals to KLH immunization. The anti-KLH IgG2 responses were at least 1.8 fold lower than those measured in CD-1 mice. In rats, there was no clear difference in the antibody response to KLH between the two strains. Although some differences in the response between mice strains was observed, the optimal immunization regimen and sampling timepoints were similar in strains tested. However, given that the selection of animal strain can impact the magnitude of the responses this needs to be taken into account in data interpretation, especially if immunosuppression is observed or expected. Therefore it is recommended to optimize the TDAR in each animal strain in order to establish the expected range of response in untrained animals prior to testing compounds in this model.
167 Assessment of Keyhole Limpet Hemocyanin-Specific T Cell-Dependent Antibody Responses in Beagle Dogs
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T-cell-dependent antibody response (TDAR) assessment is implemented in non-clinical safety testing to evaluate potential test article-mediated effects on immune function. Robust ELISA-based assays to analyze keyhole limpet hemocyanin (KLH)-specific antibodies (Abs) in serum from beagle dogs immunized with KLH were developed. Assay optimization included determination of sample collection times (up to 28 days), capture reagent concentrations, initial reference serum (pooled serum from dogs immunized with 5 mg KLH) and detection Ab dilutions, incubation times, reference serum stability, assay precision and acceptance criteria, and method robustness. In brief, KLH (0.4 µg/well) was adsorbed overnight onto a microtiter plate followed by incubation with an initial reference serum dilution of 1:200 which was titrated (3x) to a final dilution of 1:11,809,800. Bound KLH specific-Abs were detected using alkaline phosphatase-conjugated goat anti-dog IgM or IgG. After substrate addition and colorometric analysis, an endpoint titer (EPT) was calculated using a linear interpolation method. For assay precision and acceptance criteria calculation, four 11-point dilution curves of reference serum were assayed on 3 plates per day over a 3 day period by 2 analysts. Precision estimates demonstrated that the assay is precise and robust. Flexibility of the assay was demonstrated by acceptable incubation ranges of 1.1-5.5 hours for blocking, 2.2-5.5 hours for serum binding, and 1.1-16.5 hours for secondary-antibody incubation steps. Stability of reference serum was throughout 6 freeze/thaw cycles. Implementation of the assay procedure demonstrated that intramuscular immunization with 5 mg KLH resulted in increased percent fusion of BMdM. The formation of MGC was optimized by modification of culture conditions, including alteration of the growth surface and treatment with either macrophage colony-stimulating factor (M-CSF) or granulocyte-macrophage colony-stimulating factor (GM-CSF). Ongoing studies investigating identification of MGC that regulate MGC formation. An increased understanding of this mechanism will provide additional targets to control fusion. Further development of this controlled in vitro model will facilitate future investigation of MGC inflammatory activity and contribution to pathogenesis of granulomas.

168 In Vitro Whole Blood Assay in Preclinical Safety: Correlation with IR in Clinics

Infusion related reactions (IRR) are adverse events potentially triggered by the immune system during IV administration of therapeutics. Difficult to predict, they require prompt and accurate clinical management to avoid severe complications and for treatment discontinuation. Monochlonal antibodies (mAb) have a potential for IRR caused by cytokine release but the TGN1412 case showed the limitations of animal models for prospective risk assessment with respect to the translatable to humans. To fulfill regulatory expectations, in vitro human cell-based assays such as the whole blood assay (WBA) have since become hazard identification tools for screening and in vivo tests to establish safety of a WBA with 11 commercialized mAbs and routinely used it for Roche-developed mAb. After several years, we compiled sufficient clinical data to evaluate its predictivity for IRR. Fresh undiluted human blood from 30 healthy individuals was incubated for 24h with 0.1 to 10µg/ml test mAb and subsequent release of TNF, IL-6, IL-8 and IFN was measured. Erbitux® was used as a negative comparator to establish cut-off values discriminating "positive" vs. "negative" response. MabCampath® known to induce IRR in greater than 90% recipients, was included as positive control. A risk score relating to cytokine release upon first dosing in humans for the tested mAb was calculated from the incidence and magnitude of cytokine levels relative to Erbitux. Once in clinical phases, the effective rate of IRR was used to define the assay performance. Its predictivity for clinical IRR was established based on data from 15 Roche mAbs in Ph.0/1, showing 82% accuracy, 100% sensitivity and 75% specificity. We concluded that our WBA provided safety-relevant data, enhancing decision making for clinical risk mitigation. Analyzed with immune expertise the assay successfully predicted the IRR potential of mAbs, allowing critical sites to better prepare and thereby improve treatment outcome. Therefore we will continuously update this promising first evaluation with new project data.

169 Macrophage Fusion into Multinucleated Giant Cells In Vitro
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Multinucleated giant cells (MGC) have been observed in a variety of granulomatous conditions, including microbial infections (e.g., tuberculosis), foreign body reactions to implants (e.g., medical devices), foreign body reaction to inhaled particles (e.g., engineered nanomaterials), and disorders of unknown etiology (e.g., sarcoidosis). Generally, MGC are morphologically classified based on the number and arrangement of nuclei. The two major types of MGC are foreign body giant cells and Langhans giant cells. These MGC are formed by the fusion of macrophages, often in response to persistent, foreign microorganisms or materials. Although MGC are known to be associated with granulomas, their involvement in the development of these conditions has not been well described. This is in part due to a lack of well-characterized models of MGC populations. The objective of this study is to develop an in vitro model of macrophage fusion in order to study MGC function. Previous reports have shown that MGC formation is induced by interleukin-4 (IL-4). Therefore, we investigated a model of IL-4-induced fusion in murine bone marrow-derived macrophages (BMDM). As expected, IL-4 treatment resulted in increased percent fusion of BMDM. The formation of MGC was optimized by modification of culture conditions, including alteration of the growth surface and treatment with either macrophage colony-stimulating factor (M-CSF) or granulocyte-macrophage colony-stimulating factor (GM-CSF). Ongoing studies investigated identification of MGC that regulate MGC formation. An increased understanding of this mechanism will provide additional targets to control fusion. Further development of this controlled in vitro model will facilitate future investigation of MGC inflammatory activity and contribution to pathogenesis of granulomas.

170 A Comparison of Cell-Counting Methods in Rodent Pulmonary Infusion Toxicity Studies
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Pulmonary toxicity studies use bronchoalveolar lavage (BAL) to assess lung responses to particulates. The BAL cellular fraction BAL is counted, using an automated (i.e., Coulter Counter® or flow cytometer) or manual (i.e., hemocytometer) method, to determine inflammatory cell influx. The goal is to compare different, commonly used, counting methods to determine which is optimal for examining cell influx after particular inhalations. Inhalation exposures consisted of carbon nanotubes (CNT) at 5 and 0.5 mg/m3 x 5/6x x 19d in mice and mild and stainless steel welding fume (WF) at 30 mg/m3 x 3/6x x 4d in rats. High dose CNT inhalation resulted in marked lung cytokotoxicity (3.5-fold increase in BAL fluid lactate dehydrogenase (LDH)) 1d post-inhalation. BAL cell counts by the automated counter (Coulter Counter®) indicated a 3.2-fold increase in cellular influx. Conversely, the hemocytometer method showed a slight decrease in total cells (0.84) compared to sham, which agreed with flow cytometry results (0.81 decrease). Similar changes were observed in 28 and 84d post at time points associated with significant increased LDH levels (2.5 and 2.0-fold, respectively). At the lower CNT dose, LDH increased minimally (1.4 fold) and the three methods generally agreed at 0.93, 1.20, and 1.00 versus sham for flow cytometry, automated counter, and hemocytometer, respectively. Similarly, a high cytokotoxic (>5-fold increase in LDH) stainless steel WF exposure produced fold changes over sham for the automated (4.0) and manual (1.2) methods at 1d post that were also seen at 7d. By 28d, however, when cytokotoxic was near baseline (1.4 fold change), the two methods gave similar results, with fold changes of 1.6 and 1.8 for the automated and the manual methods, respectively. After exposure to non-cytotoxic mild steel WF, there was agreement between the automated counter and manual methods with a fold change of 1.1 over sham for both. The results suggest a threshold of cytokotoxicity magnitude may be important in determining whether an automated or manual method of cell counting is best suited for BAL studies.

171 Development of a Flow Cytometry-Based Method to Measure Neutrophil Activation in the Cynomolgus Monkey

During pre-clinical safety assessment of human pharmaceuticals, the data generated from standard toxicity studies may prompt the need for further immunotoxicology testing. When changes to the immune system such as white blood counts or structural changes to lymphoid tissue are observed, it is not always obvious which complication is the first line of defence when the body is challenged with foreign pathogens. However, standard sub-acute and sub-chronic toxicity studies are often not of sufficient duration to detect changes in innate immune function. In this study we have developed a method that can provide an indication of potential changes in neutrophil function that is easily incorporated into a standard toxicology study without the need for extra blood sampling and requires minimal additional analyses over and above what is normally performed in toxicology studies. When neutrophils from a healthy subject are stimulated, they undergo a shape change which is easily identified using flow cytometry and looking for increased cell size on the forward scatter plot. If
normal neutrophil function is impaired by the test compound the magnitude of this change should be reduced. In our validation we stimulated neutrophils from cynomolgus monkeys with GRO-alpha at 2 concentrations (30 and 100nM) and FMLP tested at 1µM. Stimulations were performed in a 96 well plate and each condition tested in duplicate. After stimulation, a proportion of the cells in each well were isolated for shape change analysis and the remainder used to measure CD11b expression (neutrophil activation marker). Results show that GRO-alpha or FMLP increased an increase in neutrophil size that typically ranged from 15% to 75%. The %CV between replicates was typically <25%. An increase in neutrophil size also correlated with increased expression of CD11b. The addition of inhibitors to block GRO-alpha or FMLP activation, markedly reduced the increase in neutrophil size.

172 Comparing the Sensitivity of the T-Dependent Antibody Response (TDAR) to Cyclosporin A (CsA)

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The TDAR was confirmed to be one of the most predictive indicators of immuno- toxicity by the NTP studies in the late ‘80s. The primary goal of these studies was to compare the sensitivity of the KLH TDAR in male cynomolgus monkeys (cyms: Chinese origin) and in male C57CD(SD) rats to repeat dose exposure to CsA. The approach was to engage in ‘data mining’ from prior validation studies. The conditions for eliciting the KLH TDAR in both cyms and rats were optimized. Male cyms were dosed orally to doses of 25, 50, and 100 mg/kg/day CsA for 28 days, which yielded Cmax values (ng/ml) of 492, 1,615, and 2,583, and were immunized on day 4. The cyms were bled on days 4 (before KLH), 7, 9, 11, 13, 16, 19, 22, 25, and 28, and the sera were analyzed for anti-KLH IgM (days 7, 9, 11, 13) and for anti-KLH IgG (all days) and anti-KLH IgG titers (all days) and anti-KLH IgG titers (all days) and anti-KLH IgG titers (all days). The results demonstrated a dose-related (DR) suppression of IgM titers – 6%, 20%, & 55% - and of IgG titers – 77%, 87%, & 89%. Male cynos were exposed orally to doses of 25, 50, & 100 mg/kg/day CsA for 28 days, which yielded Cmax values (ng/ml) of 1,425, 8,921, & 54,902, and were immunized on day 5. The results demonstrated a dose-related suppression of IgM titers – 6%, 20%, & 55% - and of IgG titers – 77%, 87%, & 89%. Male rats were dosed to doses of 3, 10, & 30 mg/kg/day CsA for 28 days, which yielded Cmax values (ng/ml) of 1,425, 8,921, & 54,902, and were immunized on day 5. The results demonstrated a robust enhancement in the KLH TDAR at the low dose CsA – 308% IgM and 680% IgG, and a DR suppression at the middle and high doses CsA – 45% & 76% IgM, and 52% and 81% IgG. A similar trend was observed in the rat sRBC AFC TDAR with an enhancement at the low dose CsA – 181% - and a DR suppression at the middle and high doses of CsA – 72% and 100%. These studies demonstrate that an optimized KLH TDAR can yield DR effects in cynos, and that the KLH TDAR and the sRBC TDAR demonstrate similar trends in rats – enhancement at low doses, and suppression at higher doses.

173 Optimization of an In Vitro Method for Stimulation of Cynomolgus Monkey T Cells Using Anti-CD3 and Anti-CD28 Antibodies


Evaluation of T cell activation is essential for immune modulators that are developed to target T cells. The purpose of this study was to optimize an in vitro method to evaluate T-cell activation. Because T-cells require at least two signals to become fully activated – engagement of the T-cell receptor (CD3) and a co-stimulatory molecule (CD28), we compared two anti-CD3 antibodies in combination with an anti-CD28 antibody, and used an anti-Ki-67 antibody to detect activation-induced proliferation by flow cytometry. Sterile polysyrène 96-well round bottom plates were coated with 50 µl of 3 to 10 µg/ml mouse anti-monkey or human CD3 (clone FN-18 or OKT 3). Peripheral blood mononuclear cells (PBMCs) from naïve cynomolgus monkeys were isolated, and 105 PBMCs were plated for each well. Anti-human CD28 (clone 28.2) was added to reach a final concentration of 1 µg/mL. Samples were incubated at 37°C and 5% CO2 for 48 or 72 hours. Cells were then stained with anti-CD45, anti-CD3 (clone SP34-2), and anti-Ki-67 antibodies and analyzed by flow cytometry. Ki-67 expression was observed in cultures with OKT 3 and anti-CD28 stimulation. FN-18 in combination with the anti CD28 antibody induced dose dependent T cell activation: increased Ki-67 expression was observed in cultures with FN-18 concentrations from 3 and 10 µg/ml. The stimulation index (Ki-67% of stimulated wells – Ki-67% of control wells) ranged from 15.3% to 69.3% in cultures for 48 hours increased from 17.3% to 76.1% in culture for 72 hours. In conclusion, cynomolgus monkey T cells can be fully activated in vitro with antibodies directed at CD3 and CD28. Usage of the correct anti-CD3 clone is critical for T-cell activation. Culture for 48 hours is adequate for the evaluation using Ki-67 expression as the indicator for T cell activation.

174 Fatty Acid Amide Hydrolase (FAAH) Blockade Ameliorates Experimental Colitis by Mediating microRNAs and Attenuating T Cell Activation

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Crohn’s disease (CD) and ulcerative colitis (UC) are two major forms of inflammatory bowel disease (IBD) caused by effector immune cells that lead to high morbidity and health care cost. Fatty acid amide hydrolase (FAAH) is an enzyme that is crucially involved in the modulation of intestinal physiology through anandamide (AEA) as well as other endocannabinoids. However, the effect of FAAH inhibition on experimental colitis progression has not been investigated. Here, we investigated the effect of FAAH inhibitors (10mg/kg body weight) on DSS induced experimental colitis. We evaluated the immune cell modulation and miRNA changes after FAAH treatment. FAAH effectively lessened overall clinical score, reversed colitis-associated pathogenesis and the body weight after DSS induced colitis. FAAH treatment reduced the percentage of activated CD4+ T cells in the spleen, mesenteric lymph nodes (MLN) and peyer’s patches (PP) as compared to mice that received DSS alone. Similarly, the percentage of macrophages, neutrophils, natural killer (NK1.1), and NKT cells in the PP of colitis mice declined after FAAH treatment. FAAH also reduced the concentration of inflammatory cytokines and chemokine as compared to DSS alone. Microarray analysis of microRNA (miR) revealed 26 miRs in MLN and 217 miRs in PP showed > 1.5 fold difference after FAAH treatment. Among them, eight miRs having anti-inflammatory properties were validated by RT-PCR. Pathway analysis also validated differentially regulated miRs targeted mRNA associated with inflammation. These results suggest that FAAH ameliorates colitis by reducing activated T cells as well as other immune cells at sites of inflammation, through regulation of miRs. These results suggest that FAAH inhibitors may be developed as a novel therapeutic agent for IBD (This work was supported in part by NIH grants R56DK087836, P01AT003961, R01AT006888, R01ES019313, R01MH094775, and P20GM103641).

175 Estimation of Sibutramine and Phenolphthalein in Slimming Products Available in UAE

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Slimming products have gained popularity due to its easy availability and quick source to curb obesity which is a growing problem worldwide in all age groups. Reports indicate that chronic use of slimming products have adverse effects on health as they often contain illicit compounds like sibutramine and phenolphthalein which are banned by the FDA. The objective of the present study was to detect and estimate the concentration of sibutramine and phenolphthalein in the slimming products marketed in UAE. The analysis was done for a total of 60 samples from 10 countries using GC-MS. The samples were classified as herbal and non-herbal depending upon their nature. The sample cost per gram was calculated and related with sibutramine and phenolphthalein concentration. The results revealed that 60% samples contained sibutramine and/or phenolphthalein, and the highest concentrations were found in samples from China (2.5±0.45 mg/g and 27±0.42 mg/g, respectively). Samples from Germany and France contained phenolphthalein alone, whereas samples from USA, Thailand, Italy and England showed presence of both, sibutramine along with phenolphthalein. There was no significant difference in concentration of sibutramine (herbal: 1.7±0.61 mg/g; non-herbal 2.0±0.84 mg/g) and phenolphthalein (herbal 15±1.5 mg/g; non-herbal 16±0.4 mg/g) among the herbal and non-herbal slimming products. Slimming products from Iran, Lebanon and Morocco were free from these adulterants and were available at a lower price in the local market than products from other countries. The present study indicates that regular use of adulterated slimming products could result in various health problems. Moreover, the cost of the product does not guarantee the safety of the product since expensive products were found to have to higher concentrations of both the compounds than the cheaper ones.
Free radicals (RLs) exert beneficial and harmful effects depending on their concentration; at low concentrations RLs are involved in cell signaling, while high concentrations generate oxidative stress. In previous studies it was shown that antioxidants with phenolic structures induce higher levels of cancer cells death than in normal cells. The algae *Thalassia testudinum* has polyphenolic structures with antioxidant properties, suggesting that it could have regulatory effects on cell viability. Therefore we evaluated if the lyophilized of *T. testudinum* reduces viability of normal human keratinocytes (NHEK) and transformed keratinocytes (HaCaT) depending on the amount of RLs that are captured by the lyophilized of *T. testudinum* (Antioxidant activity). The lyophilized of *T. testudinum* reduced the viability (MTT assay) of HaCaT cells by 70% when administrated as a suspension in water, while in NHEK cell the viability decreases only in 39%. The induction of apoptosis and necrosis index (Flow cytometry of annexinV/propidium iodide) showed an induction of 50% of apoptosis in HaCaT cells, whereas in NHEK cells only an increase of 9% was induced. The amount of RLs produced by HaCaT cells (Flow cytometry of superoxide anion) was three times greater than that produced by NHEK cells. The antioxidant activity of the *T. testudinum* lyophilized in NHEK cells was 6% capture of the superoxide anion, whereas in HaCaT cells the superoxide anion capture was up to 20%. This results suggest that the antioxidant effect of *T. testudinum* induces apoptosis in transformed keratinocytes while do not change the viability of the normal keratinocytes, which indicates that the regulation of viability in the transformed cells is dependent on RLs signaling, and thus, a good therapeutic target.

**Analytical and Hazard Characterization of a Gasoline-Range Hydrocarbon Fuel Manufactured from Plant-Based Sugars**


**Health, Royal Dutch Shell, Houston, TX and Technology Development, Virent, Madison, WI.**

**Sponsor:** D. Steup.

We report here results of basic mammalian toxicity and ecotoxicity tests conducted on Virent BioForm® gasoline, a gasoline-range hydrocarbon substance manufactured from plant-based sugars using novel BioForming® technology. This process features catalytic chemistry, which converts plant-based sugars into a full range of hydrocarbon products identical to those made from petroleum, including gasoline, diesel, jet fuel, and chemicals for plastics and fibers. The tested substance consisted of C9 – C10 hydrocarbons with a 55 – 95% boiling range of approximately 72 – 343 degrees F. It was comprised of 25 wt% paraffinic and isoparaffinic, 2.8 wt% olefinic, 4.4 wt% naphthenic and 67.5 wt% aromatic hydrocarbons. The mammalian toxicity testing battery consisted of *in vitro* acute oral toxicity in rats, skin and eye irritation in rabbits, Buehler skin sensitization testing in guinea pigs, *in vitro* microuncluse testing in human lymphocytes and Ames mutagenicity testing. Additionally, *in vitro* skin and eye irritation tests in reconstructed human epidermis and corneal epithelial tissue, and peptide reactivity tests were conducted for comparison to the *in vivo* results. Tests to discern potential biodegradability and ecotoxicity in daphnia were also conducted. Mammalian toxicity test results revealed this substance to be comparable to petroleum-derived gasoline blending streams. It was a moderate skin irritant (primary irritation index 3.5), but otherwise non-irritating to eyes, not acutely toxic, not mutagenic or clastogenic, and non-sensitizing. The *in vitro* test results were consistent with the findings in the animal studies. The substance was readily biodegradable and similarly toxic to daphnia as other gasoline blending streams.

**Differential Cytotoxicity of Freeze-Dried Thalassia testudinum in Normal and Transformed Human Cells**


**Toxicology, CINVESTAV, Mexico City, Mexico and Biomedical Research, Center of Chemical Reproductive Health, Edinburgh, United Kingdom and Syngenta Ltd, Bracknell, United Kingdom.**

Invertebrate GABAergic receptors are targets for insecticides. GABAergic receptor isoforms are widely distributed in various mammalian tissues. Dependent upon target specificity, bioavailability and exposure, adverse effects due to action on central, peripheral or enteric nervous systems may be observed in animal models dosed with GABAergic receptor antagonists. These effects could reflect activity on the GABAergic receptor isoforms present in these tissues. To investigate this hypothesis we used RNAseq in situ hybridisation to explore differential expression of GABAergic α1-6 and GABA β 1-3 in toxicity target tissues (FFPE) in rat and dog. There was widespread expression of all isoforms except α6 in rat and dog brain, albeit there were species differences in the intensity of expression. In rat duodenum and adrenal only β3 was strongly expressed in the myenteric and submucosal ganglia and medulla, respectively. However α3 and α5 were weakly expressed in these tissues and the other isoforms were largely negative. These results indicate that toxicity in the rat and dog could be caused by direct effects on certain GABAergic receptor isoforms. An understanding of such differential target expression may be used to aid selection of compounds with reduced affinity for isoforms with potential for causing adverse effects.

**Revised Oral Toxicity of Butyl Methoxydibenzoylmethane (Avobenzone), a UV filter for 4 Weeks in Rats**


**Pharmacy, Inje University, Gimhae, Republic of Korea.**

Butylmethoxydibenzoylmethane (avobenzone) is used as a UV filter. Ministry of Food and Drug Safety (MFDS) allows limited concentration of 5% for use of cosmetic formulations of avobenzone. A repeated oral toxicity study of avobenzone (0, 100, 450, 1000 mg/kg/day, n=98) was performed for 4 weeks in male and female Sprague-Dawley rats. After 4 weeks oral treatment, body weight changes, food consumption, organ weights, hematology, clinical chemistry, urine analysis were determined. And, toxicokinetic study was also done using liquid chromatography-MS/MS (LC-MS/MS). Plasma was collected 1 day, 15 days, 28 days (100, 450, 1000 mg/kg/day, each group n=3) post-dose. There were no significant differences of body weight change and food consumption between avobenzone treatment groups and control. In the hematology, hemoglobin (HGB) and hematocrit (HCT) were increased in male high dose (1000 mg/kg/day) compared to control. Neutrophil (NEU) level was decreased in female high dose (1000mg/kg/day), whereas mean corpuscular volume (MCV) level was increased in female group of 450 and 1000 mg/kg/day. In the biochemistry, creatinine (Crea), total cholesterol (T-Chol), albumin (Alb), alanine aminotransferase (ALT), aspartate aminotransferase (AST) concentration were increased in male, female high dose (1000 mg/kg/day). And there were significant differences for levels of calcium (C), blood urea nitrogen (BUN), total protein (TP), albumin (Alb), alanine aminotransferase (ALT), alkaline phosphatase (ALP), γ-glutamyl transpeptidase (r-GTP), total bilirubin (T-BIL) in female high dose (1000 mg/kg/day). Toxicokinetic study of avobenzone showed slight plasma accumulation in both male and female for 4 weeks. Based on the alterations of kidney function (incrcement of Crea, BUN, and Alb), the no observed adverse effect (NOAEL) of avobenzone was temporarily determined 100 mg/kg/day because of pending histopathological examination.

**Safety Evaluation of a Whey Protein Fraction Containing a Concentrated Amount of Naturally Occurring TGF-β2**


**CiToxLAB, Evreux, France, Armor Protéines, Saint-Brice-en-Cogles, France, Nestlé Research Center, Lausanne, Switzerland and Lyon Poisons Center, Lyon, France.**

TM0601p is a whey protein isolate derived from cow milk, containing a concentrated amount of transforming growth factor β2 (TGF-β2), and is intended for nutritional use in infants and adults. *In vivo* and *in vitro* studies have been performed to evaluate the safety of this product. In a 13-week toxicity study, treatment of adult Sprague-Dawley rats by gavage at up to 2000 mg/kg/day did not result in any significant findings other than minor non-adverse changes in urinaly parameters in females. The no-observed-adverse-effect level (NOAEL) was established as 2000 mg/kg/day. In a juvenile toxicity study, rat pups received 600 mg/kg/day by gavage from postnatal day (PND) 7 to PND 49. Transient lower bodyweight gain in the pre-weaning period was attributed to gastrointestinal effects of the viscous test material; following weaning, bodyweight gain was comparable to the vehicle control. Reduced esophialpohus changes and increases in urinary parameters (females) were recorded in treated pups at PND 49, and higher thymus weights were recorded in males only at the end of the recovery period (Day 77). None of the findings were considered adverse. There were no other significant findings and the NOAEL was established as 600 mg/kg/day. No evidence of genotoxicity was seen in the bacterial reverse mutation test or the *in vitro* micronucleus test. Overall the results obtained present a reassuring safety profile for TM0601p.
Drugs and compounds which absorb UVB, UVA or visible light in the range of 290-700 nm and show significant partitioning in the skin after dermal or oral administration need to be tested for phototoxicity. To date, no nonclinical in vivo assay has been formally validated. As recommended in the ICH guideline S10, “Guidance on photosafety evaluation of pharmaceuticals” (2012) an in vivo assay was developed using standardized sunlight, normalized based on the UVA part (320 to 400 nm) of the applied spectrum causing minimal erythema (grade ≤1) and minimal erythema dose MED) at UVA doses between 5 and 20 J/cm². As standardized light source the Oriel Solar Simulator (ASTM E982 and IEC 904-3) was selected. When using sunlight, the amount of UBV as main cause for erythema should be attenuated in order to achieve the recommended UVA dose at MED. Combinations of the AM 1.5 Global filter providing the total spectrum when the sun is at a zenith angle of 45.2° and/or a WG 305 filter attenuating the midrange UBV spectrum were validated in the guinea pig model for the assessment of phototoxality and phototoxicity. The experimental group was induced with nine open applications of 5% tetrachlorosalicylanilide (TCSA) in polyethylene glycol 400 and challenged with 5% TCSA in ethanol. Each application was followed by solar radiation. One control group was dosed with vehicle and one group was not dosed.

Skin reactions were scored prior to dosing, 24 hours post-induction and 24, 48, and 72 hours post challenge. Results showed that the filters did have an effect on the MED in terms of UVA dose. With the AM filter and without the WG filter the MED was approximately 1.5 J/cm², with both filters the MED was 3 J/cm² and without the AM filter and with the WG filter the MED was 5 J/cm². Comparable challenge responses were seen for all three set-ups. It was concluded that by using the solar simulator in combination with WG 305 filter an UVA dose of 5 J/cm² as MED was achieved. The UVA dose did not influence the sensitivity of the assay.

### Adaptation versus Adverse Cell Responses in Oxidative Stress: Role of Nrf2 and NF-kB

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Oxidative stress is the result of an imbalance between the free radical load and the adaptable quenching mechanisms present in a cell. An adaptive response to oxidative stress involves most importantly the nuclear actions of Nrf2 and NFkB with up-regulation of several defense proteins. However, excess of free radicals may swamp the adaptive mechanisms and lead to adverse consequences such as lipid peroxidation, altered activity of enzymes, cell proliferation, differentiation, apoptosis or necrosis. Therefore, understanding Nrf2 mechanisms is of great interest in modifying the response of cells to oxidative stress and the potential use of this information in safety assessments. In this study, we present a comprehensive computational model of cellular mechanisms that participate in oxidative stress disposition. This includes quenching mechanisms, oxidative phosphorylation, lipid peroxidation, protein oxidation, mitochondrial pore opening and ASK1-JNK pathways. We demonstrate quantitatively how cellular mechanisms adapt to oxidative stress through Nrf2 and NFkB mechanisms. In particular, we show how initial priming of a cell with a low dose of free radicals can enable it to handle a much larger dose of free radicals without adverse consequences. We also show the transition from adaptive response to adverse effects, and within the latter response, from apoptosis to necrosis, with increasing levels of oxidative stress. The model can be used for interpreting new oxidative stress data and for Nrf2 target modulation.

### Provisional Advisory Level (PAL) Development for Fentanyl Derivatives

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PAL values developed for hazardous materials by the US EPA represent general public emergency exposure limits for oral and inhalation exposures corresponding to three different severity levels (1, 2, and 3) for 24-hr, 30-d, 90-d, and 2-yr durations. PAL 1 represents the threshold for mild effects; PAL 2 represents the threshold for serious, irreversible or escape-impairing effects; PAL 3 represents the threshold for lethal effects. PALs have not been promulgated nor have they been formally issued as regulatory guidance. They are intended to be used at the discretion of risk managers in emergency situations when site specific risk assessments are not available. Fentanyl is a potent opiate receptor agonist and analgesic; PAL values for fentanyl were developed in 2011. Derivatives of fentanyl include carfentanil, α-methylfentanyl, 3-methylfentanyl, and possibly Kokolok-1. These chemicals are listed as either Schedule I or II controlled substances by the US DEA and are 50 - 10,000-fold more potent than morphine. A suspect fentanyl derivative was used as a catalyst for incapacitating the 2002 Moscow hostage crisis in which over 100 people died. Insufficient data were available to develop inhalation PAL values. Oral PAL estimates were based on sedation in chimp groups carfentanil and rats given 3-methylfentanyl. The oral PAL 2 and 3 values for fentanyl derivatives are 0.007 and 1.1 mg/L, respectively, for 24 h. Remaining oral PAL values are not recommended due to insufficient data. The PAL values were approved by the Expert Consultation Panel for PALs in June 2014. The EPA, through ORD, funded and managed the research described here. It has been subjected to Agency’s administrative review and approved for publication. The views expressed in this paper are those of the authors and do not necessarily reflect the views or policies of the Agency. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

### Detection of QTc Interval Prolongation Using Jacketed External Telemetry in Cynomolgus Monkeys: Influence of Animal Housing Conditions

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Introduction: Jacketed External Telemetry (JET) has been developed for continuous ECG recording in conscious animals without the need for restraint or surgical implantation. The aim of this study was to pharmacologically validate JET system recently installed at CDSER and investigate the influence of different housing conditions on the ability to detect drug-induced ECG changes.

Methods: The study was performed in 2 phases. In each phase 6 cynomolgus monkeys (3 females, 3 males) were used. Monkeys of the same gender were group-housed (3 monkeys/pen) in Phase 1 and singly-housed in Phase 2. The females were orally administered vehicle on all dosing days, while the males were administered vehicle followed by ascending doses of moxifloxacin (15, 45 and 135 mg/kg) with an interval of 3-4 days between each successive dose. A blood sample was taken for biomonitor at 4h post-dose on each dosing day. Lead II ECG data was collected for at least 1h prior to dosing until 24h post dose via JET system. QT interval was corrected for heart rate (QTcR). Results: HR in singly-housed males were similar over the 24 hour period compared to the group-housed conditions. Administration of vehicle had no notable effect on HR and ECG in the females. Moxifloxacin caused an increase in QTcR interval. In Phase 2, a clear increase in QTcR was seen only after administration of 45 and 135 mg/kg (increase of 11% and 21%, respectively versus time-matched vehicle). In Phase 2, a clear increase in QTcR was observed only after 135 mg/kg (increase of 17%). No differences were found in the concentration of the moxifloxacin between the phases. Conclusion: The JET system was able to detect the QTcR prolongation induced by moxifloxacin and our data suggests that group-housing of the monkeys may improve the sensitivity of detection of drug-induced ECG changes.

### Derivation and Comparison of Occupational Exposure Limits (OELs) for Hydrocarbon Vapor Mixtures Exposed from Bakken and Non-Bakken Crude Oil

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The use of hydraulic fracturing has led to dramatic increases in North American crude oil production. These technologies facilitate recovery of crude oil from geological formations, such as the Bakken formation, that are not amenable to oil recovery using conventional drilling techniques. Increases in production have increased the amount of crude oil transported from production regions, primarily by rail. In the wake of several train derailments involving Bakken crude oil, it has been hypothesized that Bakken crude oil may be more flammable than crude oils produced in other regions due to differences in hydrocarbon content, particularly volatile hydrocarbons. Given this hypothesis, it is unclear whether the toxicity of volatile hydrocarbon mixtures (VHMs) released from Bakken crude oil are comparable to crude oils produced in other regions or whether protective action levels for inhalation of VHMs from Bakken and non-Bakken crude oil are similar. Instantaneous air samples of emissions from Bakken and non-Bakken crude oils were collected using evacuated canisters and analyzed for volatile organic compounds (VOCs) using the US EPA GC/MS TO-15 Method. Occupational exposure limits (OELs) for VHMs released from each type of crude oil were calculated using a modi-
Microcystins (MC), secondary metabolites produced by cyanobacteria, increasingly occur in surface waters used by humans for drinking water and as food sources. Currently >100 structural variants (congeners) have been described, which, due to the variable L-amino acids and side-chain modifications differ in their physicochemical properties. They are also used to increase the longevity of the fragrance. Phthalates can cause asthma, endocrine disruption, reproductive abnormalities, low birth weight, kidney and liver cancer. The content of phthalic acid esters in perfumes and their associated human health risk has not been studied before. Hence, this study aimed to identify and estimate the amount of four phthalate esters viz., Dibutyl phthalate (DBP), Diethyl phthalate (DEP), Di (2-ethylhexyl) phthalate (DEHP), Dimethyl phthalate (DMP) present in commonly available perfumes and their possible health risk. Materials and methods: Ten brands of perfumes with 2 batches were used. The commonly available perfumes, frequency and volume of their use were selected based on a survey. The four phthalates were identified and estimated in perfumes by comparing RI value with their respective standards using High Pressure Thin Layer Chromatography. Estimated daily exposure rate and Hazard Indices (HI) were calculated to assess the possible health risk associated with phthalate esters. Results: All the four phthalate esters were found in perfumes. The estimated concentration of DEP, DEHP, DBP, and DMP were ranging from 0.81-1.05, 16.35-17.41, 0.05-0.40, 0.077, 0.06-0.34/74 mg/mL, respectively. The calculated Hazard indices of DEP, DEHP, DBP, and DMP were 0.0006, 0.03, 0.12, and 0.003, respectively. Conclusion: The calculated Hazard Indices for phthalates in different brands of perfumes were found to be below 1, which indicates that the daily exposure of phthalates does not pose any health risk. However, further studies are warranted to assess the human health risk associated with long term phthalates exposures through perfumes.
Among the plethora of illicit drugs, marijuana stands out as the most commonly used all over the world. A plethora of adverse effects have been associated with acute and chronic use of marijuana. In many of these cases, the mechanism mediating these adverse effects remain enigmatic. To investigate the effects of marijuana on paraoxon (PON1), an enzyme that plays an important role in cardiovascular disease, rats were administered 12.5, 25 and 50 mg/kg body weight of hexane extract of marijuana for 4, 6, 8 weeks. Control animals received the vehicle (olive oil). PON1 activity towards paraoxon (PONase) and phenylacetate (AREase) in plasma was 32, 26 and 16% of respectively. PON 1 activity towards paraoxon (PONase) and phenylacetate (AREase) in plasma and lipoproteins were determined. A dose- and time-dependent decrease was observed in the PONase activity in all the compartments. At the highest dose of the marijuana extract, PONase activity in plasma, HDL and VLDL was 36, 31 and 15% of control respectively. In contrast to PONase, AREase increased in the plasma, but the increase was only dose-dependent during the first 2 weeks. In the HDL, an ameliorating effect on AREase was observed, whereas only a slight decrease was observed in the VLDL. Since decreased PON1 activity is a cardiovascular risk factor, the decreased PON1 activity observed in this study might explain in part the cardiovascular abnormalities reported in marijuana users.

191 E-Cigarette Liquid Flavorings alter Airway Epithelial Cell Structure and Function C. Sherwood1,2,3, R. Lantz1,5, and S. Boitano1,3,4,5 1Arizona Respiratory Center, Arizona Health Sciences Center, Tucson, AZ, 2Southwest Environmental Health Sciences Center, University of Arizona, Tucson, AZ, 3Bio5 Institute, University of Arizona, Tucson, AZ, 4Pharmacy, Arizona Health Sciences Center, Tucson, AZ, and 5Cellular and Molecular Medicine, Arizona Health Sciences Center, Tucson, AZ.

Electronic (e-) cigarettes are battery powered nicotine delivery systems that atomize the contents of a fluid-filled cartridge upon inhalation. Many of the chemical constituents in e-cigarette liquids (e.g., flavorings) are distinct from conventional cigarettes and have not been tested as inhalation toxicants. Lung disease is not unprecedented following inhalation exposure to flavoring compounds (e.g., propylene glycol). The airway epithelium provides the first line of defense from inhaled particulates, pathogens, and toxicants by providing an established barrier to protect underlying tissue; secretion of molecules involved in direct and indirect innate immunity; and a “mucociliary escalator” to remove harmful substances out of the airway. In this study we hypothesized that distinct e-cigarette liquid flavorings would compromise mechanisms key in airway epithelial innate immunity with the potential to cause lung disease. We used high-capacity Real Time Cellular Analysis (RTCA) to screen for e-liquid constituent toxicity on immortalized human bronchial epithelial cell (16HBE14o-) to determine cell signaling toxicity profiles following exposure of 16HBE14o- cells to certain e-liquid constituent flavorings and compounds that caused compromised airway epithelial signaling effects, menthol, and the chocolate flavoring 2,5-Dimethylpyrazine were selected for airway physiology studies using well-differentiated primary mouse tracheal epithelial cell cultures. Menthol caused a breakdown in airway epithelial barrier structure and function and 2,5-Dimethylpyrazine increased apical ion flux that was due in part to activation of Cystic Fibrosis Transmembrane Conducatance Regulator. These findings indicate that distinct e-liquid flavorings compromise airway epithelial function and thus may have detrimental consequences for airway health.

192 Initial Risk Assessment of β-Bromostyrene A. Ono1, K. Kobayashi1, M. Matumoto1, M. Emu1, T. Nishimura1, M. Hiraiz-Koizumi1 and A. Hirose1 1Chemistry, Federal University of Agriculture, Abeokuta, Nigeria, 2Chemistry, Federal University of Agriculture, Abeokuta, Nigeria, 3Biochemistry, Lagos State University, Lagos, Nigeria, 4Biochemistry, Laode Akintola University of Technology, Ogbomoso, Nigeria, 5Biological Sciences, Covenant University, Ota, Nigeria and 6Chemical Sciences, Bells University of Technology, Ota, Nigeria.

β-bromostyrene is used in fragrances include soap, detergent, creams, lotions, & perfume. To evaluate information of the screening toxicological data set of β-bromostyrene, the 28-day repeated toxicity study (CrI: CD (SD) rats) and in vitro genotoxicity tests (Ames and chromosomal aberration tests) were conducted. The test results of the both genotoxicity studies indicated negative. In the repeated-dose study, a decrease in spontaneous movement was observed in all 500 mg/kg animals after the first dosing day. Body weight showed a tendency to decrease in 500 mg/kg males and females in the initial administration period. On the blood biochemical examination, increases in total cholesterol and phospholipid were detected in 500 mg/kg males. A decrease in HDL was observed in 500 mg/kg of both sexes. Increased liver weights were observed in 125 and 500 mg/kg of both sexes, and centrilobular hepatocellular hypertrophy was observed in 500 mg/kg of both sexes. Eosinophilic body of tubular cell of the kidney was observed in more than 125 mg/kg males, and renal tubular degeneration was observed in 500 mg/kg males. Hypertrophy of the follicular cell of the thyroid was observed in more than 125 mg/kg/day females and in 500 mg/kg males. All of observed changes were disappeared after the recovery period. Based on these results, the no-observed-effect-level of β-bromostyrene was judged to be 30 mg/kg/day for both sexes. Most of toxicological profiles are similar to those of styrene, which is known as a pulmonary tumorigen in mice inhalation studies and was categorized as IARC group 2B. However, effects of β-bromostyrene were not reported for styrene. Further studies for evaluating the endocrine modulating effects would be needed.

193 Western Diet Accentuates Cellular Damage in Benzo(a)pyrene (B(a)P)-Induced Colorectal Cancer K. Harris1, S. R. Pulliam1, M. S. Niaz1, E. Okoro2, Z. Guo3, M. K. Washington1, S. E. Adunyah1, and K. A. Ramad1 1Biochemistry & Cancer Biology, Meharry Medical College, Nashville, TN, 2Pharmacology, Meharry Medical College, Nashville, TN and *Pathology, Vanderbilt University, Nashville, TN.

The role of fat content in diet has been linked to the development of several types of cancer. The objective of this study was to investigate the effect of dietary fat type on (an environmental colon carcinogen) induced colon cancer in an adult male rat model, the Polyposis In the Rat Colon (PIRC) kindred type. Groups of PIRCs (n = 5) were fed with AIN-76A regular diet (RD) or Western diet (WD) and received 25, 50, and 100 μg B(a)P/kg body weight. Oral gavage for 60 days. Rats that were fed with the diets alone, but no B(a)P served as controls. Subsequently to exposure, rats were sacrificed; colons, liver and other tissues were retrieved and preserved in 15% formalin for observation of gross pathological changes. Blood samples were collected and concentrations of cholesterol, triglycerides, glucose, leptin, reactive oxygen species (ROS) and oxidative damage to DNA were measured. Rats that received WD + B(a)P showed increased levels of cholesterol, triglycerides, and leptin in comparison to RD + B(a)P groups and controls. Glucose levels stayed the same between WD and RD for 25, and 50 μg B(a)P/kg body wt., but showed a significant increase (p < 0.001) at 100 μg B(a)P/kg body wt. + WD. Maximal levels of ROS and oxidative DNA damage in blood were noticed in WD + B(a)P rats compared to RD + B(a)P rats and controls. The colon tumor numbers showed a B(a)P dose-response relationship. Adenomas with high grade dysplasia were prominent in B(a)P + WD rats compared to B(a)P + RD rats. Our findings revealed that WD potentiates the development of colon tumors induced by B(a)P through proinflammatory action, characterized by an increased generation of ROS and oxidative damage (This research was supported by funding from NIH grants F31ESO24079-01, S901CA142845-04, T32HL07773-19, S525GM059994-11, and Southern Regional Education Board).
analysis revealed binding affinities between purified human hemoglobin and the hydrocarbons decane, toluene, tetradecane, and octane, with toluene demonstrating the tightest binding (KD = 1.9 μM at room temperature). Ethylbenzene did not demonstrate any appreciable binding to hemoglobin. The apparent increases in RBC membrane surface area and oxidative stress, as well as the hydrocarbon/hemoglobin interactions, indicate potential degradation in oxygen transport and capacity that could lead to potential hypoxic tissue conditions that could impact human performance during fuel use in operational activities.

**195** Nasal Effects in a 28-Day Gavage Study Likely Attributable to the Gavage Technique

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In a recent study 28-day gavage study in rats administered chemical X which is a fluoroursurfactant at doses of 0, 10, 50 and 200 mg/kg/day, we reported that the histopathological changes observed in the noses of rats were likely attributable to the gavage technique and therefore administration-route specific. Besides low incidences of usually focal olfactory degeneration and atrophy, commonly observed as background changes in all groups, in a few 200 mg/kg/day males and females and 50 mg/kg/day females, the olfactory changes were more extensive and occurred primarily in the posterior-ventral regions of the nose. The posterior-ventral distribution of these nasal lesions is most consistent with secondary test-article related changes resulting from reflux of the test material into the nose in association with gavage dosing. The test material has been shown to be a mild eye irritant and this low grade of irritancy is likely a factor in the generally mild nature of the reflux nasal lesions seen in this study as well as the lack of these same lesions at lower dose levels. Conversely, the posterior-ventral distribution of olfactory lesion in this study is inconsistent with reports of compounds that produce primary systemic olfactory toxicity. With systemic olfactory toxicants, the dorsomedial olfactory regions are generally reported to be the most sensitive regions. The dorsomedial distribution of these systemically-induced olfactory lesions has usually been attributed to the high activity of metabolizing enzymes in the olfactory epithelium and/or bowman’s glands. Based on the above considerations, the low incidences of predominantly posterior-ventral nasal lesions observed following gavage administration of 200 or 50 mg/kg/day chemical X were likely due to reflux of the gavage material, as the distribution of the lesions is consistent with this mode of action. Therefore, these lesions represent a route-specific effect that is not relevant to human exposure scenarios. As such, these nasal lesions are not relevant to human hazard characterization or risk assessment.

**196** Development of Oregon’s Drinking Water Guideline Values for Four Cyanotoxins for Use during Harmful Algae Blooms

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Cyanobacteria are photosynthetic bacteria that are naturally occurring in marine and fresh surface waters. Some species of cyanobacteria can produce toxins, called cyanotoxins, which can be harmful to human and animal health. Under certain environmental conditions, cyanobacteria can proliferate to form large blooms. When composed of potentially toxin-producing species of cyanobacteria, also called blue-green algae, these blooms are commonly referred to as harmful algae blooms (HABs). When HABs occur in sources of public drinking water, drinking water facility operators and their customers seek guidance on how to respond to potential health threats in their water. In the absence of federal drinking water standards, the Oregon Health Authority (OHA) developed provisional drinking water guideline values for the four cyanotoxins detected most often in Oregon’s fresh waters – anatoxin-a, cylindrospermopsin, microcystins, and saxitoxins. In most cases, OHA used a threshold toxicity approach, applying uncertainty factors to no observable adverse effect levels (NOAELs) or lowest observable adverse effect levels (LOAELs) in published literature to develop tolerable daily intakes (TDIs). These TDIs protect against acute non-cancer health effects. OHA used TDIs and standard assumptions about body weights and water intake rates to develop acute drinking water guideline values. In the case of saxitoxins, there were insufficient data to develop a TDI and OHA adopted a drinking water guideline value shared by New Zealand and Brazil. Oregon’s acute drinking water guidelines are 3 μg/L for anatoxin-a, 1 μg/L for microcystins, 1 μg/L for cylindrospermopsin, and 3 μg/L for saxitoxins.

**197** Derivation of a Provisional Tolerable Intake for Intravenous Exposure to Silver Nanoparticles

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Silver nanoparticles (AgNPs) have been increasingly implemented into consumer and biomedical products due to their antimicrobial properties. Currently, in vitro effects of AgNPs are not yet well characterized for all routes of exposure and are associated with adverse endocrine, hepatic, and immunological outcomes. The aim of this project is to demonstrate how one might derive a tolerable intake (TI) value for intravenous (i.v.) exposure, a clinically-relevant route of exposure for some products, to size-specific AgNPs. A primary literature review was performed to determine the most relevant i.v. in vivo studies to serve as the basis of the TI. From this process, a 28-day, repeat dose i.v. toxicity study in rats injected with AgNPs (20nm diameter) was identified as being the most relevant and experimentally robust study for performing a dose-response assessment. The study identified a benchmark dose (BMD05) of 1.0 mg/kg/day for an array of immunotoxicological endpoints and this value was selected as the point of departure for derivation of the TI. To derive the provisional TI, a modifying factor (MF) of 300 was applied to this BMD05 to account for interindividual variability (10), potential interspecies difference in potency (10), and the lack of a BMD or no-observed adverse effect level (NOAEL) from a chronic toxicity study (3). The provisional TI for long-term i.v. exposure to 20nm AgNPs was determined to be 3 μg/kg/day based on the results of the 28-day study. This provisional i.v. TI is not necessarily protective for other sizes of AgNPs, AgNPs with various coatings, or following administration by other routes of exposure.

**198** Uncertainties in Selecting the Critical Effect for Toxicity Assessment of Tungstate

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Identification of the critical effect is a key component of chemical hazard identification and toxicity assessments. The U.S. EPA defines the critical effect as the first adverse effect or its known precursor that occurs in the most sensitive human-relevant species as the dose rate of a chemical increases. The critical effect is identified through a process of evidence integration that includes evaluation of 1) quality of available human, animal, and mechanistic studies; 2) magnitude and statistical and biological significance of observed effects; 3) consistency of evidence across dose levels, studies, and levels of biological diversity; and 4) biological plausibility for the association between exposure and effect, including relevance of animal evidence to humans. The oral database for tungstate includes studies of subchronic-duration exposure in rats and mice showing, as candidate critical effects: 1) increased incidence of inflammation and goblet cell metaplasia in the glandular stomach of gavaged rats, and 2) decreased bone marrow cellularity and immune responses in mice exposed through drinking water. Elevated incidence of stomach lesions was dose-related and statistically significant at ≥ 78 mg W/kg/day in both male and female rats (NOAEL = 46 mg W/kg/day), but uncertainties associated with this endpoint include lack of confirmation in other gavage studies, as well as biological relevance to humans experiencing low intermittent rather than large bolus exposures. In mice, decreased bone marrow cellularity (LOAEL = 250 mg W/kg/day, NOAEL = 49 mg W/kg/day) and decreased splenic response to bacterial toxin injection (LOAEL = 125 mg W/kg/day, NOAEL = 78 mg W/kg/day) are of clear biological significance, but other immune system changes observed at lower doses are of uncertain relevance due to a lack of dose- and/or duration-relatedness and questionable adversity of the changes. This abstract does not necessarily reflect U.S. EPA policy.

**199** A State of the Science Copper Reference Dose for Soil Remediation

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Copper (Cu) is an essential micronutrient that has a U-shaped dose-response curve, with both chronic deficiency and excess resulting in adverse effects. Although acute excess intake can produce transient GI symptoms, chronic intake of excess Cu leads to Cu accumulation in the liver, and the resulting oxidative stress can lead to cirrhosis, followed by kidney and CNS damage. The dose-response relationship for Cu, therefore, presents a challenge for risk assessment. The current USEPA refer-
ence dose (RID) (0.04 mg Cu/kg/d) is based on an abstract describing an acute poi-
soning event at a 1950s cocktail party. More recently, a review by an expert panel
indicated steep dose-response curves and a narrow margin of safety between Cu
essentiality and excess; this effort, however, was not intended to identify individual
points of departure that could be used to derive copper RID values. Given the avail-
ability of more recent data that is both more robust and relevant, an effort was un-
dertaken to develop a chronic RID that reflects the current state of the science and
is appropriate for deriving soil remediation standards. Since such standards assume
risks and hazards based on chronic exposures, we focused our review of the Cu tox-
icology literature on repeated exposure scenarios and measures of chronic toxicity
where possible. Four critical Cu toxicity studies were identified (two human, one
monkey, one rat), and points of departure were derived. Benchmark dose modeling
was performed when possible; otherwise NOAEL and LOAEL values were used.
Candidate RIDs based on typical risk assessment practices for human and animal
studies led to similar values, ranging from 0.1 to 0.35 mg/kg/d. Based on the overall
weight of the evidence from the above studies, an RID of 0.1 mg/kg/d is proposed based on evidence that 0.13 mg/kg/d is safe to adults and 0.29 mg/kg/d has
been reported to be safe for infants.

Titanium dioxide (TiO2) is characterized as a poorly soluble particulate (PSP)
with low toxicity. It is well accepted that low toxicity PSPs such as TiO2 induce lung tumors in rats when deposition overwhelms particle clearance mechanisms.
Epidemiologic studies of workers in TiO2 production have not shown evidence of
elevated lung cancer risk, and despite the sensitivity of rats to PSPs, the relevance
of PSP-induced tumors to humans has not been completely discounted. TiO2 is
listed as a possible human carcinogen, but currently, no environmental toxicity
criteria for TiO2 are available. Because TiO2 particles agglomerate, TiO2 is pre-
dominantly found as inhalable course particles (PM10) rather than as fine (PM2.5)
or ultrafine (nanoscale) particles in the environment. We derived cancer-based
toxicity values for fine TiO2 based on data from chronic inhalation studies in rats
using multiple approaches including different dose-metrics (regional deposited
dose and lung surface area burden), different modeling techniques (bench-
mark dose modeling, smoothing spline regression, and bilinear modeling), and
different extrapolation approaches. Based on empirical evidence and mechanistic
support for a mode of action (MOA) for TiO2-induced lung tumor in rats, involv-
ing chronic inflammation and cell proliferation, we derived reference concentration
(RC) values, ranging from 14.7 to 220 μg/m3. Despite the questionable relevance
of the particle overload MOA in rats for humans and the empirical evidence of a
threshold, certain regulatory applications (Proposition 65 in California) necessitate the development inhalation unit risk (IUR) values. Hence, we derived IURs based on rat lung tumors ranging from 0.0027 to 0.0045 mg/m3). Risk-based concentra-
tions at a 10-5 theoretical risk and continuous exposure (70 years lifetime,
24 hours per day) are lower than the RC values but above background. These
toxicity values should be useful for regulators interested in setting health-protective
standards for exposure to fine TiO2.

201 Threshold of Toxicological Concern (TTC) for Anticancer
Compounds

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Pharmaceutical companies develop increasingly specialized treatments to treat late
stage cancers in patients whose disease condition is progressive and fatal. In order to
accelerate the availability of potentially life saving treatments, anticancer agents are
allowed expedited regulatory review and modified requirements for preclin-
tical toxicity testing. Limited data packages in early development can present
challenges for occupational health and product quality in terms of determining
safe levels for occupational exposure and for product impurities at multi-prod-
uct manufacturing facilities. The present analysis established an endpoint specific
Threshold of Toxicological Concern (TTC) for developmental and reproductive
toxicity for anticancer agents. A comprehensive database was created consisting of
NOAELs for critical endpoints of 112 anticancer agents categorized by mechanism of
action ranging in 5 distinct acting (i.e., cytotoxic) anticancer agents with 134
NOAEL values and 59 indirect acting (i.e., non-cytotoxic) anticancer agents with
167 NOAELs for developmental and/or reproductive toxicity. The 5th percentile
NOAEL was 0.01 mg/kg/day and 0.003 mg/kg/day for reproductive and develop-
mental endpoints, respectively. The 5th percentile NOAEL for the combined re-
productive and developmental endpoints was 0.005 mg/kg/day. A human exposure
threshold value of 3 μg/day was derived from the 5th centile NOAEL assuming
standard uncertainty factors and adjusted to a 60 kg human. The analysis shows the
threshold is protective for highly potent groups of anticancer agents, such as those
that affect hormones. There was no difference between the thresholds of direct
acting (i.e., cytotoxic) and indirect acting (i.e. non-cytotoxic) anticancer agents
for developmental and reproductive toxicity. It was confirmed that the 1.5 μg/
day threshold for genotoxic impurities is protective for developmental and repro-
ductive toxicity for pharmaceutical agents designed to mechanistically target high
proliferating cells.

202 Use of Categorical Regression Modelling in the Health
Assessment of Guanidine Compounds

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Several salts of guanidine are used commercially, pharmacologically, in several lab-
oratory applications, and for other purposes. Guanidine nitrate is a high produc-
tion volume chemical (U.S. EPA, 1990); 65 million pounds were produced at the
former Army ammunition plant in Desoto Kansas (Aguirre, 1998). There are no
existing health assessments for guanidine compounds, and the literature search re-
vealed no reported-dose human or animal studies (subchronic or chronic duration)
for guanidine compounds. However, guanidine chloride has been used in the man-
agement of Lambert Eaton Myasthenic Syndrome (LEMS) in humans, and thus
the appearance of side effects in treatment was used as a basis for the identification
of toxicological effects for determining reference values. Case reports that accurately
reported the dosimetry and side effects that might potentially identify points or
departure (PODs) or reveal additional side effects were tabulated and a categorical
regression model (CatReg) was used to calculate points of departure. The Cloglog
model with linear dose and linear model with linear dose, provided the best fit by AIC score. The Cloglog model pro-
vides a potential POD of 2 mg/kg day, which is the lower 95% confidence interval
of the 10% effective response concentration (ERCL10) (the CatReg equivalent of a
BMDL at a BMIR of 10%). The sub chronic 12 mo., and chronic 84 mo. regression
results rounded to the same 2 mg/kg-day POD.

203 Provisional Advisory Levels (PALs) for Agent BZ and
EA3167

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PAL values developed for hazardous materials by the US EPA represent general
public emergency exposure limits for oral and inhalation exposures at three sev-
ery levels (1, 2, and 3) for 24-hour, 30-day, 90-day, and 2-year durations. PAL
represents the threshold for mild effects; PAL 2 represents the threshold for serious,
irreversible or escape-imparing effects; PAL 3 represents the threshold for lethal
effects. PALs have not been promulgated or formally issued as regulatory
guidance, but are intended to assist emergency response decision-making, and to
serve as criteria for determining re-use and re-entry into affected areas, such as after
transport/storage accidents, natural disasters and subversive activities. The US EPA
through its Office of Research and Development funded and managed the research
described here. It has been subjected to Agency's administrative review and ap-
proved for publication. BZ and EA3167 are strong anticholinergic agents that bind
cNS and PNS muscarinic receptors, causing tremors, hallucinations, sedation, and
increased heart rate and blood pressure. They were developed as military incapac-
itants, but were not used by the US in war; BZ (called QNB), is now an in vitro
tool in neurological studies. Oral and inhalation PALs were derived for BZ using
human and animal studies. Studies were unavailable for EA3167, but the BZ 24-
hour oral PAL values were adopted based on structural and toxicological similarity.
The 24-hour oral PAL 1, PAL 2, and PAL 3 for BZ and EA3167 are 3.5, 11, and
1900 μg/L; the BZ 30-day oral PAL 3 is 1900 μg/L. The 24-hour BZ inhalation
PAL 1, PAL 2, and PAL 3 are 0.83, 5.4, and 23 μg/m3; the 30-day PAL 2 and PAL
3 are 1.2 and 3.7 μg/m3. Other PALs were not developed due to insufficient data.
The views expressed in this paper are those of the authors and do not necessarily
reflect the views or policies of the Agency. Mention of trade names or commercial
products does not constitute endorsement or recommendation for use.
Provisional Advisory Level (PAL) Development for Chloroacarone


PAL values developed for hazardous materials by the US EPA represent general public emergency exposure limits for oral and inhalation exposures corresponding to three different severity levels (1, 2, and 3) for 24-hr, 30-d, 90-d, and 2-yr durations. PAL 1 represents the threshold for mild effects; PAL 2 represents the threshold for serious, irreversible or escape-impairing effects; PAL 3 represents the threshold for lethal effects. PALs have not been promulgated nor have they been formally issued as regulatory guidance. They are intended to be used at the discretion of risk managers in emergency situations when site specific risk assessments are not available. The United States Environmental Protection Agency through its Office of Research and Development funded and managed the research described here. It has been subjected to Agency’s administrative review and approved for publication. Chloroacarone is highly irritating, and PAL values are based on clinical signs of irritation in rats. Chloroacarone oral PAL 2 and 3 values are 39 and 120 mg/L, respectively, for 24-hr, and 13 and 63 mg/L, respectively, for 30-d. Chloroacarone inhalation PAL 2 and 3 values are 0.43 ppm and 1.3 ppm, respectively, for 24-hr. Chloroacarone PAL values for other severity levels and durations were not derived due to insufficient data. The PAL values were approved by the Expert Consultation Panel for PALs in June 2004. The views expressed in this paper are those of the authors and do not necessarily reflect the views or policies of the Agency. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Reference Exposure Levels for Benzene

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Prolonged or repeated exposure to benzene is associated with both blood cell proliferation and reduction in blood cell numbers. The adverse health effects of benzene result from the ability of its metabolites to affect rapidly dividing cells, especially in bone marrow where enzymes detoxifying its metabolites are at lower levels than in liver. Children may be more sensitive to benzene because so many tissues undergo rapid cell division and differentiation to stimulate and maintain growth and development. Recent data from Chinese workers showed that adverse effects of benzene are dependent on several enzymes including CYP2E1, NQO1, GSTT, GSTM, and EPHX1. The risk for benzene poisoning (total white cell count < 4000/μl or white cell count of 4000-4500/μl and platelet count < 80,000/μl) varied up to 20-fold based on the levels of enzymes present. OEHHA reevaluated its chronic Reference Exposure Level (REL) for benzene using data from a study of 250 Chinese workers where adverse effects on seven categories of peripheral blood counts were observed at average workplace levels of 0.57 ppm (~2 mg/m3) benzene. Staff used a benchmark dose approach with grouped dose response data for B cells from the study and OEHHA’s peer-reviewed methodology to derive a chronic REL of 1 ppb (3 μg/m3). This REL is lower than OEHHA’s earlier value due to use of a more sensitive key study in workers and a larger intraspecies uncertainty factor (UF) to protect children. Childhood development in benzene poisoning based on genotype led us to use an intraspecies UF of 60, twice the OEHHA default of 30. The acute REL was also updated. A decreasing monotonic dose response was seen for early nucleated cell counts 2 days after benzene exposure, compared with an increase in non-nucleated cell counts 3 days after benzene exposure. This result supports benzene poisoning as a predictor of neurologic performance than Cxt adjustment, and that the dose-response relationship for impairment after 24 hours of exposure was shifted to the right on the dose axis, compared with 1 hour of exposure (Oshiro et al. 2011). For the chronic inhalation MRL, the 2000 value is based on a 35-ppm LOAEL for color vision impairment in Croatian photogravure printers (Zavalić et al., 1998a, 1998b), but reevaluation of the data supports a NOAEL of 35 ppm and a LOAEL of 156 ppm for color vision impairment in these workers. Newer studies identify a 45-ppm NOAEL for self-reported symptoms, psychomotor performance, color vision, and hearing in Croatian photogravure printers (ATSDR, 2003, 2004, 2008; Seeger et al. 2004, 2005; Zupanic et al. 2002). This NOAEL may provide a better basis for the MRL, because it is the highest NOAEL below LOAELS (ranging from 50 to 156 ppm) identified in more than 20 studies evaluating neurotoxic effects in toluene-exposed workers.

Oral Risk Assessment of m- and p-Cresols in Drinking Water

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m-Cresol and p-Cresol are isomeric methylbenzene alcohols that are used as disinfectants, preservatives, industrial solvents and in the production of synthetic resins. They extract as inseparable contaminants in water using US EPA Method 625. This poster derives a Total Allowable Concentration (TAC) for m- and p-Cresols in drinking water under NSF/ANSI Standard 61 (Drinking Water System Components: Health Effects). US EPA established an oral reference dose (RfD) of 0.05 mg/kg/day in 1990 that can be used for TAC derivation, but a number of high quality studies have been published since then with effects at doses lower than those used by U.S. EPA. Cresols are not mutagenic, but are clastogenic in vitro but not in vivo. Two chronic studies in rats and mice demonstrate some potential for carcinogenicity following oral exposures at high doses of cresols (700-1,040 mg/kg/day). Reproductive/developmental toxicity studies demonstrate no treatment-related impacts on fertility or development following oral exposure at doses less than 175 mg/kg/day. Among the newer studies published since development of the U.S. EPA RfD are two developmental toxicity studies in rabbits. A NOAEL of 5 mg/kg/day can be assigned based on maternal toxicity demonstrated by clinical signs of toxicity and mortality at cresol doses at above 50 mg/kg/day. Using the approach recommended by the U.S. EPA, a human equivalent dose (NOAELHED) of 2 mg/kg/day was estimated from the 5 mg/kg/day NOAEL. An oral RfD of 0.007 mg/kg/day was derived from the NOAELHED using total uncertainty factors of 200 (3x for intra- and inter-species extrapolation, respectively, 3x for subchronic to chronic extrapolation, and 3x for database deficiency). Based on this value, along with a 20% relative source contribution factor, a body weight of 70 kg, and 2L/day drinking water intake, a TAC of 50 μg/L was estimated for m/p cresols (combined). A margin of safety of 1,400 between the TAC and the NOAELHED indicates that exposure to m- and p-cresol isomers in drinking water at levels no greater than TAC is unlikely to result in adverse health effects.

No Significant Risk Level (NSRL) Derivation for Pulegone

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Pulegone is a constituent of many essential oils, such as pennyroyal and peppermint oils, which are used as flavoring agents for foods and beverages, as fragrance ingredients, or as herbal medicines. Recently, pulegone has been listed as a carcinogen on the State of California’s Proposition 65 based on its IARC categorization as a Group 2B carcinogen. While IARC’s monograph has yet to be published, the carcinogenicity of pulegone in F344/N rats and B6C3F1 mice has been evaluated by the National Toxicology Program (NTP). The NTP study reported statistically increased incidences of combined urinary bladder tumors in female rats and of combined liver tumors in male and female mice. While many rodent tumors are of questionable relevance to humans, industry is required to comply with the Proposition 65 labeling requirements unless they demonstrate that their products contain pulegone at a level below an applicable NSRL. OEHHA has not yet derived a NSRL for pulegone; therefore, the NTP study was used to establish a NSRL. In the NTP study, high mortality and morbidity occurred in male and female rats. Consequently, rat tumors were not considered in the establishment of the NSRL. Tumor incidences in male mice were within concurrent and historical control
ranges and did not exhibit dose-response, so these tumors were not considered for NSRL determination. Liver tumors in female mice exhibited a non-linear dose-response and were statistically significantly increased at the high dose only. Using this data set, benchmark dose (BMD) multistage modeling was performed to derive a NSRL of 30 μg/day.

**209 REACH Registrants Fail to Use Available Dermal Uptake Data in Their Derivation of Dermal DNELs**

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The EU chemicals legislation REACH requires Derived No-Effect Levels (DNELs) to be derived for all relevant routes (inhalation, dermal, and/or oral). DNELs should represent an external exposure, thus dermal absorption data are needed to derive dermal DNELs. Our aims were to investigate (1) to what extent REACH registrants use available scientific data on dermal uptake to develop dermal DNELs and (2) if the selection of such data appears to be biased. We have previously compiled dermal uptake data for substances in the Swedish list of occupational exposure limits (OELs, Johanson & Rauma, Arbete o Hälsa 42:2). Based on the previous study we identified 108 chemicals that (a) ought to have a skin notation according to the ECETOC criteria (skin absorption contributes >10% to the systemic dose compared to inhalation at the OEL, n=106) and/or (b) already have a skin notation in the Swedish OEL list (n=70). The 108 substances were cross referenced with the ECHA database of registered substances, in order to investigate: whether they were registered, had dermal-DNELs, which dermal uptake data and absorption percentage had been cited, if the 108 substances, 46 were found in the ECHA database. Although all have a potential for significant dermal uptake, only 28 have a long-term dermal DNEL for systemic effects, and whereof 21 have dermal uptake data included in the ECHA database. For the 21 substances, we identified 60 scientifically published dermal uptake studies, 20 of these studies were also cited by registrants (covering 12 substances). For 12 substances both we and registrants identified absorption percentages from at least one study. Although cited numbers often differed, no clear trend in direction or magnitude of difference was discernible. We conclude that many REACH registrants fail to use available scientific data. However, there does not seem to be any selection bias resulting in higher dermal DNELs.

**210 Examination of Multiple Dose-Response Analysis Methods for Estimating Dermal Cancer Risks for PAH Mixtures**

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Risk assessments for polycyclic aromatic hydrocarbon (PAH) mixtures are typically limited to analysis of individual chemical components due to the lack of toxicity information on the whole mixture. Often, the challenge to performing a mixture-based quantitative dose-response assessment results from a limited toxicological database, disparate study designs, or single-dose studies. We sought to test alternative dose-response methods to develop probabilities of skin tumor response for an example PAH mixture (i.e., built-up roofing asphalt), by exploring several means to derive potency estimates (i.e., dermal slope factors), guided by US EPA’s (2000) mixtures risk assessment guidelines. We gauged the respective strengths and shortcomings of each alternative approach, articulating the assumptions inherent in each, and assessed the consistency of outcomes among methods. We proceeded through a series of analyses to understand the nature of the tumorigenic response of the example asphalt mixture (and its components) from available animal bioassays. Further, we tested the impact of assuming parallelism of the dose-time-response curves for asphalt mixtures and benzo[a]pyrene (BaP) to extrapolate the dose-response relationship from BaP to asphalt mixtures (for which only single-dose studies are available). We primarily used US EPA’s Multistage Weibull (MSW) Time-to-Tumor model to analyze the probability of neoplastic lesions at specified times. Thus, our dose-response analysis provided a means to assess the cancer potency of whole mixtures (based on single-dose studies) by relating the shape of the dose-response curve to BaP (where multi-dose studies existed), while following generally accepted US EPA practices, with some modifications to allow for analysis of disparate data sets.

**211 Carcinogenic Risk from Dermal Exposure to Benzo[a]pyrene**


Benzo[a]pyrene (BaP) is a polycyclic aromatic hydrocarbon (PAH) with mutagenic and carcinogenic properties that is found in the environment primarily as a result of incomplete burning of materials that contain carbon. High soil concentrations are found at hazardous waste sites associated with creosote/wood treatment, coal gasification, and petrochemical industries. The general population can be exposed to BaP dermally via contact with soil and sediment contaminated with PAHs. Occupational exposure to complex mixtures of PAHs including BaP, such as coal tar, coal tar pitches, unrefined mineral oils, shale oils, and soot has been demonstrated to be associated with increased risk of skin cancer. Previous EPA efforts to characterize the risk from BaP exposure via contaminated soil have focused on systemic absorption and did not address direct effects on the skin. As dose-response data demonstrating increased incidence of benign and malignant skin tumors in mice following repeated dermal exposure to BaP are available, EPA’s Integrated Risk Information System has developed a draft cancer slope factor for dermal BaP exposure to estimate increased risk of skin cancer. Male mouse incidence data from a well reported, lifetime, low dose bioassay were modeled using the multi-stage-Weibull model. As no established methodology exists to adjust for interspecies differences in dermal toxicity at the point of contact, several methods were explored to extrapolate from mice to humans. Ultimately, the BMDL10 for the occurrence of skin tumors in mice exposed dermally to BaP was adjusted to its human equivalent by allometric scaling, in order to account for more rapid metabolism, distribution, and clearance in mice. As evidence supports a mutagenic mode of action, linear low-dose extrapolation is recommended, using the proposed dermal slope factor of 0.006 per μg/day. 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.

**212 Testing the Validity of US EPA’s Proposed Dermal Slope Factor for Benzo[a]pyrene: Genetic Alteration Signatures in Common Skin Cancers**

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USEPA’s revised toxicological assessment of Benzo[a]pyrene (BaP) was released for public review on September 26, 2014. EPA’s Science Advisory Board will evaluate the document in the coming months. The newly revised document proposed a Dermal Slope Factor (DSF) of 0.006 (μg/kg)-day-1. The DSF in usual units is 480 (mg/kg-day)-1, which is far higher than the proposed oral slope factor of 1 (mg/kg-day)-1. If EPA’s proposed DSF were true, it would predict that a large fraction of non-melanoma skin cancers in the US are caused by low level dermal exposures to PAHs. These exposures would include exposures to urban soils, to coal tar containing pharmaceuticals, to grilled and charcoal broiled meats, among others. To test this assumption, a literature review was performed to summarize the state of scientific knowledge regarding altered genetic signatures in UV- and PAH-induced non-melanoma skin cancers. UV-induced cancers, both in rodents and humans, display a high frequency of specific mutations in the p53 tumor suppressor gene and a genetic signature of C to T and CC to TT mutations at dipyrimidine sites. Skin cancers in rodents induced by dermal exposure to BaP contain a distinct and separate mutational signature in the H-ras oncogene, the result of A to G transversions at hot-spot codons. Although low levels of p53 mutation have been reported in PAH-induced mouse skin cancers, the researchers do not implicate them as causal events in mouse skin tumorigenesis. Furthermore, mutations observed in the H-ras oncogene contain the UV mutational signature, not the PAH signature. The BaP dermal slope factor has far reaching implications, because BaP is EPA’s indicator PAH, which is used to assess the risks posed by all PAH mixtures using USEPA’s Relative Potency Factors for other potentially carcinogenic PAHs. The potential impact of the proposed DSF on human health risk assessments is discussed.

**213 Oral Risk Assessment and Acceptable Drinking Water Levels for the Commonly Used Polyvinyl Chloride Plasticizer Acetyl Tributyl Citrate**

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General population exposure to acetyl tributyl citrate (ATBC) may occur from the widespread use of this non-phthalate ester plasticizer for polyvinyl resins in products that contact drinking water, in medical plastics, cosmetic products, and children’s toys. Exposure can also occur through ingestion of food and drugs, as ATBC is permitted as a food additive, food contact substance, and pharmaceutical excipient. An oral risk assessment incorporating new chronic data and current risk
assessment methodology was undertaken to establish allowable drinking water levels. Absent any human health effect data, hazard identification relied on laboratory animal data, which identified treatment-related effects after oral ATBC exposure including body weight loss and liver and kidney effects. In a combined chronic/carcinogenicity dietary exposure study in rats, treatment-related effects beginning after 52 weeks of exposure included reductions in body weight at ≥ 300 mg/kg-day not attributable to reduced palatability and without increases in neoplastic lesions up to the highest dose (1000 mg/kg-day). In a two-generation reproductive toxicity study in rats, treatment-related effects were limited to consistently lower body weights in F1 male rats at 300 mg/kg-day. The generalized toxicity evidenced as reduced body weight in chronically fed rats was selected as the critical effect with a resulting Human Equivalent NOAEL of 32 mg/kg-day. Using a total uncertainty factor of 30x (3x inter-species, 10x intra-species), an oral RD of 1 mg/kg-day was determined for ATBC, consistent with the 2005 tolerable intake of 1 mg/kg-day. U.S. EPA Exposure Decisions: “Free criteria for assessing available exposure information” was then used to define a Reference Dose (RfD) as “the level of exposure to a chemical for which there is a high degree of confidence that there is no appreciable risk of adverse effects to human health.” U.S. EPA calculated a RfD for the ATBC metabolite, 2,4-Dichlorophenoxyacetic acid (2,4-DPAA), as 0.075 mg/kg-day. U.S. EPA Exposure Decisions: “The Human Equivalent NOAEL of 0.075 mg/kg-day was used. The immature mouse evaluated in the key study was considered to represent a susceptible population with respect to immune system response. Due to insufficient quantitative data in humans, hazard identification relying on laboratory animal data identified toxic effects associated with oral exposure to 2,4-DP including body weight loss, developmental immunotoxicity, liver effects, and parental and fetotoxicity without specific effects on reproduction. In two-year bioassays, an increase in syncytial alteration of hepatocytes was reported in all dosed male mice but no tumor incidences were increased in rats or mice. A dose-related decrease in delayed-type hypersensitivity observed in Sprague-Dawley rats following pre-natal and 12 weeks post-natal exposure to ≥ 3 mg/kg-day 2,4-DP via drinking water was the critical effect based on the magnitude of the response and the appropriateness of the study design, recognizing that indirect assessment of potential immunotoxicity in other studies (including studies with early post-natal life exposure to 2,4-DP) found no evidence of immunotoxicity. As benchmark dose modeling did not identify a reliable point of departure for derivation of the Reference Dose (RfD), the Human Equivalent NOAEL of 0.075 mg/kg-day was used. The immature mouse evaluated in the key study was considered to represent a susceptible population with respect to immune system response. Thus, inter- and intra-species uncertainty factors (UF) were each set at 3x. Using a 10x composite UF, an oral RD of 0.008 mg/kg-day was determined for 2,4-DPC.

Diuron is a substituted urea compound used globally as an herbicide. Urinary bladder tumors were induced in rats after chronic dietary exposure to diuron likely through a cytotoxicity-mediated mode of action (MOA). The present study estimates potency for activation of key functional pathways in this MOA identified from global gene expression profiling at 7 d and 20 wk and examines their concordance with apical key events over time, including urothelial cell cytotoxicity, proliferation, hyperplasia and ultimately tumors. The most sensitive urothelial pathways at 20 wk involved immune cell responses based on lower quartile (Q1) pathway transcriptional benchmark dose levels (BMD<sub>50</sub>) of 4.5 mg/kg-d, consistent with the apical benchmark dose (BMD<sub>α</sub>) of 4 mg/kg-d for urothelial cytotoxicity observed with scanning electron microscopy. Xenobiotic metabolism/detoxification and oxidative stress pathways were active at Q1 BMD<sub>α</sub> of 25-31 mg/kg/d, consistent with the BMD<sub>α</sub> for urethelial simple hyperplasia of 22 mg/kg/d at 20 wk and the suspected tumor threshold of 23-32 mg/kg/d at 2 y. A similar metabolic and oxidative stress response with comparable potency was seen at 7 d (Q1 BMD<sub>50</sub>), of 18-25 mg/kg-d without ultrastructural or histopathological evidence of urothelial alterations. Using a case study approach, we demonstrate that the transcriptional effects following short-term exposure correlate in a dose responsive manner to key MOA events and tumorigenicity. Combined with previous and ongoing research with conazoles, phthalates and phenobarbital in mouse liver, our case studies encompass multiple species, target tissues, MOA, chemical classes and exposure durations of 2 d to 20 wk, thus demonstrating the utility of this approach as a chemical testing and prioritization strategy. This abstract does not reflect EPA policy.

Diuron Phthalate (DIP) is a plasticizer used in consumer products. The California Office of Environmental Health Hazard Assessment (OEHHA) added DIP to their list of chemicals known to cause cancer in 2013. Sources include liver adenoma and carcinoma observed in male and female rats and mice to be related to PPAR α activation as the plausible mechanism of action and determined this mechanism to be relevant to humans. Oral exposure data were modeled against tumor incidence using EPA Benchmark Dose Model Software (BMDS) v.2.4. The lower limit of the benchmark dose (BMDL) ranged from 164 to 314 mg/kg/d for all 5 of the sex/species combinations. The data from the male rat liver resulted in the highest p-value, with a low AIC and low scaled residual. The BMDL for this data set was 212 mg/kg/d. This data set was further evaluated in the BMDS and animal cancer slope factors were scaled to human using the allometric scaling designated by OEHHA and no significant risk levels (NSRLs) were calculated using a lifetime risk of 10-5. The calculated NSRLs ranged from 341-494 μg/day (average 430 μg/day). DIP and DEHP share a PPAR α-dependent mechanism of action, OEHHA promulgated an NSRL for DEHP (310 μg/day) using an additional 10 fold species sensitivity factor to account for rodents being more sensitive relative to humans. By the same rationale, the oral NSRL for DIP is 4305 μg/day. Consumer products containing plasticizers and intended for adults generally have dermal exposure. Dermal absorption of DIP is reported to be 0.3% for short term exposures and 4% for exposures up to 7 days; oral absorption was 50% (European Chemicals Agency, 2003). OEHHA has calculated dermal-specific NSRLs using a ratio of oral/dermal exposure. The dermal NSRL for DIP would be 717,447 μg/day for short term exposures and 53,388 μg/day for longer exposures. Use of route specific safe harbor values is recommended for more accurate evaluation of chemical exposures, and therefore risk, from consumer products.
High Throughput Screening (HTS) assays have generated toxicological data on environmental protection agency, research triangle park, NC. H. Ryan1 and J. D. Field1,2 Consumer Product Safety Directorate, Health Canada, Ottawa, ON, Canada and Safe Environments Directorate, Health Canada, Ottawa, ON, Canada. Sponsor: M. Khan.

In recent biomonitoring studies, the main metabolite of diethyl phthalate (DEP) was detectable in >99% of the Canadian population and at levels higher than those of any other phthalate. DEP is used extensively in cosmetics and as a fragrance ingredient may legibly be captured under the term ‘parfum’ on product labels in Canada, thus making it difficult to avoid. While DEP does not appear to produce the characteristic suite of lesions and malformations that are collectively referred to as the rat phalate syndrome, its safety has come under increased scrutiny in other jurisdictions. In July 2014, a Chronic Hazard Advisory Panel (CHAP) commissioned by the U.S. Consumer Product Safety Commission issued their final report. The CHAP concluded that DEP exposure from consumer products is currently negligible, but did not specifically evaluate exposures from other sources. In order to establish a point of departure for risk assessment, a benchmark dose for DEP was computed based on a reduction in testosterone levels observed in F0 parental male rats exposed to DEP through dietary administration in a two generation reproduction study. The rat dose was converted to a human equivalent dose by alomometric scaling and a provisional tolerable daily intake (pTDI) of 0.9 mg/kg bw/d was derived based on the application of uncertainty factors to the lower limit of a one-sided 95% confidence interval on the modelled benchmark dose. Although the reduction in testosterone observed in male rats is not considered a robust finding and may not be an appropriate endpoint for higher-tiered assessment, this pTDI confers a high level of health protectiveness as it is based on the most sensitive end-point identified for phthalates in the animal literature.

MassDEP first derived a UR value for PCE in 1990; updated in 2008 to 1x10^-5 per ur/l using newer bioassay data and derivation methods. EPA finalized their final PCE UR on IRIS of 3x10^-5 per ur/l for MCL, providing an alternate UR of 1x10^-5 per ur/l based on rat mononuclear cell leukemia (MCL). However the basis for EPA's final UR, liver tumors only, is inconsistent with the basis of the URs previously derived by MassDEP and EPA (2008, 2011 drafts), based on MCL, and is 30-fold lower than previous estimates. In consideration of updating our regulatory value, we disagreed with elements of EPA’s final decision to use liver tumors instead of MCL and ultimately decided on an alternative approach consistent with our state’s protocols. Following review of PCE assessments by EPA (2012, 2011) and NRC (2010), evaluation of individual animal bioassay data on severity of MCL, statistical methods for multiple comparisons, MCL literature and recent epidemiology studies, we conclude that the MCL endpoint from the JISA 1993 study has internal and external validity and is an appropriate basis for the PCE UR. Furthermore, EPA (2012) provided sufficient scientific justification to support use of MCL for the primary IRIS UR for PCE. Like EPA, we used the improved PCE PBPK model (Chiu and Ginsberg 2011) for dose extrapolation. For the MCL dose metric, we made a different decision than EPA for balancing contributors to uncertainty in cross-species extrapolation. We selected total metabolism (TM) estimating the presumed active moieties but with more quantitative uncertainty, instead of the better estimated but less specific PCE AUC in blood. Using TM and multistage dose-response model, we derived a UR of 3x10^-5 per ur/l based on MCL; 3-fold lower than EPA’s MCL UR and 10-fold higher than EPA’s liver UR. Our analysis highlights the importance of considering chronic exposure to PCE in the health risk assessment of TCE. This poster summarizes the results of an updated literature review on the toxicity of TCE, an evaluation of recently published studies, and a risk assessment conducted on methods and health endpoints.
with current methodology. An updated Public Health Goal (PHG) is derived for TCE in drinking water based on a one-in-one-million combined lifetime extra risk of kidney cancer, liver cancer, and NHL calculated from meta-analyses of multiple epidemiological studies. The health-protective concentration for non-cancer effects of TCE in drinking water is derived from reduced thymus weight observed in female B3CGF1 mice exposed to TCE in drinking water for 30 weeks. Overall, the updated PHG is developed to protect individuals from adverse effects of TCE, including cancers, that may occur at any age level.

223 Derivation of Draft Noncancer Reference Exposure Levels for Ethylene Glycol Mono-N-Butyl Ether

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Ethylene glycol mono-n-butyl ether (EGBE) has gained widespread use in industrial and consumer applications due to its properties as a solvent. It is well-known for its hemolytic properties (i.e., red blood cell damage resulting in regenerative anemia) in rodents. However, airborne EGBE exposures in humans are more often associated with eye, nose, and upper respiratory tract irritation, with no or only minor hemolytic effects at high doses. In work and home environments, major human exposure routes of EGBE are through inhalation and skin contact. The critical effects of airborne EGBE exposure from short to long term exposures are eye irritation, respiratory obstruction, and epithelial degeneration of upper respiratory airways. We are deriving acute, 8-hour, and chronic Reference Exposure Levels (RELs) for inhalation exposure following OEEHA’s Air Toxics Hot Spots risk assessment guidelines. The acute REL is based on a whole-body human exposure study with trigeminal-mediated sensory irritation as the critical endpoint. A LOAEL of 98 ppm is identified. To account for individual variability, Uncertainty Factors (UFs) for interspecies and intraspecies toxicokinetic and toxicodynamic differences are assigned V10 and 10, respectively. A NOAEL-to-NOAEL UF of 10 is applied. Thus, we generate a proposed acute REL of 1.6 mg/m3 (0.33 ppm). For 8-hour and chronic RELs, the critical endpoint is histopathological changes in upper respiratory epithelium of rats exposed to EGBE for 2 years. Using US EPA benchmark dose methodology, a Point of Departure (POD) of 7.6 ppm is calculated. Following time-adjusted exposure and Human Equivalent Concentration calculation, the 8-hour and chronic values are 0.95 and 0.48 ppm, respectively. An interspecies UF of V10 and an intraspecies UF of 10 (to account for the potential differential susceptibility of children to respiratory irritants) for both 8-hour and chronic exposures are applied. Thus, the proposed 8-hour and chronic RELs are 0.15 mg/m3 (0.032 ppm) and 0.077 mg/m3 (0.016 ppm) respectively.

224 Mode of Action of the Effect of Trans Fatty Acids (TFAs) on LDL-Cholesterol and Implication for Dose Response

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The Food and Drug Administration (FDA) published a Federal Register notice tentatively determining that partially hydrogenated oils (PHOs), the primary dietary source of industrial TFAs – (TFAs) are no longer generally recognized as safe (GRAS) when used in food. This determination was based on the conclusion that there is no threshold intake for iTFA that would not increase an individual’s risk of coronary heart disease, based primarily on research showing iTFAs increase LDL-cholesterol (LDL-C) at high levels of intake. LDL-C is one of three surrogate endpoints for coronary heart disease risk recognized by the FDA. A Mode of Action (MOA) analysis was conducted for the relationship between iTFA dose and LDL-cholesterol. The MOA evaluation identified two key events: (1) increased VLDL levels, and (2) decreased LDL receptor activity. However, unlike classical MOAs, these two key events occur in parallel, rather than sequentially, presumably because fatty acids are nutrients regardless of configuration. The data were evaluated using the ILSI/IPCS MOA framework, based on the modified Hill criteria. The data for evaluating the temporal and dose responses for key events relative to the change in LDL-cholesterol were very limited, lacking sampling at early time points for key events. The result of this analysis and a holistic evaluation of the data indicates that the data overall are consistent with a nonlinear dose response and this is supported by physiology. LDL-cholesterol levels vary widely within an individual between fed and fasted states, indicating that there is a wide variability within the normal range. In addition, there is a large degree of regulation and feedback controls on every aspect of TFA transport, utilization and clearance, reflecting significant investment in maintenance of homeostatic levels. This analysis illustrates the utility of bringing a risk assessment perspective to the understanding of nutritional issues.

This work was sponsored by the ILSI North America PHO Task Force.

225 Derivation of Inhalation Reference Values for Hexamethylenediamine Using an Exposure Model Based on the Dihydrochloride Salt

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Hexamethylenediamine (HMDA) is used in the fiber and plastics industry as an intermediate in the production of nylon, high-strength resins, and polyamide adhesives. As a toxicant, HMDA acts primarily as a respiratory irritant with effects occurring in the upper respiratory tract, although systemic effects have been noted at high concentrations. Reference values (ReVs) are chemical-specific air concentrations derived to protect human health. Acute and chronic ReVs were developed for HMDA based on an inhalation study conducted by the National Toxicology Program, which used the salt of HMDA, hexamethylenediamine dihydrochloride (HDDC). For the acute evaluation, rats and mice were exposed to 0, 10, 30, 89, 267, and 300 mg HDDC/m3 for 2 weeks. The critical effect identified in the study for the most sensitive species was nasal lesions in rats, and the human equivalent point of departure (POD) was determined using dosimetric adjustments. Uncertainty factors were applied to account for variation in sensitivity within the human population, toxicodynamic differences between rats and humans, and any deficiencies in the scientific database. For the chronic evaluation, rats and mice were exposed to 0, 1.6, 5, 16, 50, and 160 mg HDDC/m3 for 13 weeks. The critical effect identified in the study for the most sensitive species was hyaline degeneration in the olfactory epithelium in mice. The data provided in this study were suitable to benchmark dose (BMD) modeling, and dosimetric adjustments were made to the 95% lower limit of the BMD(10) to determine the human equivalent POD. Uncertainty factors were applied to account for similar uncertainties as in the acute study, plus an additional factor for the use of a subchronic study in a chronic evaluation. The ReVs were initially calculated for HDDC and then adjusted for HMDA. The resulting proposed acute ReV is 27 ug/m3 and the chronic ReV is 2.9 ug/m3 for respirable HMDA ≤ 10 μm in diameter. These values will be submitted for public comment before being finalized.

226 Adequacy of Available Data to Derive a Provisional Inhalation Toxicity Value for Carboxyl Sulfide

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The adequacy of epidemiologic, toxicologic, and mechanistic data was assessed for deriving a provisional inhalation reference concentration (RfC) for carboxyl sulfide (COS). U.S. EPA’s 1994 Guidelines for deriving RfCs note that the minimum database for a low confidence RfC should have at least one subchronic-duration inhalation animal study that examines a comprehensive suite of endpoints (including respiratory effects) and identifies effect levels. No comprehensive animal inhalation toxicity study is available for COS, but peer-reviewed EPA-sponsored subchronic-duration inhalation studies in rats examined a battery of neurotoxicity endpoints, and identified a LOAEL of 300 ppm and LOAEL of 400 ppm based on lesions in multiple areas of the brain and changes in auditory evoked potentials. Significant dose-dependent decreases in cytochrome oxidase activities were also seen at 200 to 400 ppm in regions of the rat brain that developed lesions, indicating a possible mechanistic role of mitochondrial dysfunction in COS-induced neurotoxicity. Considerations supporting the derivation of a provisional RfC for COS based on neurological effects in the absence of a comprehensive inhalation study include: 1) acute inhalation studies of COS identifying central nervous system dysfunction and brain lesions as the primary effects of COS exposure; and 2) that COS is a metabolite of a known neurotoxicant, carbon disulfide, the IRIS RfC for which is based on neurological effects in humans. One-generation reproductive/developmental toxicity studies in rats exposed to ≤ 182 ppm found no effects on reproduction or development, suggesting that these are not sensitive endpoints for this chemical, and also found no gross or microscopic pathology of 33 tissues (including brain) in offspring, suggesting, with some uncertainty, that systemic effects other than neurotoxicity are also not likely a concern. This abstract does not necessarily reflect U.S. EPA policy.

227 Derivation of a No-Significant-Risk-Level (NSRL) for N,N-Dimethyl-p-Toluidine (DMPT)

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DMPT is listed on the State of California’s Proposition 65 List as a chemical known to the State to cause cancer. This listing is based on the results of a National Toxicology Program (NTP) 2-year oral carcinogenicity study which found clear
evidence of carcinogenic activity in F344/N rats based on increased incidences of hepatocellular carcinoma (HCC), and hepatocellular adenoma (HCA) or HCA/ HCC (combined) in both sexes and increased incidences of nasal cavity neoplasms in male F344/N rats, as well as clear evidence of carcinogenic activity in B6C3F1/N mice based on increased incidences of HCA, FHC, and hepatoblastoma in both sexes and alveolar/bronchiolar neoplasms in females. Unless a business is able to demonstrate that DMPT in a product is below a specific NSRL, then they must comply with the Proposition 65 clear and reasonable warning requirement. The State of California has not published a NSRL for DMPT. Evaluation of tumor inci- dence data identified three tumors that were most relevant for NSRL derivation: HCA/HCC combined in male and female F344/N rats, nasal cavity neoplasms in male F344/N rats, and alveolar/bronchiolar neoplasms in female mice. The U.S. EPA’s Benchmark Dose Software (version 2.5) was utilized to model tumor data for each tumor type and estimate cancer slope factors. The estimated cancer slope factor for alveolar/bronchiolar adenoma or carcinoma in female mice was the largest among those assessed, and thus this NSRL was derived for 1,1,1-Trichloroethane. ATSDR has used a NSRL of 11.5 μg/dL for DMPT, in accordance with the guidelines of California EPA. This NSRL value can be used as part of an exposure and safety assessment to evaluate compliance with Proposition 65 requirements for products sold in the State of California.

228 Identification of Critical Effects for Derivation of Oral Minimal Risk Levels (MRLs) for Polychlorinated Biphenyl Ethers (PCBDE) M. Odin1, K. Zaccaria2 and H. Pohl3. 1SRC, Inc., North Syracuse, NY and 2ATSDR, Atlanta, GA.

The number of human and animal studies evaluating toxic effects of PCBDE exposure has substantially increased since the last ATSDR Toxicological Profile update in 2003. These studies have filled data gaps and provide a robust database for generation and support of new and updated toxicity assessments for PCBDE. New human studies provide qualitative support for effects of PCBDE on thyroid hormone levels, male and female reproduction, and most convincingly, on various aspects of development, including birth outcome, reproductive and endocrine development, and especially neurological development, but do not provide a basis for quantitative assessment. New animal studies provide support for derivation of new oral MRLs substantially lower than the previous MRLs, and also allow derivation of an acute oral MRL for decaBDE, which did not have one previously. Critical effects for MRL development identified from the recent animal literature are as follows: 1) for acute oral exposure to decaBDE, a NOAEL of 1.34 mg/kg and a LOAEL of 2.22 mg/kg for altered neurobehavior in 2-4 month old mice following a single exposure to decaBDE on PND 3; 2) for intermediate oral exposure to decaBDE, increased serum glucose at ≥0.05 mg/kg/day and related pancreatic changes at ≥1 mg/kg/day (altered insulin regulation, morphological changes in pancreas) in rats exposed for 8 weeks; 3) for acute oral exposure to lower brominated PCBDE, reduced maternal serum T4 and reproductive impairments such as delayed estrus and reduced estrous cycle lengths; 4) for intermediate oral exposure to decaBDE, reduced serum testosterone to ≤0.001 mg/kg/day and related testicular changes (increased number of apoptotic cells and multinucleated giant cells in the testes) at ≥0.03 mg/kg/day in male rats exposed for 8 weeks.

229 An Approach to Deriving Oral Minimal Risk Levels (MRLs) for PFOA and PFOS L. Ingerman1, G. L. Diamond1, S. Chou2, D. Jones2 and P. Ruiz2. 1SRC, Inc., North Syracuse, NY and 2ATSDR, Atlanta, GA.

ATSDR previously considered derivation of oral MRLs for perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS) in 2009. At that time, the Agency determined that it would be difficult to define points of departure for MRL derivation based on either the available human data or the available animal studies. This was due in part to the lack of consistency between effects observed in humans and those observed in laboratory animals as well as the large interspecies differences in the toxicokinetics of perfluoroalkyls for which mechanisms are not completely understood. A large number of epidemiological studies have been published since 2009 that provide much stronger evidence for the identification of targets of toxic effects. However, because these studies lacked clearly identified NOAELs and LOAELs, they were not considered suitable for derivation of MRLs. ATSDR is considering deriving intermediate-duration oral MRLs based on the findings of increased liver weight in two studies of monkeys administered PFOA or PFOS via capsules for 6 months (Butenhoff et al. 2002; Searat et al. 2002). Because using the administered dose is problematic due to species differences in the toxicokinetics of the two compounds, particularly the difference in elimination half-times, ATSDR is exploring the alternative approach of using an internal dosimetric (blood PFOA or PFOS levels) as the point of departure for the MRLs. This point of departure could then be converted to an equivalent exposure dose in humans, defined as the continuous ingestion dose that would result in steady-state concentrations of PFOA or PFOS equal to the serum concentration selected as the point of departure. A single compartment, first order model in which elimination kinetics are represented by observed serum elimination half-times for PFOA or PFOS and an assumed apparent volume of distribution and gastrointestinal absorption fraction (both based on studies conducted in monkeys) could then be used to calculate human equivalent doses.


The U.S. Environmental Protection Agency office of Remediation and Technology Innovation (OSRRT) through an interagency agreement with Oak Ridge National Laboratory (ORNL) has modified the Regional Screening Level (RSL) exposure parameters based on parameters presented in the 2011 Exposure Factor Handbook. This action was based on a 2014 directive from the Office of Solid Waste and Emergency Response. This policy changed the guidance in the RSLs “...to reduce variability and uncertainty in the exposure assumptions used by regional superfund staff to characterize exposures to human populations for human health risk assessments”. Forty-one exposure factors were altered and 16 changes were recommended. Changes impact RSLs for air, soil, water and biota. Landuse sce- narios impacted are: adult resident, child resident, workers, adult recreator and child recreator. Screening levels have become more protective for residential soil, industrial soil, tapwater and residential air exposure due to EFH updates. Industrial air screening levels are not impacted by the EFH updates.

231 Fall 2014 Review and Update of Drinking Water Standards and Health Advisories (DWSHA) Tables D. J. Stewart1, C. Baut2, C. S. Wood2 and S. S. Kucherenyua. 1University of Tennessee, Knoxville, TN, 2Oak Ridge National Laboratory, Oak Ridge, TN and 3U.S. Environmental Protection Agency, Washington, DC.

The fall 2014 DWSHA tables, sponsored by the U.S. Environmental Protection Agency’s (EPA’s) Office of Water (OW), summarize enforceable standards, Maximum Contaminant Levels (MCLs) and Maximum Contaminant Level Goals (MCLGs), and non-enforceable Health Advisories (HAs) for drinking water con- taminants. The tables provide MCLs, MCLGs, Reference Doses (RfDs), non-cancer HAs for different durations of exposure (One-day, Ten-day, and Lifetime), Drinking Water Equivalent Levels (DWELs), oral cancer slope factors (CSFs), and 10–7 – 10–6 cancer risk levels. Resulting from a systematic review initiated in 2012, the fall 2014 DWSHA tables were updated to ensure that 1) the benchmark values are consistent with the most current EPA assessments and 2) the tables reflect EPA’s new carcinogen lifetime value calculation policy which requires that a Lifetime noncancer HA is presented along with the cancer risk level drinking water concentrations (10–4 – 10–6 Cancer Risk). Risk managers may then determine whether the noncancer Lifetime HA or the cancer risk level drinking water concentrations for any particular contaminant provide a more meaningful scenario-spe- cific risk reduction. Benchmark changes were made or validated in the fall 2014 DWSHA tables for 106 drinking water contaminants. Specifically, RfDs, DWELs, Lifetime HAs, one/ten-day HAs, and cancer risk levels were updated for 8, 11, 45, 5, and 7 contaminants, respectively. These changes are documented in 46 Data Source Summary Documents (DSSDs), which are hyperlinked to the contaminant names in the fall 2014 DWSHA tables online. Additional global changes to the tables include: removal of letter or narrative carcinogenicity descriptions, addition of cancer slope factors, and replacement of 10–4 cancer risk values with 10−4 – 10−6 ranges. Every fall, the DWSHA tables will be reviewed and updated. If more current benchmark information is available, new DSSDs will be added as applicable.
Global regulatory bodies call for integration of scientific evidence from epidemiological, mechanistic and pharmacokinetic studies, to characterize health risks from chemical exposures. However, guidance is not available for evaluating and selecting epidemiological inputs to quantitative risk assessment (QRA), as these decisions can influence risk values and associated uncertainties. Epidemiological considerations that influence QRAs are the quality of study designs and methods, disease outcome(s) and exposure metric(s) used, and the potential for confounding and other biases. Combining evidence across epidemiological studies may increase the precision of inputs to risk assessment, but poses interpretational challenges in the face of significant heterogeneity of results due to different study designs and exposure metrics. Epidemiological evidence of a possible exposure threshold presents additional challenges, as current regulatory default paradigms rely on linear no-threshold (LNT) dose-response default models for carcinogens, which assume risk is proportionate to cumulative exposure—even at low (possibly endogenous) concentrations. To illustrate each of these, and the impact on QRAs based on different handling of epidemiological inputs, we consider the results from the four most recent epidemiological studies on the relationship between formaldehyde exposure and myeloid leukemia (ML) risk. No consistently increased mortality from ML was observed with cumulative, average, or highest exposure; however, one study reported increased ML risk with “peak exposure” but not with increasing numbers of peaks. When latency was considered, few ML’s occurred within 20 years of exposure (by any metric), precluding precise risk estimation. Given the limitations of the human exposure data, the lack of positive animal evidence for leukemia, and affirmative mechanistic evidence that exogenous formaldehyde cannot reach the bone marrow, a valid QRA based on integration of the evidence could not be performed.

Studies to understand potential effects of hydraulic fracturing (HF) on human health and the environment are currently being performed at state and federal levels, but none have included a human health risk assessment. One of the key challenges of conducting an HF risk assessment is the lack of established human health criteria for many of the compounds used in HF fluids. As part of a risk assessment focusing on potential drinking water risks from HF chemicals, we developed a hierarchy for the selection of toxicity criteria, including development of criteria de novo when no criteria have been developed by an authoritative agency. Our approach, which involved the development of health-protective drinking water concentrations, preferentially used Maximum Contaminant Levels (MCLs) followed by tap water Regional Screening Levels (RSLs) as health-protective drinking water benchmarks. If MCLs or RSLs were not available, we identified chronic oral criteria developed by other regulatory agencies and developed health-protective drinking water concentrations using the exposure assumptions that underlie the RSLs. When no established criteria value was available, we identified repeated dose oral toxicity data for the compound or its surrogate and derived a chronic oral toxicity value de novo using a methodology consistent with US EPA’s approach for developing Reference Doses (RfDs). Using this approach, we evaluated 97 chemicals found in 12 typical HF fluid systems, as well as 82 flowback chemicals. Because of the lack of established criteria, we needed to develop health-based drinking water concentrations for more than 50 HF fluid chemicals; in contrast, MCLs and RSLs were largely available for flowback chemicals, with only 3 compounds requiring the derivation of toxicity criteria de novo. The hierarchical selection and development of health-based benchmarks, which is presented for all 179 HF-related chemicals in this poster, allowed for a comprehensive evaluation of potential drinking water risks from HF chemicals.
236 Identification of Chemical Vascular Disruptors during Development Using an Integrative Predictive Toxicity Model, Zebrafish, and In Vitro Functional Angiogenesis Assays

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Chemically induced vascular toxicity during embryonic development can result in a wide range of adverse prenatal outcomes. We constructed an adverse outcome pathway (AOP) for developmental vascular disruption based on molecular initiating events correlated with abnormal embryonic vascular development in mice. ToxCast high-throughput screening data for 25 assays mapping to the AOP were used to rank order 1060 chemicals for their potential to disrupt vascular development. A subset of 38 predicted vascular disrupting chemicals (pVDCs) or non-pVDCs, including pesticides, flame retardants, and endocrine active compounds, were selected for targeted testing in zebrafish (Danio rerio). To test computational predictions, embryos generated by Tg(fli1:EGFP) and Tg(fli1:EGFP) zebrafish reporter lines were used to visualize and quantify trunk, cranial, and hyaloid blood vessel formation during development. The same chemicals were also evaluated in a functional angiogenesis assay comprised of a human endothelial cell and fibroblast co-culture system. In general, chemical rankings correlated among the predictive signature and zebrafish and in vitro tubulogenesis assays, although each assay platform revealed chemically-specific effects. As expected, positive chemicals in the VEGFR2 ToxCast assay disrupted angiogenesis in at least one assay platform used for signature evaluation. Taken together, this assay suite meets a critical need to assess predictions generated by computational models of developmental vascular toxicity. This abstract does not necessarily reflect EPA policy.

237 Synthetic Hydrogels for Screening Vascular Disrupting Compounds

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The large quantity of known environmental and synthetic compounds calls for a robust screening assay to identify potentially toxic compounds. Many of these compounds have been characterized as putative vascular disruption compounds (pVDCs) using assays of human vascular tissue in a dish. However, current assay systems typically measure two-dimensional (2D) endothelial tubule formation as a measure of vascular disruption. These assays are often performed on ECM-mimicking materials such as Matrigel, which is composed of hundreds of unique proteins and exhibits lot-to-lot variability that may reduce reproducibility. Recently, we have observed endothelial tubulogenesis and sprouting in assay platforms comprising well-defined, synthetic hydrogels. Here we report the analysis of de novo tubule network formation and EC sprouting using a high throughput, multivariate approach. Human induced pluripotent stem cell-derived endothelial cells (iPSC-ECs) were encapsulated in a synthetic hydrogel composed of poly(ethylene glycol) (PEG) and tethered cell attachment and cell-degradable crosslinking peptides. iPSC-ECs exhibited invasion that was correlated with the concentration of cell adhesion peptides and degradable peptide crosslinks. Immunocytochemistry of encapsulated cells demonstrated that iPSC-ECs expressed CD31 and exhibited morphologically distinct sprouting behavior consistent with endothelial cell sprouting, iPSC-EC invasion was potently inhibited by VEGF signaling inhibitors while survival was unaffected by inhibition, which suggests that VEGF is required for maximal iPSC-EC invasion but not for cell survival in hydrogel arrays. The screening approaches described here are capable of quantitating single-cell invasion, sprout length, survival, and tubule network formation simultaneously and thus can efficiently distinguish the particular influence of pVDCs on multiple EC functions.

238 Acute Inhalation Toxicity of Volatile Organic Compounds—Application of an Improved Cell-Based In Vitro Procedure

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Cell based in vitro methods to assess the biological effects of inhalable compounds are based on “air-lifted interface” (ALI) cell culture protocols where cultures from cell lines, primary cells or ex vivo sources such as PCLS (precision cut lung slices) are exposed efficiently to airborne material. They have been applied in approaches to test toxicity and biological action of environmental, workplace or chemical compounds. The basic ALI procedure has been successfully prevalent as a short-exposure test (1 hour exposures) for chemical gases. However, although a good predictability for highly toxic gaseous compounds or non-toxic inert gases could be demonstrated, it was not clear until now, if highly hydrophobic or non-toxic volatile organic compounds (VOCs) can also be assessed by cell based methods in vitro under these conditions with regard to a relevant estimate of their acute inhalation toxicity characteristics in vitro. Therefore, a four compound test substance matrix was set up from the data base of the European chemicals agency (ECHA) including Vapocoolant (hydrophilic low toxicity), styrene (hydrophobic, low toxicity), toluene (hydrophobic, low toxicity) and styrene (hydrophobic, low toxicity) could be derived from all substances and reflected the relative high and low acute in vitro inhalation toxicity potency. The results demonstrate that it was possible to study the acute inhalation toxicity by cell based methods even for low toxic, hydrophobic airborne chemicals and thereby characterize the in vitro approach as a useful alternative method for screening purposes in the sense of the “3Rs”.

239 Characterization of Aerosolized Zinc Oxide Exposures to Lung Epithelial-Macrophage Cocultures at the Air-Liquid Interface (ALI) Using the NACIVT System

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The successful implementation of animal-alternative, in vitro lung toxicity screening assays to replace or reduce in vivo inhalation testing is critical for product development. To continue the development and characterization of in vitro aerosol methodologies using the Nano-Aerosol Chamber for In Vitro Toxicology (NACIVT) system, rat lung epithelial L2 cells and NR8383 macrophages were co-cultured on an ALI. Aerosolized test atmospheres of fine-sized ZnO particles were generated in a glass cylindrical chamber using a jet-mix device (sampled as source particle concentration) and, through a connection tube, delivered at 25 mL/min to the cells and/or sampling filters maintained in the temperature-humidity-controlled deposition chamber. In 15 test atmospheres generated at 24-140 mg/m3 (MMAD[GSD]=0.85-1.0um[2.5-2.7]), 23-49% of the unipolar-charged ZnO deposited in the NACIVT system (23-62 ug/cm2) while only 9.5-22% of those uncharged were collected (7.8-14 ug/cm2). The mass deposited was proportional to the aerosol concentration at r2=0.86 and 0.84, respectively. Preliminary data showed that after a 1-hr airborne particle exposure (equivalent to 28 ug deposited/cm2), cellular metabolic activities (measured by XTT assay) of the co-cultures (n=3-4/group) at 0, 24, 48, and 72 hr post-exposure (PE) were reduced to 77±7, 44±8, 10±3, and 10±3% (mean±SD) of the time-matched air-exposed controls. A 24-hr PE exposure at 8.3±1×105, 28±2, or 57±1×105 ug particulate/cm2 reduced metabolic activity to 80±4, 85±6, 48±9, and 34±3% of control values, respectively, suggesting a concentration-response relationship. In conclusion, these ongoing characterization studies have provided important information for evaluating the system’s ability to simulate aerosol particulate exposures using both chemical and biological end points using in vitro toxicity characteristics in vitro. Therefore, a four compound test substance matrix was set up from the data base of the European chemicals agency (ECHA) including Vapocoolant (hydrophilic low toxicity), styrene (hydrophobic, low toxicity) and styrene (hydrophobic, low toxicity) could be derived from all substances and reflected the relative high and low acute in vitro inhalation toxicity potency. The results demonstrate that it was possible to study the acute inhalation toxicity by cell based methods even for low toxic, hydrophobic airborne chemicals and thereby characterize the in vitro approach as a useful alternative method for screening purposes in the sense of the “3Rs”.

240 Targeted Omics Analyses and Metabolic Enzyme Activity Assays Demonstrate Maintenance of Key Mucociliary Characteristics in Murine Cells Cultured for 6 Months

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The respiratory tract is one potential route of exposure to toxic chemicals. In vitro risk assessment of inhaled drugs and toxicants is typically performed in short term exposure assays using cell lines derived from the respiratory epithelium. 3D primary respiratory epithelium have emerged as more physiologically relevant models due to the conservation of key morphological features and functions, however their short shelf life limits their application to mostly acute exposure. Murine is a commercially available 3D respiratory epithelium system that can potentially maintain functional attributes for up to 1-week. Here we provide some key breakthroughs that are characteristics of the respiratory epithelium morphology, function, and xenobiotic metabolism over a short (1 month) and a long (6 months) period. Targeted proteomics using a panel of 240 Air Surface Liquid (ASL) proteins, qRT-PCR
screen of 40 xenobiotic metabolizing enzymes, cilia beat frequency (CBF) and CYP metabolizing enzyme activity were measured at multiple time points. CBF was significantly decreased after a period of 140 days. ASL proteomics and xenobiotic genes qRT-PCR did not reveal any significant change in expression profile over the 6 months period. In NHBE cells from three donors. Similar xenobiotic metabolizing gene expression profile were found in Mucluar. HBECCs and EpiAirways primary cultures with the exception of CYP2A13 and CYP2B6, which were expressed at a higher level in Mucluar. Inducibility and activity of CYP1A1/1B1 and activity of CYP2A6/2A13 were maintained in one tested Mucluar donor for up to 150 days, respectively. In conclusion, Mucluar retain key characteristics of a mucociliary epithelium when maintained for a period of 6 months in culture. Mucluar is therefore a potentially useful model to test repeated sub-cytotoxic doses of toxicants over a prolonged period of time.

241 Prediction of Chemical Respiratory Sensitizers Using GARD, a Novel In Vitro Assay Based on a Genomic Biomarker Signature


Background: Respiratory sensitization is a hypersensitivity reaction of the respiratory tract that develops upon exposure to certain low molecular (LMW) chemicals. Repeated exposure to these compounds may result in the development of Occupational asthma, severely affecting the quality of life for affected individuals. To limit occurrence of allergic diseases, hazard classification of respiratory chemical sensitizers remains an area of great importance and efforts are being made to develop predictive assays for identification of such compounds. In the current study, we present a novel cell-based testing strategy for assessment of respiratory sensitizers based on a genomic biomarker signature. We present results demonstrating that the signature has a potent ability to predict respiratory sensitization. Methods: A panel of reference compounds comprising respiratory sensitizing chemicals (N=29) and controls (N=74) were used for stimulation of cells. Transcriptomes were analyzed and a genomic biomarker signature was identified using one-way ANOVA p-value filtering and further optimized using an in-house developed wrapper algorithm. We have developed the usage of the biomarker signature into a novel non animal based testing strategy for assessment of respiratory sensitization, called Genomic Allergen Rapid Detection, GARD. Results: The performance of GARD was validated using an external test set comprising respiratory sensitizers (n=12) and non-sensitizers (n=58). Classification of the unknown testing chemicals were based on supervised machine learning. The predictive performance of the assay was determined using cooper statistics and accuracy, sensitivity and specificity was estimated to 89%, 67% and 84%, respectively. Conclusion: We present a novel non-animal based testing strategy with potent ability to predict respiratory sensitization. In the light of the current absence of validated or even widely accepted methods for this endpoint, we believe that our assay fills an important gap for risk assessment of chemicals.

242 Airway Epithelial Injury, Fibroblast Changes, and Cytokine/Chemokine Profiles Induced by Exposure of Human EpiAirway-FT Tissues to Diacetyl Vapors

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Inhalation exposure to diacetyl (DA) vapors in artificial butter flavorings has been associated with the development of obliterator bronchiolitis (OB) in microwave popcorn factory workers. Inhalation of DA vapors has also been shown to cause OB-like lesions in rats. EpiAirWay-full thickness (FT) tissue was investigated as an in vitro model to assess the toxicity of DA vapors on functional human airway epithelium with a sub-epithelial collagen matrix containing fibroblasts. EpiAirWay-FT tissues were exposed to DA vapors at the air-liquid interface for 1 hr on day 0, 2 and 4. Specifically, 0 (vehicle) or 25 mM DA (50 μl) was applied to vapor cups in order to provide vapor exposure (~1000 ppm) for 1 hr to the apical surface. Culture supernatants were collected every 24 hr in order to measure changes in order to provide vapor exposure (~1000 ppm) for 1 hr to the apical surface. Culture supernatants were collected every 24 hr in order to measure changes in WSSs were prepared by machine smoking 60 of Marlboro Red (R60) or Marlboro Silver (S60) cigarettes with the ISO smoking machine regimen and using a series of impingers to capture gas and particulate phase mainstream smoke constituents in 100 mL of dimethylsulfoxide. This machine smoking method will produce WSSs of different composition, but treatments using these two WSSs do not necessarily reflect human smoke exposure from these cigarettes due to compensatory smoking practices. The airway tissue models were apically exposed to 0.1% to 1% (v/v) WSS, either in a single 4-h treatment or multiple 4-h treatments. Cytotoxicity, tissue integrity, oxidative stress, mucus secretion, and matrix metalloproteinase (MMP) secretion were measured. The endpoints, except cytotoxicity and tissue integrity, responded in time- and dose-dependent manners. The R60 treatment resulted in higher levels of response than S60 for protein oxidation, mucus secretion, and MMP secretion. Based on the lowest effect dose, better differentiation between the WSSs was observed after three daily exposures than after one or five exposures. We further demonstrated that oxidative stress was a possible mechanism for WSS-induced mucus secretion. Our study demonstrates the value of evaluating cigarette smoke toxicity using disease-related endpoints in a physiologically relevant human airway tissue model. The results suggest the potential of such systems in informing the evaluation of tobacco products.

244 Abamectin Induces Rapid and Reversible Hypoactivity within Early Zebrafish Embryos

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During early zebrafish embryogenesis, spontaneous tail contractions represent the first sign of locomotion and result from innervations of primary motoneuron axons to target axial muscles. Based on a high-content screen, we previously demonstrated that exposure of zebrafish embryos to abamectin – an avermectin insecticide – from 5-25 hpf of effects on survival and gross morphology. Therefore, the objective of this study was to begin investigating the mechanism of abamectin-induced hypoactivity in zebrafish. Similar to 384-well plates, static exposure of embryos to abamectin from 5-25 hpf in glass beakers resulted in elimination of activity at low micromolar concentrations. However, abamectin did not affect neurite outgrowth from spinal motoneurons and, compared with exposure from 5-25 hpf, developing embryos were equally susceptible to abamectin-induced hypoactivity when exposures were initiated at 10 and 23 hpf. Moreover, immersion of abamectin-exposed embryos in clean water resulted in complete recovery of spontaneous activity relative to vehicle-exposed embryos, suggesting that abamectin reversibly activated ligand-gated chloride channels and inhibited neurotransmission. To test this hypothesis, we pretreated embryos to vehicle or non-toxic concentrations of fipronil or endosulfan – two insecticides that antagonize the γ-aminobutyric acid (GABA) receptor – from 5-25 hpf, and then exposed embryos to vehicle or abamectin from 23-25 hpf. Interestingly, activity levels within embryos-exposed embryos pretreated with either antagonist were similar to embryos exposed to vehicle alone. Using quantitattive PCR and phylogenetic analysis, we then confirmed the presence of GABA receptor α1 and β2 subunits at 5, 10, and 23 hpf, and demonstrated that zebrafish GABA receptor subunits are homologous to rodent and human GABA receptor subunits. Overall, our data collectively suggests that abamectin induces rapid and reversible hypoactivity within early zebrafish embryos, an effect that is likely mediated through the GABA receptor.
At present, detection of marine biotoxins in seafood is most often done with the in vivo mouse bioassay or LC/MS-MS based analysis. The mouse bioassay requires animals, has a high rate of false positive and false negative outcomes and the LC/MS-MS technique is expensive and does not allow for the detection of unknown marine biotoxins. Therefore, there is an urgent need for the development of in vitro assays with high sensitivity, enabling the detection of marine biotoxins at their current regulatory levels. The present study investigated the suitability of a fluorescence-based assay using mouse neuroblastoma neuro-2a cells with measurement of changes in membrane potential for rapid screening of marine neurotoxins in seafood. Model neurotoxic compounds were selected based on their similarity with marine neurotoxins in terms of mode of action: diphenhydramine and veratridine as Na+ channel affecting agents, ouabain and digoxin as Na+/K+ ATPase pump affecting agents and kainic acid as glutamate receptor agonist. In addition to these model compounds, pure marine neurotoxins commercially available (brevetoxin, domoic acid, palytoxin, saxitoxin, tetrodotoxin) and contaminated seafood extracts were tested to provide a first proof of principle. All model compounds, pure marine neurotoxins and two extracts from mussels or fish contaminated with either saxitoxin or tetrodotoxin elicited changes in membrane potential in neuro-2a cells detected by the fluorescent probe bioxonol. These data suggest that this fluorescent-based bioassay represents a promising alternative for reducing and ultimately replacing the in vivo assays currently used for the screening of both known and unknown marine biotoxins in seafood.
249 Bisphenol A Effects on In Vitro Human Neural Development

Was Window of Susceptibility Dependent

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Environmental factors affect the development and progression of a host of central nervous system disorders and are likely due to a continuum of exposure in windows of susceptibility (WOS) during and post neural development. Human stem cell based assays may prove to be an accurate in vitro model, mimicking different WOS. Our objective was to characterize bisphenol-A (BPA) effects during different in vitro neural developmental stages, starting with dividing and migrating neural progenitor (NP) cells to early post mitotic neuroblasts expressing HuC/D (WOS1) all the way to post mitotic maturing neurons that have homogenous MAP2+ extending neurites (WOS2). In our lab, we have generated differentiated neurons from NP cells by 14 DIV and based 2 separate WOS on the following: expression of post mitotic neuronal cell marker HuC/D increasing from 3 to 64% over 0-14 DIV (WOS1) and MAP2+ homogenous cultures from 14-28 DIV (WOS2). Continuous exposure to 10 μM BPA during WOS1 inhibited total cell viability. In contrast, a lower dose of BPA (0.1 μM) reduced viability during WOS 2. BPA did not inhibit HuC/D+ neural cell differentiation in WOS1 at any dose tested but in WOS2 10 μM BPA decreased HuC/D+ cells. Branching and pruning is a vital step for neuronal target search during developmental growth and reduced branch points might indicate more retraction and pruning of neurites during development. Branch point per neuron was inhibited starting at 10 and 0.1 μM BPA for WOS1 and WOS2, respectively. We have developed a multiplex high content WOS assay that incorporates events during the continuum of human neural development and BPA effects differed respective of the WOS assayed.

250 Thirdhand Smoke Stresses Mitochondria in Mouse Neural Stem Cells: An Indicator of Possible Impairment of Brain Development in Response to THS Exposure

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Toxics like cigarette smoke adversely affect brain development in young children. While the effects of mainstream and sidestream cigarette smoke are well known, no data are available on how thirdhand smoke (THS), the residue left on indoor surfaces after smoking occurs, affects brain development. Stem cells offer an excellent alternative to animal studies for evaluation of such effects. We used mouse neural stem cells (mNSC), derived from neonatal mouse brain, to evaluate the impact of THS on brain development. mNSC were exposed to solutions of THS extracted from cotton fabric exposed to smoke from 133 cigarettes over 11 months. The THS, that did not inhibit HuC/D+ neural cell differentiation in WOS1 at any dose tested but in WOS2 10 μM BPA decreased HuC/D+ cells. Branching and pruning is a vital step for neuronal target search during developmental growth and reduced branch points might indicate more retraction and pruning of neurites during development. Branch point per neuron was inhibited starting at 10 and 0.1 μM BPA for WOS1 and WOS2, respectively. We have developed a multiplex high content WOS assay that incorporates events during the continuum of human neural development and BPA effects differed respective of the WOS assayed.

252 Triphenyl Phosphate-Induced Developmental Toxicity in Zebrafish: Potential Role of the Retinoic Acid Receptor

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Using zebrafish as a model, we previously reported that developmental exposure to triphenyl phosphate (TPP)–a high-production volume organophosphate-based flame retardant (OPFR) – results in dioxin-like cardiac looping impairments that are independent of the aryl hydrocarbon receptor (AHR), suggesting that disruption of retinoic acid receptor (RAR) – a nuclear receptor that regulates vertebrate heart morphogenesis – mediates this developmental toxicity. Therefore, the objective of this study was to investigate the role of RAR in mediating TPP-induced developmental toxicity in zebrafish. Using a high-content screening (HCS) assay, we first revealed that static exposure of zebrafish from 5-72 hours post-fertilization (hpf) to TPP in the presence of non-toxic concentrations of an RAR antagonist (BMS493) significantly enhanced TPP-induced toxicity (relative to TPP alone), even though identical non-toxic BMS493 concentrations mitigated retinoic acid (RA)-induced toxicity. Therefore, using real-time PCR, we quantified the relative change in expression of cyp26a1 – a major target gene for RA-induced RAR activation in zebrafish – within embryos exposed to vehicle, RA, and TPP, and found that RA and TPP exposure resulted in a ~5-fold increase and decrease in cyp26a1, respectively, relative to vehicle-exposed embryos, suggesting that TPP may be an inverse agonist for zebrafish RARs. To address whether this mechanism of action was relevant to humans, we then exposed Chinese hamster ovary (CHO) cells stably transfected with chimeric human RARo-, RARγ-, or RARα to TPP in the presence of RA, and found that TPP significantly inhibited RA-induced luciferase activity in a concentration-dependent manner, suggesting that TPP may also be an antagonist or inverse agonist for all three human RARs. Overall, our findings suggest that TPP – an understudied yet widely used OPFR – may be an inverse agonist for zebrafish RARs, a mechanism of action that likely has relevance to humans.

253 The Comparative Study of Zebrafish Embryonic Toxicity Test and Mouse Embryonic Stem Cell Test to Screen Developmental Toxicity of Human Pharmaceutical Drugs

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Both zebrafish embryonic test (ZET) and mouse embryonic stem cell test (mEST) have been shown to be useful to assess developmental toxicity of various chemicals including human pharmaceutical drugs. However, the comparative study about the sensitivity and specificity of these methods using the same set of human pharmaceutical drugs is scarce. In this study, we assessed developmental toxicity tests of 39 chemicals (22 teratogens or compounds with reproductive toxicity; Atraxatavint, Methotrexate, Dexamethasone, Valproic acid, Retinoic acid, Diclofenac, Theophylline, Thio-TEPA, Topiramate, Clarithromycin, Hydroxyurea, Busulphan, Cytarabine, Thalidomide, Dipyridamole, Omeprazole, Saccharin, Penicillin, Acetate, Famicotidine, Fexofenadine, Lanoprazole, Sodium cyclamate, and 2 ONO compounds). Chemicals that showed abnormality at any endpoint were categorized as ZET. The sensitivity and specificity of ZET was 59% and 82%. The assessment of developmental toxicity using mEST was performed according to the ECVM international validation study. The sensitivity and specificity of mEST was 50% and 82%. The low sensitivity of ZET is likely due to the
lack of detection of teratogenicity of hydrophobic drugs including Hydroxyurea, Busulphan and Cytarabine. These drugs were judged as teratogen in mEST. The simplicity of ZET makes it possible to assess developmental toxicity of human pharmaceutical drugs in a high-throughput manner. Complementary test using mEST especially for hydrophobic drugs may provide a better prediction of developmental toxicity of human pharmaceuticals.

254 Organophosphate Flame Retardants Affect Development and Neurotoxicity in Alternative Models

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There is limited information on the potential health effects of organophosphate flame-retardants (OPFRs) used as brominated flame-retardants (BFRs) replacements. The effects of 9 OPFRs: triphenyl phosphate (TPP), isopropylated phenyl phosphate (IPP), ethylhexyl diphenyl phosphate (EHDPP), butylated phenyl diphenyl phosphate (BDDPP), triethyl phosphate (TEP), isodecyl diphenyl phosphate (IDDP), tri-O-cresyl phosphate (TOCP), tris(1,3-dichloro-2-propyl) phosphate (TDCPP) and trichloroethyl phosphate (TCEP) with 2 BFRs tetrabromobisphenol A (TBBPA) and brominated diphenyl ether (BDE 47) were evaluated using alternative model systems. In mouse embryonic stem cells, none of the compounds (0.03-100 µM) affected the expression of differentiation marker (goosecoid) in the absence of cytotoxicity. Neural proliferation and neurite outgrowth (NOG) are more sensitive in human stem cell-derived neuroprogenitor cells (hNPI) and neurons (hN2) than in rat primary neuronal cultures. 6/11 FRs affected proliferation in hNPl and 10/11 altered NOG in hN2 cells at <30 µM; however, these induced cytotoxicity in parallel. Only IPP and BDDPP selectively impaired NOG in rat cultures (in the absence of cytotoxicity). In primary cultures of rat cortical neurons grown on microelectrode array plates, four OPFRs (BDDPP, IPP, EHDPP, TCP) selectively decreased action potential firing rate in the absence of cytotoxicity at <10 µM. In C. elegans, while only BDE-47 decreased feeding at <10 µM, a total of 6 compounds (BDE-47, IPP, BDDPP, TPP, EHDPP, IDDP) affected growth and 4 (BDDPP, BDE-47, TTP, IDDP) affected reproduction in the 1-10 µM range; reproduction was the most sensitive end-point. In the developing zebrafish (0.4 µM – 120 µM), TPP, IPP and TDCPP were as toxic as TBBPA, while TCEP and IDDP were the least toxic. For some endpoints, the effects of the OPFRs were equal to or stronger than the BFRs suggesting that OPFRs should be further tested for hazard characterization.

255 Using the Drosophila melanogaster Genetic Reference Panel to Identify Toxicity Pathways for Toluene


Mechanistic information is needed to link effects of chemicals at molecular targets in high-throughput screening assays to adverse outcomes in whole organisms. This study was designed to use the inbred Drosophila Genetic Reference Panel (DGRP), lines of flies with full genome sequences, to identify adverse outcome pathways associated with toluene. We generated a profile of behavioral response to toluene across 123 DGRP lines and mapped the genetic basis of this response. Single flies were placed in 5 mm dia glass tubes and exposed to 750 ppm of toluene vapor (n=24-50/line); paired controls received air. Real-time locomotor activity was observed at 10-minute intervals for 1 hr before, 4 hr during, and 15 hr after exposure. Toluene reduced activity during exposure. This acute effect differed quantitatively across DGRP lines, demonstrating variation in susceptibility to this narcotic vapor. We carried out a genome-wide association analysis for sensitivity to toluene using the DGRP web portal (http://dgrp2.gnets.ncsu.edu/). Mean activity counts during exposure were adjusted for effects of Wolbachia infection and major inversions, and then used to fit a mixed-effects model accounting for relatedness among the lines to estimate the effects of individual sequence variants. We tested 1,891,456 DNA variants with minor allele frequencies > 0.05 and P-values < 10-5. We performed separate single marker analyses for toluene, and the response to toluene (toluene-air). We found 11 variants in 6 genes associated with the acute effect of toluene, 21 variants located in or near 13 genes associated with activity in toluene, and 19 variants in or near 13 genes associated with activity in air. Whether pathways associated with these genes are consistent with the narcotic effect of toluene remains to be determined. This abstract does not reflect U.S. EPA policy.

256 Developmental Toxicity of Phase I and II ToxCast Chemicals to Caenorhabditis elegans

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Alternative animal models using lower organisms share many advantages with in vitro assays, while also exhibiting complex whole organism responses to chemical exposures. Over 950 unique compounds in the ToxCast Phase I and II libraries were screened using a high-throughput C. elegans larval growth and development assay. Changes in the size of individual nematodes were measured using COPAS Biosort flow cytometry after 48-hour exposures to chemicals over seven concentrations (0.5-200 µM). Activity of chemicals at each concentration was classified using a previously determined effect size threshold, which indicates a biologically significant decrease in nematode larval development and growth. Using this threshold, LECs were defined as the lowest concentration at which the mean size of exposed nematodes was less than the effect size threshold and remained below this threshold for subsequent, higher concentrations; 63% (603/959) of Phase I and II compounds were active for at least the highest concentration tested, with a higher percentage of active compounds in the Phase I library (71% [207/293] versus 59% [396/676] in Phase II). The 35 most active compounds disrupted C. elegans development at the lowest concentration tested including a number of organotins, avermectin insecticides and anthelmintics, organophosphates, and organochlorines. Activities in the C. elegans growth assay were compared to those from two zebrafish embryonic development studies, as well as mammalian data available in the US EPA’s ToxRefDB for many of the Phase I compounds. Concordance between C. elegans and one zebrafish study was higher (79%; n=292) than between rat and rabbit developmental outcomes (58%; n=200). Concordance was more modest at 59% for 959 Phase I and II compounds between C. elegans and a second zebrafish study. Using data from 200 chemicals tested in all four species, balanced accuracies of 45-53% were observed when using nematode or zebrafish data to predict rat or rabbit developmental effects.

257 Comparative Effects of Brominated and Organophosphate Flame Retardants on Caenorhabditis elegans Feeding, Reproduction, and Growth

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Flame retardants, some of which are considered to be global contaminants, are found in many consumer products, from furniture to plastics. In 2013, more than 2 million metric tons of flame retardant compounds were used worldwide. With the phasing out of brominated flame retardants (BFRs), primarily due to concern with developmental toxicity, the production, use, and exposure to alternative compounds such as organophosphate flame retardants (OPFRs) are on the rise. However, limited toxicity data is available to estimate potential adverse health effects of OPFRs. In this study, the toxicological effects of nine OPFRs (triphenyl phosphate, TPP, isopropylated phenyl phosphate, IPP, ethylhexyl diphenyl phosphate, EHDPP, butylated phenyl diphenyl phosphate, BDDPP, triethyl phosphate, TEP, isodecyl diphenyl phosphate, IDDP, tri-o-cresyl phosphate, TOCP, triis(1,3-dichloro-2-propyl) phosphate, TDCPP) and trichloroethyl phosphate, TCEP, were compared to four BFRs including three brominated diphenyl ethers (BDE-47, BDE-99, DE-71), and tetrabromobisphenol A (TBBPA). Three medium-throughput, quantitative assays were used to determine the effects of these chemicals on the feeding, growth, and reproduction of the nematode Caenorhabditis elegans. In general, BFRs were more toxic than OPFRs. For example, LECs for reproduction were 13 µM for BDE-47 and BDE-99, and 0.79 µM for DE-71; whereas EHDPP had an LEC of 1 mM. Two exceptions were TPP and BDDPP, which were as potent as the BFRs in the reproduction assay with LECs of 4 and 6.3 µM. In general, BFRs were more toxic than OPFRs. For example, LECs for reproduction were 13 µM for BDE-47 and BDE-99, and 0.79 µM for DE-71; whereas EHDPP had an LEC of 1 mM. Two exceptions were TPP and BDDPP, which were as potent as the BFRs in the reproduction assay with LECs of 4 and 6.3 µM. TCEP was the least potent of all of the compounds tested with only slight effects observed in all three assays. All other OPFRs elicited effects on at least one C. elegans endpoint. These results suggest that AP flame retardants continue to be cause for concern, and should be tested in traditional animal models in vivo for further hazard characterization.
The increased prevalence of environmental toxins calls for new testing platforms for fast, inexpensive high-throughput screening. We are developing freshwater planarians as such systems for developmental neurotoxicology. The regenerative capabilities of planarians allow development to be induced at will through amputation with full regeneration occurring within 7-10 days. As the planarian brain has similar structure and molecular compartmentalization as the vertebrate brain (Buttarelli et al., 2008), neurodevelopmental defects found in planarians could be directly relevant to human development. Using a combination of robotics and automated image analysis, we have developed a high-throughput screening platform which uses a series of behavioral and structural/cellular readouts to quantify neurotoxicity. As a proof of concept, we studied two known neurotoxins: dimethyl sulfoxide (DMSO), a popular solvent, and chlorpyrifos, a common pesticide. Glucose was used as a negative control. Sublethal concentrations of either toxin, determined through initial mortality assays, were analyzed for their effect on neuronal function using automated behavioral assays in adult and regenerating worms. For both neurotoxins, increasing concentrations resulted in reduced motility and altered behavior compared to control worms. These behavioral defects appear to result from impaired brain regeneration as quantified using structural and cellular markers in bright field and fluorescence imaging. We found that concentrations comparable to (Selderslagh et al., 2009) or lower than (Crompton et al., 2000) those found in other systems impair planarian behavior and regeneration, signifying our assay is highly sensitive. In conclusion, using planarians, we hope to direct new guidelines to minimize human exposure for a large family of toxins in a realistic time frame.
morpholino knockdown of p53 and estrogen receptors (α, βα, βb), did not prevent the necrosis, nor did co-treatment with pifithrin-α (p53 inhibitor). Overall, our studies suggest that cell death by tamoxifen occurs via an MMP-induced mechanism, and that non-classical endocrine disrupting effects occur in addition to the well-characterized anti-estrogenic effects. Funding sources: NIH T32 ES007060, P30 ES000210

263 Using the Larval Zebrafish Locomotor Assay in Functional Neurotoxicity Screening: Light Brightness and the Order of Stimulus Presentation Affect the Outcome


We are evaluating methods to screen/prioritize large numbers of chemicals using 6 day old zebrafish (Danio rerio) as an alternative model for detecting neurotoxic effects. Our behavioral testing paradigm simultaneously tests individual larval zebrafish under sequential light and dark conditions in a 96-well plate. Controlling the duration and intensity of light, we can manipulate activity, assessing changes in locomotion during light-dark transitions, and responses to both light and dark. Manipulating different variables allows us to explore whether the light level affects our ability to detect effects of chemical exposure on locomotor activity. Our testing paradigm employed a wide range of light levels (0.01 lux to 51.3 lux) and assessed the activity of larvae dosed acutely with ethanol (a known neurotoxic chemical and disruptor of locomotor activity). The highest ethanol concentration (2%) affected activity regardless of the light level. The lower ethanol concentrations (1% or 0.5%) showed the largest hyperactive effects during the lowest light levels (i.e., dark or at 0.01 lux). At higher light levels (0.5 lux or 51.3 lux), a different pattern emerged showing no difference in activity between control and 0.5% ethanol, and little to no hypoactivity in the 1% group. Moreover, the order of stimulus presentation also affected the outcome: when 0.5 lux followed 0.01 lux, there was no difference in activity among the 1%, 0.5% and control groups, but if that same light level (0.5 lux) followed a much brighter light presentation (51.3 lux), the 1% ethanol group exhibited marked hypoactivity. To sum, the light level and the order of stimuli presentation will affect the sensitivity of the zebrafish larval locomotor assay and the direction of the effect (i.e., either hyper- or hypo-activity). This abstract does not reflect EPA policy.

264 The Effects of Developmental Deltamethrin Exposure on Aggression in Adult Zebrafish

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Pyrethroids are generally considered to be a safer alternative to other classes of insecticides. However, developing organisms are more susceptible to the adverse effects of pesticides and little is known about the long-term neurobehavioral effects of developmental neurotoxicity resulting from pyrethroid exposure. The zebrafish model was used to test the hypothesis that developmental exposure to low doses of pyrethroid pesticide deltamethrin leads to persistent behavioral alterations. Zebrafish embryos were treated with deltamethrin at doses below the LOAEL (0.25 and 0.5 μg/L), during the embryonic period (3-72hpf) using a static non-renewal water exposure. After 72hpf, embryos were transferred to clean water and reared until adulthood. Aggression was measured in adult zebrafish using the mirror-induced aggression assay. We found that dominant adult male zebrafish that had been developmentally exposed to deltamethrin were more aggressive than dominant control fish. This phenomenon was not observed in female zebrafish. In addition, in adult male zebrafish, a 2-week exposure to fluoxetine was able to reduce aggression, indicating the involvement of serotonin in the mediation of this behavioral phenotype. In control male zebrafish, the magnitude of aggression was found to be positively correlated with transcript levels of the serotonin transporter a (r=0.948, p=0.001), serotonin transporter b (r=0.772, p=0.05), and the dopamine receptor d2 (r=0.810, p=0.02). This correlation was not present in adult male fish that had been developmentally exposed to deltamethrin (r=0.0143, p=0.96), (r=0.186, p=0.49), and (r=0.104, p=0.70) respectively. Our data suggest that exposure to deltamethrin during the embryonic period results in sex specific behavioral effects that persist into adulthood and that these effects are associated with monoaminergic system dysfunction. NIEHS R56ES018863, T32ES007148, R01ES015991

265 Development of a Zebrafish Model to Identify Adverse Outcome Pathways Linking Thyroid Hormone Disruption to Developmental Neurotoxicity

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Thyroid hormones (THs) are critical for proper fetal neurodevelopment, and can influence a number of neurodevelopmental processes. Many environmental contaminants can disrupt TH-signaling and regulation, and thus are suspected to cause developmental neurotoxicity (DNT) through disruption of TH-mediated pathways. However, the identification of mechanisms linking TH disruptors to altered neurodevelopment has been limited by gaps in the understanding of the functional roles of TH-signaling components in specific neurodevelopmental processes. As a first step towards addressing these gaps, we are examining the spatiotemporal expression patterns of TH signaling molecules, such as transporters, deiodinases and thyroid hormone receptors (TRs) in zebrafish embryos using quantitative real-time PCR and whole mount in situ hybridization across early neurodevelopmental stages. Phenotypes associated with developmental hyperthyroidism and hypothyroidism are being evaluated using pharmacological treatment with T4 and T3 and a conditional thyroid ablation line. Preliminary results demonstrate that the expression of deiodinases increases throughout the first five days of zebrafish development while TRs and transporters show minimal or no changes in expression. Experimental manipulation of developmental TH levels during the first 5 days post fertilization causes morphological and behavioral abnormalities. Characterization of TH-mediated neurodevelopment in zebrafish will facilitate the identification of adverse outcome pathways through which TH-disruption impacts neurodevelopment and establish the zebrafish as a model for screening environmental chemicals for DNT risk. This work is funded by the USEPA (grant RD83550 to PJL) and by an NIEHS-funded predoctoral fellowship to RMW (T32 ES007059).

266 Effects of Bisphenol A (BPA) Exposure on Dopaminergic Gene Expression and Behavior in Zebrafish (Danio rerio)

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Bisphenol-A (BPA) is used worldwide in polycarbonate plastics and epoxy resins. It is found in the linings of food and beverage cans, plastic bottles, plastic medical devices, and many other commercial products. The leaching of BPA monomers from these products into food and water has resulted in widespread human exposure. The endocrine disrupting effects of BPA exposure is well documented in rodent models; in addition, BPA exposure has also been found to affect neurobehavioral development in mice. However, few studies have elucidated the molecular mechanisms underlying the effects of BPA exposure on the central nervous system. This study characterizes the effects of developmental BPA exposure on the dopaminergic system and locomotor activity using the developing zebrafish (Danio rerio) model. Zebrafish embryos were exposed to 0, 10, or 25 μM BPA using a static non-renewal bath exposure for 96 hours post fertilization (hpf). The transcript levels for genes involved in dopaminergic neurotransmission were quantified in zebrafish embryos using RT-qPCR. There is a dose-dependent increase in transcript levels of the dopamine receptor d1 (drd1), dopamine receptor d2 (drd2), dopamine transporter (dat), and tyrosine hydroxylase (th) at 96hpf following BPA exposure. The swim activity of 2-week old larval zebrafish was quantified to characterize the locomotor effects of the dopamine system dysregulation in 2 week old larval zebrafish. A significant increase in swim activity was observed in zebrafish treated with 10 μM BPA. In order to determine if the changes in swim activity resulting from developmental BPA exposure was mediated by the upregulation of the DA receptors, 2 week old larval zebrafish were treated with SCH-23390, a drd1 antagonist. Treatment with SCH-23390 significantly reduced the hyperactivity observed in zebrafish exposure to 10 μM BPA. These data indicate that embryonic exposure to BPA causes dysregulation of the dopaminergic system, which results in persistent changes in swim activity of larval zebrafish.
Zebrafish are excellent tools for studying later life effects of embryonic exposure to environmental contaminants for several reasons: their short generation time is ideal for full embryo-to-adult experiments in relevant time-scales, their ex utero development and transparent embryos allow for easy evaluation of exposure levels that do not cause immediate overt effects, their easy maintenance and breeding and high fecundity allow high throughput experimentation with many replicates. Moreover, zebrafish have recently become a highly popular tool for the study of developmental neurotoxicity, utilizing a rapidly growing list of behavioral tests, developed for both juvenile and adult fish, which allow the assessment of particular effects. We are using such tests to assess the developmental neurotoxicity of PCB-126 (3,3',4,4',5-pentachlorobiphenyl), the most toxic dioxin-like PCB congener, which acts through the aryl hydrocarbon receptor (AHR) pathway. Dioxin-like compounds are highly toxic, causing reproductive and neurodevelopmental problems. Although the effects and the associated mechanisms following acute exposure to dioxins are well studied, the full potential for later-life health effects that result from early-life low level exposure to such compounds is not well understood. We exposed zebrafish embryos to either vehicle control (DMSO) or a low concentration of PCB-126 (0.3 nM) for 20 hours (4-24 hours post fertilization), and then reared the fish to adulthood (3 months) in clean water. We conducted behavior tests at several juvenile stages and a number of different tests after the fish reached adulthood. Two important issues arose during adult testing: 1) the importance and significance of repeating a specific test with the same animals over time; and 2) the need to demonstrate a given effect using multiple assays. We discuss these issues in light of our assay results. [Support: WHOI Postdoctoral Scholar Program, NIH grant P01ES021923, and NSF Grant OCE-1314642]

**PS 267** When Zebrafish “Misbehave”—Learning about Delayed Effects of Low-Level Embryonic Contaminant Exposure from Adult Zebrafish Behavior

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Flame retardants (FRs) represent a diverse variety of chemicals most of which, despite widespread use and exposures, continue to be poorly understood in terms of their toxicity. This study used our advanced high-throughput screening platform in embryonic zebrafish to measure FR bioactivity. Forty-four FRs were targeted with special focus on aryl and chlorinated OPFRs and their major metabolites. In addition, several PBDEs and metabolites were examined, as well as the most heavily used FR tetrabromobisphenol A (TBBPA) and other polyhalogenated FRs. Dechlorinated embryos were exposed to each FR from 6-120 hours post fertilization (hpf). Each chemical was tested at doses spanning four orders of magnitude (0.64 nM-6.4 μM) at 32 replicates per dose. Embryos were evaluated for survival and 20 developmental malformations at 24 and 120 hpf. Neurobehavioral effects were measured using two photomotor response assay tests: 1) spontaneous motion of 24 hpf embryos under light stimulation; and 2) total movement of 120 hpf larvae under light and dark stimulation. Of the 44 FRs, 31 elicited significant adverse effects in all three assays, while only two, tetrabromobenzoxazole and TBBPA-dibromopropyl ether were without effect. Compared to controls, 38 of 44 FRs significantly altered photomotor responses measured as hypo or hyper activity at both 24 and 120 hpf. Heatmap cluster analyses revealed that the aryl and chlorinated OPFRs caused hypoactivity in embryonic and larval fish and an inability to acclimate to light stimuli. In contrast, the PBDE metabolites 6-OH-BDE-47 and 5-OH-BDE-47 caused significant hyperactivity upon exposures to dark stimuli, whereas 2,4,6-trichlorophenol and 3-OH-BDE-47 produced hypoactivity. Taken together, data generated suggest that many FRs in use and considered as replacements may impair neurodevelopment. Additional work is needed to better understand toxicity mechanisms and develop FRs with structural attributes that minimize hazard.

**PS 268** Zebrafish As Biosensors: Advanced Morphological-Behavioral Testing Platform Reveals Neurodevelopmental Defects in Embryos and Larvae Exposed to Comprehensive Suite of Flame Retardant Chemicals

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The use of organophosphate (OP) flame retardants is growing rapidly and studies have found human exposure to be significant. Although the adverse neurobehavioral effects following developmental exposure to OP pesticides such as chlorpyrifos (CPF) have been widely demonstrated in humans, rodents and zebrafish, the potential for OP flame retardants to produce similar neurotoxicity is relatively unexplored. This study was conducted to compare the effects of developmental exposure of the OP flame retardants TPP and TDCPP to CPF. Zebrafish were exposed to either 0.3 or 0.05 μM of CPF, TPP, or TDCPP from 0-5 days post fertilization. The DMSO (0.03%) vehicle served as control. Following exposure, zebrafish larvae were placed in standard tank water for 24 h. Then they were tested in an alternating light-dark locomotor test, wherein two cycles of 10-min of illumination and 10-min of dark followed an initial 10-min dark habituation period. Locomotor activity was tracked and analyzed using a Daniovision chamber and Ethovision software. All 3 OP compounds were found to cause hyperactivity in the larval swim test. However, the timing of the hyperactivity over the course of the session differed between the OP insecticide and OP flame retardants. During the initial habituation period, both CPF doses caused significant hyperactivity (0.03 μM p<0.05, 0.3 μM p<0.01). In the first light/dark cycle only the higher CPF dose caused significant hyperactivity (p<0.05). During the second half of the test session no CPF hyperactivity was seen. In contrast, TPP and TDCPP were not seen to cause hyperactivity during habituation or during the first light cycle. During the second cycle the higher TPP dose (p<0.05) and both TDCPP doses (p<0.05) caused significant hyperactivity relative to control. We have found that OP flame retardants do have significant neurobehavioral effects on development. Further assessment will determine the persistence of these effects into later life stages.
Persisting Effects of a Flame Retardant Metabolite, 6-OH-BDE-47, on Larval and Juvenile Zebrafish Swimming Behavior

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Exposure to polybrominated diphenyl ether (PBDE) flame retardants is known to cause neurodevelopmental toxicity and altered behavioral responses in developing rodents and fish. However, no studies have looked specifically at neurodevelopmental toxicity of the oxidative metabolites, OH-BDEs. It is unclear whether the developmental neurotoxicity of PBDEs is due to actions of the parent compounds or their metabolites. OH-BDEs have a structural similarity to endogenous thyroid hormones (TH), and are known to affect TH regulation at multiple endpoints. Since TH is a key developmental signaling molecule, TH dysregulation during early development could lead to persistent effects. The objective of this study was to characterize the effects of low-dose developmental exposure to 6-OH-BDE-47, on zebrafish (Danio rerio) behavior during larval and juvenile life stages.

Zebrafish were exposed to 6-OH-BDE-47 (10M-50M) during embryo-larval development (4hpf to 6dpf), and then assessed for swimming behavior at 6 dpf and 45 dpf. Exposure doses were below the overt toxicity threshold. Larval behaviors were assessed using a Daniovision chamber that tracks fish swimming activity during alternating light and dark periods. Later, as juveniles, fish were assessed by tests of sensorimotor plasticity, predatory escape response, and spatial learning. Noldus Ethovision program was used for data analysis in all tasks. Exposed larvae and juveniles showed persistent effects on swimming activity, hyperactivity, and startle habituation test. These results indicate that early exposures to a PBDE metabolite can have lasting behavioral effects on activity and fear response. These data demonstrate that leflunomide affects dopamine synthesis, which can manifest itself in abnormal behavioral output. Our data also illustrate the versatility of leflunomide as a model for testing interactions between environmental compounds and neurodevelopment.

The Antirheumatic Drug, Leflunomide, Interferes with the Dopamine Synthesis Pathway

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Leflunomide belongs to a class of drugs known as disease-modifying antirheumatic drugs and is used to treat rheumatoid arthritis. Leflunomide exerts a therapeutic effect via its metabolite, teriflunomide, which inhibits dihydroorotate dehydrogenase, an enzyme in the de novo pyrimidine biosynthesis pathway. Studies have shown that zebrafish exposed to leflunomide lack melanin, which is derived from tyrosine in a pathway common to melanin and the neurotransmitter dopamine. Therefore, we hypothesized that leflunomide may also target dopamine biosynthesis. To test this hypothesis, zebrafish embryos were exposed to leflunomide (250 nM -2.5 μM) and transcript levels of enzymes involved in dopamine synthesis were measured. At 24 and 48 hpf, zebrafish were treated and the non-specific hydrolase, the penultimate step in dopamine synthesis, was downregulated. Tyrosinase, an enzyme bridging dopamine and melanin synthesis pathways, was also downregulated. Next, we used a zebrafish transgenic line expressing GFP under regulatory control of the dopamine transporter to test whether leflunomide affected the number or distribution of dopaminergic neurons. We found that leflunomide altered GFP expression in the ventral diencephalon. Notably, these observations correlated with dramatically reduced behavioral activity that persisted until at least two months post-exposure. These data demonstrate that leflunomide affects dopamine synthesis, which can manifest itself in abnormal behavioral output. Our data also illustrate the versatility of zebrafish as a model for testing interactions between environmental compounds and neurodevelopment.

Developmental Neurotoxicity of Polybrominated Diphenyl Ether-47 in Caenorhabditis elegans

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Polybrominated Diphenyl Ethers (PBDEs) were used as flame retardants for over 30 years and can still be found in many household materials. Concern over their use mounted due to their persistence in the environment and build up in biological tissues. Contamination through dust particles leads to human exposure with children ages (2-7) having the highest body burden of PBDEs. Increasing epidemiological evidence suggests children with high PBDE blood levels score lower on cognitive tests and display hyperactive behaviors. Additionally, children birthed from mothers with high PBDE blood levels show similar neurodevelopmental affects. This study aimed to assess the toxicity of PBDE-47 on the developing nervous system in Caenorhabditis elegans (C. elegans). C. elegans are a useful model organism for neurotoxicity due to their low volume of neurons and a short developmental life cycle. Embryonic stage (eggs) and developing larvae (L1-stage worms) of the pan-neuronal GFP expressing C. elegans strain were chronically exposed to PBDE-47 (1.25, 2.5, and 5μM) followed by fluorescent microscopy and behavioral analysis. Results revealed a dose dependent decrease in the size of the nerve ring and increase in neuronal GFP expression in worms treated at the egg stage followed by maturation to larval stage 4 (L4). Additionally, the number of eggs hatched is decreased with increasing dose. Similarly, when L1 stage worms were treated and allowed to mature through L4, there was a significant concentration dependent reduction in the nerve ring area, while GFP expression remained unchanged. Treatment with PBDE-47 also caused atypical thermotaxis behavior. Results suggest that PBDE-47 can alter the development of the central nervous system in C.elegans and that future studies should aim to identify specific neurons affected.

Expression of Cytochrome P450 Isozymes in Primary Cortical Cultures from Rat Frontal Cortex

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The need to screen large numbers of chemicals for developmental neurotoxicity has spurred the development of high throughput/ high content assays for neurite outgrowth, synaptogenesis and electrical activity using primary cultures of cortical neural tissue. While these assays are capable of screening large numbers of chemicals, the ability of these cell cultures to metabolize chemicals is unknown, which hampers interpretation of results and necessitates metabolite testing in these assays. Therefore, the present study examined mRNA expression for cytochrome P450 isozymes in primary cortical neural cultures on Days in Vitro (DIV) 1 and 14. Primary cultures of rat frontal cortex were prepared from 0-24h old rat pups and plated onto 30 nm dishes. On DIV 1 and 14, total RNA was isolated. In addition, total RNA was isolated from 0-24h old rat pup liver tissue for comparison to the neural mRNA expression patterns. mRNA expression levels were quantified using hydrolysis probe real-time qPCR assays for each of the cytochrome P450 gene targets: Cyp1a1, Cyp2c11, Cyp2d4, Cyp2e1, Cyp3a1, Cyp3a2, Cyp3a23/3 and Cyp4x1. β-actin mRNA expression was consistent in liver and brain samples of equal RNA input, thus all values were normalized to β-actin mRNA expression. While readily expressed in rat liver tissue, there was little to no expression of Cyp2e1, Cyp3a2, Cyp3a23/3 or Cyp1a1 in cortical cultures. Cell-based P450 glo assays confirmed the lack of functional Cyp1a and Cyp3a. When normalized to β-actin, Cyp2d4 and Cyp2x1 were expressed on DIV1 and 14, but at levels much lower than in rat liver tissue. By contrast, normalized gene expression of Cyp2c11 and Cyp4x1 in cortical cultures on DIV 1 was the same, and by DIV 14 was increased, compared to its expression in the liver. While protein levels and functional activity of these Cyps remain to be confirmed, these data indicate that primary cortical cultures may have limited metabolic capability compared to rat liver tissue. (This abstract does not represent Agency Policy).

Screening for Developmental Neurotoxicants Using In Vitro “Brain on a Chip” Cultures


Currently there are thousands of chemicals in the environment that have not been screened for their potential to cause developmental neurotoxicity (DNT). The use of microelectrode array (MEA) technology allows for simultaneous extracellular measurement of action potential (spike) rates and patterns from multiple locations in neural networks consisting of glutamatergic and GABAergic neurons and glia. Further, repeated measurements over successive days from these “brain on a chip” cultures allows the development of spontaneous activity to be monitored and the effect of chemical exposures on network development to be characterized. This experiment examined the ontogeny of spontaneous network activity in primary rat neural cells dosed with a “training set” of 5 chemicals (Bis 1, mevastatin, Na3VO4, acetaminophen, and loperamide) previously assessed in neurite outgrowth and synapse formation assays. Cells were grown on 48 well MEA plates and treated with chemical (0.01-30 μM) 2 hours after plating. Their activity was recorded for a period of 15 minutes on days in vitro (DIV) 2, 5, 7, 9, and 12. Cell viability was assessed on DIV 12. When mean firing rate (MFR) and number of active electrodes (#AE) were examined on DIV 12, networks treated with acetaminophen showed no effect on MFR, #AE or viability. Compared to controls, networks treated with Bis 1 and loperamide showed a decrease in MFR at concentrations below those that caused cell death. MFR for cells treated with Bis 1 decreased at 1 μM, and those
treated with loperamide decreased at 0.1 µM. Mevastatin and Na2VO4 had effects on MFR and #AE only at concentrations that were also cytotoxic. Mevastatin caused cytotoxicity at its lowest concentration (0.01 µM). Na2VO4 became cytotoxic at 1 µM. These results demonstrate that neural networks grown on MEA plates ("brain on a chip" culture) may provide a physiological, high throughput, high content method to screen chemicals for their potential to cause developmental neurotoxicity. (This abstract does not reflect USEPA Policy)

### 276 Novel Quantitative Methods for Characterization of Chemical-Induced Functional Alteration in Developing Neuronal Cultures

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Thousands of chemicals lack adequate testing for adverse effects on nervous system development, stimulating research into alternative methods to screen chemicals for potential developmental neurotoxicity. Microelectrode arrays (MEA) collect action potential spiking and bursting patterns from in vitro cultures of rat cortex, offering a high-content method to assess chemical-induced alterations in development of functional neural networks. However, appropriate methods are needed to analyze information-rich MEA recordings to determine the endpoints that best distinguish developmentally neurotoxic chemicals from controls. The present study evaluated a 2 step analytic approach for its ability to identify and describe alterations in developing neural networks treated with a training set of 5 compounds (acetaminophen, bis-1, domoic acid mevastatin and Na3VO4). On days in vitro (DIV) 2, 5, 7, 9 and 12 data were collected and analyzed for 17 different bursting parameters (burst rate, duration, etc.). In controls, all of these parameters showed a clear developmental trajectory. First, random forest and support vector machine classification techniques rank the effect of a given chemical on these parameters, providing a subset endpoints most affected by each chemical. Following this, linear regression analysis across DIV was conducted for each concentration and utilized to determine concentration-dependent developmental effects. For acetaminophen, a negative control, no endpoints had significant concentration-related trends. For the remaining 4 compounds, significant concentration-related trends were observed for various endpoints, with mean firing rate, #bursts/min, and correlation of activity altered for 4, 3 and 2 chemicals, respectively. This analysis approach greatly utilizes the high content data from MEAs for characterizing chemicals for potential developmental neurotoxicity. (This abstract does not reflect Agency Policy)

### 277 Alteration of Network Activity in Cortical Neurons by Triadimefon

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Triadimefon (TRI) is a conazole fungicide used to control powdery mildews on crops and as a veterinary and clinical treatment. TRI inhibits transmitter re-uptake at dopamine, serotonin, norepinephrine, and noradrenaline synapses and is a prototype TRI causes hyperactivity and repetitive behavior. Recently, we reported that other conazole fungicides similar to TRI decreased spontaneous electrical activity in cortical cultures grown on microelectrode arrays (MEAs). The present experiments used MEAs to assess effects of TRI on spontaneous network activity by measuring the mean firing rate (MFR) of action potentials (spikes), bursting (groups of spikes) parameters, and synchrony of bursts (SYNC) in primary cortical cell cultures from neonatal (0-24hr old) rats. Stable native activity from mature networks (n=11) was recorded for 30 mins (baseline) prior to exposure of TRI (0.01-100 µM) via a cumulative concentration paradigm. Compared to baseline, TRI (0.5-100 µM) decreased MFR in a concentration-dependent manner (EC50 =7.9 µM). TRI (0.01-0.05) µM caused concentration-dependent increases in the inter-burst interval from (21.9 ± 3.5 to 33.8 ± 6.6 secs) and increased mean burst duration by 51 % at 5 µM. This directly correlated with the number of spikes in bursts which increased from 9.21 ±0.77 to 15.05 ±1.57 at 5 µM, and remained elevated. Most notably, TRI caused a U-shaped change in SYNC, which first decreased (0.01-0.5 µM) by 46 %, then recovered to control levels (1-100 µM). TRI yielded a unique array of effects on cortical function that require additional study to ascertain the exact mechanisms involved. (This abstract does not reflect U.S. EPA policy.)

### 278 A Multiplexed Assay for Determination of Neurotoxic Effects on Spontaneous Network Activity and Cell Viability from Microelectrode Arrays

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Microelectrode array (MEA) recordings are increasingly being used as an in vitro method to detect and characterize the ability of drugs, chemicals and particles to cause neurotoxicity. While effects of compounds on spontaneous network activity in MEAs is easily measured by MEA recordings, compound cytotoxicity is not routinely determined, particularly within the same well from which recordings are collected. In the present experiments, primary cultures from neonatal rat cortex were exposed to six compounds (glyphosate, beta-cyfluthrin, domoic acid, tributyrin, lindane and loperamide) in multi-well MEA (mwMEA; 48 well) plates. Effects of these compounds (0.03-100 µM) on spontaneous network activity (mean firing rate; MFR) and release of cytotoxicity marker lactate dehydrogenase (LDH) were determined following 60 min exposure. Glyphosate did not effect MFR or LDH release. By contrast, tributyrin decreased MFR coincident with increased LDH release. Domoic acid and beta-cyfluthrin decreased MFR in a concentration-dependent manner without altering LDH release. Lindane and loperamide also did not alter LDH release, but caused biphasic alterations in MFR, with increases in MFR at lower followed by decreases at higher concentrations. These results demonstrate a simple and rapid method for within well determination of compound effects on network function and viability in mwMEA plates. This multiplexed assay will facilitate neurotoxicity screening. (This abstract does not reflect Agency policy)

### 279 Mouse Pluripotent Stem Cell Motor Neurons Generate Robust Neural Network Activity on Microelectrode Arrays

S. Stice1, A. Majumder1, B. Culp1, A. M. Nicholoni3 and C. Arrowood1, 276 Novel Quantitative Methods for Characterization of Chemical-Induced Functional Alteration in Developing Neuronal Cultures

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Pluripotent stem cell derived cells that are cost effective and generate robust, uniform results will enhance efforts to identify chemicals that effect the network of activity in the central nervous system (CNS). Currently, rodent neural tissue generates spiking and bursting activity after 7-14 DIV. However, sourcing primary tissues can pose logistical drawbacks including sourcing animals and animal prep variability. We have designed a scalable method of generating large numbers of prequalified CNS neurons that can be used in longitudinal microelectrode array (MEA) assays where both phenotype and network activity can be monitored temporally and spatially. A starting stock of mouse embryonic stem cells containing the motor neuron specific promoter H9d driving GFP were exposed to both retinoic acid and a sonic hedgehog agonist to generated Mouse Motor Neurons (mMNGFP+). To characterize the use of mMNGFP+ on MEAs, various cell densities were tested to identify variability in activity and also response to treatment. Bicuculline, a GABA-antagonist, was applied to modulate the observed bursting and synchrony of the culture. In response to bicuculline [50 mM], the burst activity increased and was more synchronized, as is evident from the increase in the percentage change of several metrics. Varied cell densities altered several metrics including the number of bursts and the percentage of spikes detected in bursts. mMNGFP+ generated consistent and robust MEA network activity that was responsive to dose escalation and may provide a useful, scalable and consistent neural network screen for compound affecting the CNS.

### 280 A High-Throughput MEA Assay Utilizing Rat Cortical Neurons Can Detect Both Glycine Receptor and GABAA Receptor Seizure Responses in Brucine


Neurotoxicity produces significant compound attrition during drug discovery. Currently available in vitro assays cannot predict all toxicity mechanisms due to the failure of general cytotoxicity assays to predict sublethal target specific electrophysiological liabilities. Ion channel and receptor activity assays can be used to predict some seizure potential, but these only focus on specifically measured targets for prediction and may miss responses that rely on a combination of targets. Most evaluation of seizure inducing compounds occurs later in preclinical in vivo studies which have much higher costs. Therefore, the development of a high-throughput in vitro assay to screen compounds for electrophysiological liabilities would de-risk compounds earlier at lower cost and greater reliability. Here we demonstrate the
use of a 48-well Axion BioSystems microelectrode array (MEA) along with custom data analysis algorithms to screen for neurotoxic liabilities using spike data from cryogenically preserved rat cortical neurons. We developed a panel of spike train descriptor statistics that distinguish between response fingerprints of negative and positive control compounds. We are able to further distinguish the mechanism of action for certain classes of seizurogenic compounds, including GABA receptor antagonists (e.g. picrotoxin) and glycine receptor antagonists (e.g. strychnine), by their endpoint responses. Brucine (an alkaloid from Strychnos nux-vomica) is a known glycine receptor antagonist but has also been reported to be a GABA receptor antagonist. When brucine is tested at various doses, a modulation from a glycine receptor antagonist response at the higher doses to a GABA antagonist response at the lower doses is observed. The ability of this assay to distinguish pro-convulsant mechanisms as well as resolve two separate mechanisms of action in one compound demonstrates the power of this assay to characterize neurotoxic responses.

281 Acute Insecticide-Induced Changes in Electrical Activity of Primary Cortical Cultures: Multielectrode Array Data Collection and Analysis


Multi-electrode array (MEA) technology allows the investigation of (effects on) electrical activity in neuronal cultures with a higher throughput compared to traditional electrophysiology. This study describes the acute effects of two structurally different insecticides, β-cypermethrin (CYP) and endosulfan (ES), on neuronal activity in primary cortical cultures grown on MEAs. Cortices from neonatal Wistar rats were dissociated and cultured on MEAs in a multi-well format. Neuronal activity of the cortical cultures was recorded as extracellular field potentials using a Maestro amplifier (AXIS 1.7.8; Axion Biosystems Inc.). Baseline neuronal activity was recorded at DIV10-11 from the cortical cultures for 30 min, after which they were exposed to different concentrations of CYP, ES or solvent control and activity was recorded for another 30 min. DMSO never exceeded 0.1% v/v. MEA data was analyzed using Neuroexplorer 4.0 and custom-made Excel macros. Neuronal activity can be measured from the cortical cultures within a week after isolation. Exposure to CYP inhibits neuronal activity in a concentration-dependent manner, to 29±5% of control (mean±SEM) at 10 μM CYP (n=15), with a lowest observed effect concentration (LOEC) in the (sub)micromolar range. Exposure to ES strongly induces neuronal activity in a concentration-dependent manner, to 38±55% of control (mean±SEM) at 10 μM ES (n=15), with a LOEC in the submicromolar range. These data show that insecticides CYP and ES affect spontaneous neuronal activity in vitro at (sub)micromolar concentrations. The heterogeneous cortical culture, in which numerous signalling pathways are integrated, is very suitable for screening purposes. The molecular targets affected by these respective insecticides are to be identified in additional studies. Funding: European Union [DENAMIC, FP7-ENV-2011-282957].

282 Microengineered Peripheral “Nerve-on-a-Chip” toward Preclinical Testing

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3D engineered tissues as models for toxicity screening is a growing field. For peripheral neural tissue, where appropriate readouts are bioelectrical conduction, application has lagged. Here we develop an organotypic model mimicking morpho- and physiological processes of peripheral nerves. Using photolithography, polyelectrolyte diacrylate and ParacortexTM scaffolds were cultured with rat embryonic dorsal root ganglion. Outgrowth was confined to narrow tracts and electrical activity measured using field potential and bi-polar stimulating electrodes. Treatment with tetrodotoxin (TTX) blocked Na+ channel activation, while dinitroquinoxalone-dione + amino-phosphonopentanoate (DNQX/APV) blocked synaptic activity. Tissue constructs were stained to identify neurites and cell nuclei as well as for electron microscopy. Wide-field fluorescence, confocal, and TEM imaging revealed fiber tract growing in 3D. Dense parallel fiber growth and fasciculation were observed. Fibers were relatively small (<1 μm diameter), and Schwann cells were seen encapsulating neurites. Population responses with consistent delay, amplitude, and envelope, even under high frequency (100 Hz) stimulation were seen. Distal stimulation resulted in delay of onset of the spike. TTX completely and reversibly blocked responses, while DNQX/APV resulted in no change. Intracellular recordings showed electrically-evoked responses with rise times independent of baseline voltage. Thus, population spikes are wholly propagation of compound action potentials. We demonstrate microengineered neural fiber tracts resembling morphology, physiology, and pharmacological responses of peripheral sensory tissue. Results demonstrate the feasibility of developing benchtop models with clinically analogous outcomes for predictive pre-clinical toxicity screening. J Curley, J Biomol Mater Res B 2011; 99:532. Funding: NSF CAREER (CBET-1055990), I-Corps (IIP-1439383) and DoD (W81XWH-12-1-0246).

283 High-Throughput Neurite Outgrowth Screening for Developmental Neurotoxic Substances

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BACKGROUND: Low predictivity of animal toxicity data for human safety as well as insufficient throughput of existing methods demand for new approaches to assess developmental neurotoxicity (DNT) of industrial chemicals, environmental contaminants and other substances showing human exposure (1). The disruption of biological processes, such as neurite outgrowth, is assumed to contribute to DNT. Therefore, in vitro systems based on such processes may be used for DNT screening (2). METHODS: LUHMES cell lines are non-transformed, conditionally immortalized neuronal precursors and they can be differentiated within six days to fully mature dopaminergic neurons (3). For the neurite outgrowth screen, very early differentiated cells (day 2) were treated for 24 h with chemicals of the national toxicoology program 80 compound library. Data were acquired after live cell staining by automated microscopy, followed by algorithmic data analysis. Neurite area and viability were determined simultaneously for the same pictures. The ratio of EC50 (viability)/EC50 (neurites) was calculated. Only if this was greater 4, substances were classified as DNT. Ten-point concentration-response curves were obtained in three independent screens. RESULTS AND CONCLUSIONS: Of the 75 substances, eight became classified as cytotoxic (at < 20 μM) and eight as potential DNT. Eight of the overall hits confirmed earlier published findings. Seven DNT hits appear novel and will be further characterized. Quality controls were based on five duplicate substances within the library and a defined acceptance control (narciscine) on each plate. The test proved to be robust and able to identify potential developmental toxins amongst widely varying chemical classes.

284 Quantitative Structure-Activity Relationships to Predict Biological Effects of Engineered Nanoparticles on Macrophage Innate Immune Function

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Although the potential health effects of engineered nanoparticles (ENPs) are poorly understood, epidemiological studies have made strong links between exposure to urban air particulates and susceptibility to lung infections, including communi- acquired pneumonia. Here, we used high throughput assays of macrophage phagocytosis of S. pneumoniae to investigate the effects of a library of 16 different metal and metal oxide ENPs on macrophage innate immune function. Flow cytometry analysis revealed that that ENP-mediated inhibition of pathogen phagocytosis is highly dependent on the material chemistry. ENPs known to have strong redox activity (e.g., CuO, CoO) caused robust inhibition of phagocytic function, whereas other ENPs (e.g., TiO2, SiO2) caused no effect even at high cell doses. Mouse lung infection studies using CoO, Fe3O4 and SiO2 ENPs confirmed the in vitro assays correctly predicted the relative potency by which these ENPs enhanced S. pneu monia infection in vivo (CoO>Fe3O4>SiO2). Regression-based structure-activity modeling using simple periodic table based descriptors revealed that the potency by which metal oxide ENPs inhibit macrophage phagocytosis is related to the ease with which they can participate in electron transfer reactions. The variation in the potency of these ENPs could be described with high prediction accuracy (based on leave-one-out cross validation) using two descriptors, the metal electronegativity and the ratio of the metal atoms to oxygen atoms. Our results indicate that exposure to some ENPs, at doses well below lung overload levels, can suppress lung clearance of bacteria and enhance susceptibility to infection. Furthermore, it is feasible to predict these in vivo outcomes using targeted in vitro assays coupled with understanding physicochemical properties of the ENPs. (Supported by NIEHS Grant ES019544)
Gold nanodots (AuNDs) encapsulated with polyamidoamine (PAMAM) dendrimer with high quantum yield have great potential in medical imaging and drug delivery applications. In the present study, we investigated the immunomodulating effects of three different modified dendrimers encapsulated AuNDs, in TPH-1 human macrophages, in which terminal groups of the fourth-generation dendrimers included amine, ammine and hydroxyl groups, respectively (denoted as G4NH2, G4OH, M-G4NH2). First, we compared the biodistribution of AuNDs (AuNDs-NH2, AuNDs-M, and AuNDs-OH). Three AuNDs entered the cells, distributed and stayed in the lysosome. We further investigated the effects of three kinds of AuNDs on lipopolysaccharide (LPS)-induced proinflammatory cytokines production and the associated mechanism in phorbol 12-myristate 13-acetate (PMA)-activated cells. We found that only AuNDs-NH2 enhanced LPS-induced secretion of IL-6 in cells. Therefore, we used cytokines and chemokines PCR array to further investigate whether AuNDs-NH2 also affected the LPS-stimulated cell M1/M2 polarization. Our results showed that AuNDs-NH2 up-regulated several M1 or M2c markers and down-regulated other M2 markers in LPS-stimulated cells. Thus, it is possible that the highly biocompatible AuNDs exhibit immunomodulating effects, and modulate macrophages polarization for regulating cytokines and chemokines production.

**Comparative In Vivo, Ex Vivo, and In Vitro Toxicity Studies of Engineered Nanomaterials**


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Efforts to reduce the number of animals in engineered nanomaterials (ENM) toxicity testing have resulted in the development of numerous alternative toxicity testing methods, but in vivo and in vitro results are still evolving and variable. This inconsistency could be due to the fact that there is a lack of overall consensus on the relevant dose metric and endpoint(s) for in vitro studies which are typically performed with submerged culture systems. In this study, five ENM (SiO2 (10), CeO2 (23), CeO2 (88), TiO2 (10), and TiO2 (200); parentheses indicate average diameter in nm) were instilled into CD-1 mice (in vivo) at 10 μg via oropharyngeal aspiration, or cultured with mouse lung slices (ex vivo) and alveolar macrophages (in vitro) at concentrations of 2-100 and 22-132 μg/ml, respectively. Biomarkers of lung injury and inflammation were assessed at 4 and/or 24 hr post-exposure. The results showed that small-sized ENM (SiO2 (10), CeO2 (23), but not TiO2 (10)) significantly elicited proinflammatory responses in the mouse lung in vivo. However, only SiO2 (10) significantly increased proinflammatory cytokine levels (e.g., interleukin-6) in both the lung slices ex vivo and alveolar macrophages in vitro. This inconsistency was compensated by considering appropriate exposure dose metric in the in vitro (or ex vivo) system resulting in a similar toxicity ranking of ENM observed in vivo (i.e., SiO2 (10) and CeO2 (23) to display the most toxic, ex vivo and in vitro). We conclude that exposure to ENM induced acute lung inflammatory effects in a size- and chemical composition-dependent manner. A better understanding of ENM dosimetry for in vitro systems (i.e., a delivered dose is not always equivalent to an exposure dose) will reduce the disparity between in vitro and in vivo nanotoxicology outcomes. (This abstract does not represent U.S. EPA policy).

**Inhibition of Multixenobiotic Resistance (MXR) Transporters by Silver Nanoparticles and -ions In Vitro and In Vivo**

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The P-glycoprotein (P-gp, ABCB1) and multidrug resistance associated protein 1 (MRP1), important members of the ABC (ATP-binding cassette) transporters, protect cells and organisms via efflux of xenobiotics and are responsible for the phenomenon of multidrug or multixenobiotic resistance (MDR/MXR). In this study we first evaluated the interaction of silver nanoparticles (Ag NPs, 20-200 nm) and Ag ions (AgNO3) with MXR efflux transporters using MDCKI and the P-gp overexpressing MDCKII-MDR1 cells and calcine-AM as a substrate. Next the in vivo modulation of MXR activity was studied in D. magna juveniles with the model P-gp and MRP1 inhibitors verapamil-HCl and MK571, respectively. Perfluorooctane sulfonate and bisphenol A also inhibited the efflux of calcine from daphnids. Small-sized Ag NPs and AgNO3 inhibited the MXR activity in daphnids and MDCKII-MDR1, but abcb1 gene expression remained unchanged. Both Ag NPs and dissolved ions contributed to the effects. This study provides for the first time evidence for interference of Ag NPs and AgNO3 with the MXR activity both in vitro and in vivo and MXR activity should be taken into account when Ag NP toxicity is assessed.
Silver nanoparticles (Ag NPs) have been known to demonstrate antimicrobial activity which has led to wide-spread use of Ag NPs in consumer items such as tooth paste, shampoo, infant nipples, nursing bottles, fabrics, deodorants, kitchen utensils etc. Despite the widespread use of silver products, relatively few studies have been undertaken to determine the effects of Ag NPs exposure to human health and the environment. In the present study, Ag NPs coated with polyvinyl pyrrolidone (PVP) were synthesized and evaluated for their toxicity to Hep-2 cells and expression of several apoptosis pathway genes. Ag NPs were synthesized by sonochemical method. TEM micrographs revealed that the Ag NPs capped with PVP were monodispersed, spherical with a size of ~10nm. Cytosiva Imaging system and TEM studies revealed endocytosis of NPs mainly in endocytotic vesicles and to lesser extent to the nucleus. Toxicity of NPs to the cells were then evaluated using MTT dye reduction assay which revealed that 10µg/mL Ag NPs caused more than 90% cell death. NPs effect on cell apoptosis was measured using fluorescence marker 7-AAD. FACS flow cytometer analysis showed Ag NPs caused almost 50% cell death. Ag NPs influence on the expression levels of several apoptosis pathway genes were investigated at 2.5 µg Ag NPs concentration. RNA was extracted from cells followed by cDNA synthesis and qPCR using Human Apoptosis PCR Array which included 84 genes. The results were analyzed using the online PCR array analysis tool. Most of the genes of apoptosis pathway were upregulated. Capsases and tumor necrosis factor genes were upregulated up to 26 and 10 fold respectively. Genes involved in DNA damage such as p53 and p73 and Caspase activators such as AIFM1 BAX, TP53 were also upregulated. The results indicate that NPs cause changes in gene expression at much lower concentrations compared to toxicity estimation using traditional methods. This study is supported by NSFCREST (HRD-1241701) grant.
flying groups of nanomaterials and their associated potential risks. Examining and understanding the biological interactions of nanomaterials will give a clearer indication of potential hazards posed by the material. Therefore, grouping materials based on the type and severity of the bio-physical response, or the mode-of-action, is a more coherent grouping strategy. Reduced cytochrome c was treated with different concentrations of BaSO4 and CeO2 for 24 hours at 4°C. UV-VIS was used to observe peaks at 520nm and 549nm, which correspond to the peaks of reduced cytochrome c. Concentration range 62.5 – 500 μg/ml for both showed a 2.3 fold increase in oxidation for CeO2 at 26.5 μg/ml increasing to 6.2 fold at 500 μg/ml, whereas the BaSO4 induced a 1.4 fold increase at 62.5 μg/ml increasing to 3.6 fold at 500 μg/ml. The increased oxidation by CeO2 vs. BaSO4 is also a reflection of the more severe toxic effect observed in STIS (Short Term Inhalation Studies). Bio-physical nature of nanomaterials is being used for risk assessment, some using cytochrome c (Nel et al. 2013). However, this study, developed along-side Mathilde Delaval (University Paris Diderot), has produced a more simple and robust assay. In conclusion, bio-physical interactions can reveal insights about the potential hazard that a nanomaterial possesses. It can contribute to a more coherent and useable grouping of nanomaterials, which can aid better and more efficient regulation.

294 Hemotoxicity and Stability of Luminescent Nanoparticles: In Vitro and In Vivo Evaluation

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Luminescent nanoparticles Y2O3:Eu and La2O3:Eu have potential biomedical applications for use as contrast agents and as drug delivery systems in cancer. However, their biocompatibility, stability and cytotoxic effects in vitro and in vivo have not been widely studied. The objective of this study was to evaluate the hematotoxic effect of Y2O3:Eu and La2O3:Eu nanoparticles and their photostability after the histologic standard treatment. Methods: For hematotoxicity test, red blood cells were washed and treated with different concentrations of nanoparticles and incubated by 1 h at 37°C. The hemolysis was determined by spectroscopy in the supernatant. Additionally, blood smears of each treatment were prepared to evaluate cytomorphic abnormalities. For stability of Y2O3:Eu and La2O3:Eu in histologic standard treatment, a murine melanoma model was developed by transplantation of the B16F cells in BALB/c nude mice. All the experiments were conducted according to the Mexican Animal Care Guide: NOM-062-200-1999. The nanoparticles were i.t. administered at different doses. The in vivo presence was evaluated by x-rays and ultrasound. Subsequently, histological cross-sections of the tumor were analyzed by epifluorescence microscopy. Results: In vitro hemolysis assays demonstrated that the nanoparticles were hemocompatible. The Y2O3:Eu and La2O3:Eu nanoparticles were detected in the tumor tissue by ultrasonogram and x-rays. Was observed a radiodense image with regular borders, especially for Y2O3:Eu. The nanoparticles resisted the histological standard treatment, which allowed their observation by epifluorescence microscopy in tumor slices. In conclusion, the luminescent nanoparticles are biocompatible and present good photostability.

295 Risk Assessment of Nanomaterials Using Control Banding

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In the absence of complete exposure and toxicity data on engineered nanomaterials (ENM), risk assessment using control banding can be used to support risk management decisions and to triage exposure control efforts in the work place. Control banding incorporates the available information on chemicals in a systematic evaluation of risks by using rankings for exposure and toxicity. Hazard (severity) and exposure (probability) factors are graded into 4 levels referred to as bands, each of which corresponds to a risk control strategy. The severity and exposure bands are combined into a matrix with bands that have quantitative ratings for each of the risk levels. Each level corresponds to a control strategy regarding the need for engineering controls (general ventilation, fume hoods, local exhaust, containment, seek expert advice), or other protective measures, such as personal protective equipment. Although this methodology has been recommended for nanomaterials, specific applications have not been published. A control banding case study using four ENMs (silver, aluminum oxide, titanium dioxide, carbon nanotubes) that are currently being used in a variety of consumer products is presented. To develop the severity factors, a review of toxicological and human health literature for the ENMs was conducted and information about physicochemical factors that can influence toxicity and the health endpoints associated with each ENM was summarized. The probability factors were developed for routine workplace tasks associated with the ENMs, from receipt of the material to final discharge of the processed wastewater that could result in potential exposure to the ENMs. The risk level for each task was determined based on the sum of all the points from the severity and probability factors. Overall severity or probability scores of <20 are considered low, 21–40 are medium, and >40 are high. The risk control strategies for the case study provide specific quantitative control banding examples that may be adapted and adapted to other ENMs and workplace settings.

296 Silver Nanoparticles Induce Cardiovascular Toxicity in Endothelial Cells and Zebrafish Embryos

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Silver nanoparticles have distinctive physico-chemical properties that make them attractive in a variety of applications. These uses are rapidly expanding, and thus, definitely increase the possibility of the exposure of human and other organisms to silver nanoparticles. In order to gain new insights into the toxicity of silver nanoparticles, the study of cardiovascular effects was investigated in vitro and in vivo. Since, biologically accessed silver nanoparticles eventually end up in contact with endothelial cells. Therefore, in this study, cultured human umbilical vein endothelial cells (HUVECs) were treated with silver nanoparticles for 24 h. The HUVECs were investigated using MTS and apoptosis assays. The productions of intracellular reactive oxygen species were measured. The results show that silver nanoparticles induce some level of cytotoxicity, oxidative stress, and apoptosis. In vitro study, we found that silver nanoparticles cause mortality, malformation, and heart defects in zebrafish embryos. After alkaline phosphatase staining, we also found aberrantly sub-intestinal vessels in silver nanoparticle exposed embryos. These results suggest that the exposure to silver nanoparticles can possibly cause a risk to cardiovascular system.

297 Silver Nanoparticles Induce Autophagy Dysregulation and Activation of NLRP3 Inflammasome in HepG2 Cells

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Silver nanoparticles (AgNP) have applications for several medical products due to their antimicrobial properties; however, adverse effects associated with exposure are not fully evaluated. Further studies are needed to better understand the bioactivity of AgNP and identify biomarkers of toxicity. Autophagy involves transfer and lysosomal degradation of cytoplasmic constituents, whereas inflammasomes are key signaling platforms that regulate the activity of caspase-1 and maturation of highly pro-inflammatory cytokines IL-1β and IL-18. The goal of this study was to: 1) evaluate AgNP cytotoxicity in cultured human HepG2 cells, 2) evaluate AgNP induced autophagy dysregulation and NLRP3 inflammasome activation, and 3) determine the effects of autophagy inhibition on AgNP induced NLRP3 inflammasome activation and cytotoxicity. AgNP (10, 50 and 100 nm diam.) were characterized for size and shape using TEM and DLS. Cells were incubated with AgNP (0.1, 0.5, 1, 5, 10, or 50 μg/ml) for 24 hr. Cytotoxicity was assessed by MTS assay. Fluorescence microscopy was used to detect lysosomal activity (LysoTracker DND), autophagosomes (Cyto-ID green), and inflammasomes (caspase-1). Pro-autophagic protein, LC-3B, was detected by confocal microscopy and IL-1β levels were measured by ELISA. Cytotoxicity was observed only after 24 hr post-exposure at the higher concentrations of AgNP (10 and 50 μg/ml) but not at lower concentrations (< 10 μg/ml). Autophagy and NLRP3 inflammasome activation were detected at non-cytotoxic AgNP concentrations (1 and 10 μg/ml). Blocking autophagy with 3-methyladenine (3MA) reduced AgNP induced cytotoxicity, caspase-1 activation and IL-1β secretion. In conclusion, the data demonstrated that 1) autophagy and inflammasome activation were induced at non-cytotoxic AgNP concentrations, 2) autophagy dysregulation may serve as a mechanism to induce cytotoxicity and inflammasome activation, and 3) LC-3B, active-caspase-1 and IL-1β may serve as sensitive biomarkers to detect early toxicity in AgNP-exposed cells.
The role of nanoparticle (NP) interaction with biomolecules to form a biocorona is the determining aspect of NP behavior and its consequences in the physiological environment, and must be considered when assessing potential toxicity. The adsorbed biomolecular corona decides the fate of a nanomaterial in vivo and thus its successful application in the biomedical arena, a comprehensive understanding of the dynamic interactions of the proteins with the NP is imperative. A systematic investigation on the size, surface chemistry and surface charge on time dependent adsorption kinetics and individual protein corona formation was conducted with BPEI and lipidoic acid coated 40nm and 80nm gold NP (AuNP). NP were exposed to four human hard corona proteins, human serum albumin (HSA; 40mg/ml), fibrinogen (2mg/ml), immunoglobulin (IgG; 12mg/ml) and transferrin (2.5mg/ml) at physiological concentrations, for 24h. Time evolution data over 0, 6, 12 and 24h revealed that irrespective of surface chemistry, rapid and prominent binding of HSA and IgG coronas occurred over both BPEI and lipidoic acid coated AuNP marking an increase in size, without agglomeration up to 24h, at 37°C. Interestingly, IgG exhibited size dependent binding, wherein IgG treated 80nm NP had a higher surface charge compared to the 40nm NP. In contrast, fibrinogen triggered agglomeration instantaneously upon contact with both BPEI and lipidoic acid coated NP, while transferrin induced aggregation in BPEI coated NP. These findings suggest that protein coronas at their physiological concentrations interact variably; wherein HSA and IgG coronas adsorbed strongly onto the NP surface keeping the NP well-dispersed, while fibrinogen caused rapid, strong and irreversible agglomeration. Importantly, individual protein coronas exhibited diverse cellular uptake patterns in human umbilical vascular endothelial cells, HSA and IgG coronas showed a high cellular uptake and fibrinogen coronas reduced cellular uptake.

Silver nanoparticles (Ag NPs) are most widely used nanomaterials in various consumer applications, mostly due to their antimicrobial properties. Although the release of Ag-ions, cell membrane damage and generation of reactive oxygen species (ROS) are thought to play the key role in toxic action of silver, the exact mechanisms behind the silver toxicity are still unclear. It has also been shown that the coating of silver NPs strongly modulates its toxicity (Bondarenko et al., Arch Toxicol (2013) 87:1181–1200). The yeast S. cerevisiae, a promising model organism for studies of toxicity mechanisms as it is well characterized with a lot of mutants available. In this study, we used a S. cerevisiae wild-type and its single-gene deletion mutants (from EUROSCARF) to elucidate the mode of action of Ag NPs. The mutants sensitive to oxidative stress (OS), cell wall/membrane stress and deficient in endocytosis were used. Uncoated (nAg), polyvinylpyrrolidone-coated (nAg-PVP), casein-coated (collargol, nAg-col) Ag NPs and AgNO3 were compared for their toxicity. Toxicity was evaluated in 48-h growth inhibition done-coated (nAg-PVP), casein-coated (collargol, nAg-col) Ag NPs and AgNO3 stress and deficient in endocytosis were used. Uncoated (nAg), polyvinylpyrrolidone-coated (nAg-PVP), casein-coated (collargol, nAg-col) Ag NPs and AgNO3 were compared for their toxicity. Toxicity was evaluated in 48-h growth inhibition done-coated (nAg-PVP), casein-coated (collargol, nAg-col) Ag NPs and AgNO3.
Aluminum (Al) is a vital pathogenic agent responsible for the incidence of neurodegenerative diseases. Our previous work has demonstrated that aluminum nanoparticles (NP) have an enhanced capacity to produce reactive oxygen species and consequently have widespread toxic properties. In the present work, we are investigating the cognitive abilities of aluminum NP in transgenic (TG) mice bearing susceptible genes of Alzheimer’s disease (AD). TG mice bearing arg61, App, Ps1, and Tau were used as the subjects. Mice were randomly divided into 5 groups, which were intranasal treated with 0.9% saline, Aic3 (Al ion), 50nm-sized alumina NP (Al oxide), 13nm-sized alumina NP, and nanowire alumina (6nm@400nm) at the concentration of 5mg/kg bw. Morris water maze test and open field test were used to detect the cognitive ability of the mice after 8-weeks treatment. The results showed a significant difference in cognitive abilities among the groups. Compared with control, the moved distance, movement and the velocity of Al ion-treated mice reduced significantly, while the mice treated with alumina NP at 50nm, 13nm and nanowire had not shown significance. The cognitive ability of mice treated with Al ion showed its low-dose effect as longer time in the target area and less error, but the mice treated with nano-alumina demonstrated significance size effect, the time in the target area of mice treated with 50nm size alumina decreased, 13nm size extremely decreased, while nanowire no significance. We concluded that alumina NP could induce the decline of the cognitive ability in a size-dependent manner; the trend of effect was significant demonstrated in mice bearing susceptible genes of AD. The present study may establish a path for studying the interaction of metal nanoparticles toxicity with genetic mutations for AD progression.

In Vivo Evaluation of the Pulmonary Toxicity of Cellulose Nanocrystals

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Cellulose nanocrystals (CNC), with advantageous chemical and mechanical properties, are viewed as lightweight and inexpensive alternatives to carbon nanotubes (CNTs). Over the past decade, due to their low cost, high abundance and ease of availability, CNC materials have gained prominence in a number of applications: nanofillers in polymer composites, building materials, cosmetics, filtration membranes, photonic films, food, and in the drug industry. Thus, it becomes critical to evaluate the potential health risks associated with CNC exposures. Here, we compared pulmonary outcomes caused by exposure of C57BL/6 mice to two different processed forms of CNC materials, i.e., CNCs (10 wt%; gel/suspension) and CNCP (powder). Pharyngeal aspiration with CNCS and CNCP was found to facilitate the innate inflammatory response assessed by an increase of bronchoalveolar lavage (BAL) leukocytes. Biomarkers of tissue damage were elevated to a higher extent in mice exposed to CNCP. Compared to CNCS, CNCP caused a significant increase in the accumulation of oxidatively modified proteins. The up-regulation of inflammatory cytokines was higher in the lungs after CNCS treatments. Most importantly, the elevated levels of IFN-γ, a Th1 cytokine and IL-13, a Th2 cytokine in the BAL were unique to CNCS and CNCP exposures, respectively. Moreover, CNCP materials were significantly longer than CNCs. Taken together, our data suggests that particle morphology and nanosize dimensions of CNCs may be critical factors affecting the nature of the innate immune inflammatory responses.
mental species. Safety assessment of these NMs is critical to protect the well-being of workers and consumers as these technologies become widely implemented. Current animal toxicology exposure paradigms are early in discovery and are not easily set to environmental or industrial exposure conditions. In vitro toxicity methods, able to screen rapid and deeper molecular studies have required dispersion of NMs in aqueous media before cell exposure. For this reason, an ambient aerosol exposure chamber (AEC) was developed to capture the surrounding suspended particulate and dose-life culture systems to assess the toxicity of NMs at the air-liquid interface. To validate the AEC, titanium dioxide, silica, carbon black, multiwall carbon nanotube, and silver nanopowders were aerosolized and exposed to nose and lung respiratory cell models. The proinflammatory response elicited from the cells after exposure to NMs was analyzed by IL-8 cytokine secretion at 0, 12, and 24h time points, and the results from this study were related to the findings in literature where the in vitro toxicity data was correlated to acute pulmonary inflammation in rodents. Methods for assessing the cellular inflammation, viability, and nanomaterial uptake were established and optimized during use of the AEC to best determine the toxicity of these five NMs. Results show that different NMs caused variations in IL-8 production and secretion, and all exposed cell culture models tested remained viable for the entirety of the acute exposure study.

**307 Pulmonary Microdistribution of TiO2 Nanoparticles in Rats following Single or Multiple Intratracheal Administrations Using XRF Microscopy**

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The toxicological assessment of inhaled nanoparticles (NPs) had been conducted using intratracheal administration tests where single-dose protocol was often performed, although multiple intratracheal administrations of small quantities of NPs appeared to be more representative of inhalation exposure than a large single administration. However, pulmonary microdistribution of NPs following single or multiple doses of NPs had not been investigated. Here, we quantitatively evaluated pulmonary microdistribution (per 100 μm x 100 μm mesh) of TiO2 NPs in rats following single or multiple doses of TiO2 NPs at a total dosage of 10 mg/kg, using X-ray fluorescence (XRF) microscopy. From the quantitative results of pulmonary TiO2 NPs microdistribution, (i) large variations in each lobe were observed among the rats with same dose (e.g., RSD: 53.4%, 109%, 51.0%, 87.2%, and 102% (single dose, n = 5) for right cranial, middle, caudal, accessory, and left lobe samples); (ii) the different lobes of rats with same dose showed different trends on the deposition of TiO2 NPs in lung (e.g., single dose: 0.07±0.03, 0.045 ± 0.049, 0.10 ± 0.051, 0.086 ± 0.075, 0.065 ± 0.066 ng/mesh (n = 5) for right cranial, middle, caudal, accessory, and left lobe samples); (iii) the pattern of pulmonary TiO2 NPs deposition was similar between the rats following single and multiple doses. Based on these observations, several samples are necessary for evaluating pulmonary microdistribution. In addition, it is recommended that histopathological examination should use the sections from all lobes of the lung to avoid the over- and under-estimation. This work is part of the research program “Development of innovative methodology for safety assessment of industrial nanomaterials” supported by the Ministry of Economy, Trade and Industry (METI) of Japan.

**308 Nanoparticle Surface Characterization and Clustering through Concentration-Dependent Surface Adsorption Modeling**

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Quantitative characterization of nanoparticle interactions with their surrounding environment is vital for safe nanotechnological development and standardization. A recent quantitative measure, the biological surface adsorption index (BSAI), has demonstrated numerous promising applications in nanomaterial surface characterization and biological/environmental prediction. In the BSAI approach, a nanoparticle surface is characterized through five descriptors representing possible interactive forces. This current study further advances the approach by the application of five descriptors to address the concentration dependence of the descriptors enabling better prediction of adsorption profile and more accurate categorization of nanomaterials based on their surface properties. We obtained adsorption profile from 23 types of nanomaterials and 30 organic probe chemicals. Statistical analysis on the adsorption data was performed based on three different models: the original BSAI approach, a concentration dependent polynomial Linear Free Energy Relationship (LFER) model, and an infinite dilution model based on the Langmuir adsorption model. The characteristic and descriptive abilities of these models were analyzed and compared. The new models showed superior prediction ability and clustering of the nanomaterials with different chemical profiles. These advancements in BSAI modeling showed a promising development in the application of quantitative predictive modeling in biological applications, nanomedicine, and environmental safety assessment of nanomaterials.

**309 Silicon Nanowires: Free Radical Production and Related Damage in Cellular Exposures**

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Processing and synthesis of purified nanomaterials of diverse composition, size, and properties are a recent development. Studies have demonstrated that some nanomaterials have toxic effects and this has led to toxicity research focusing on nanotechnology. Close to two million workers will be employed in the field of nanotechnology over the next ten years. The unknown effects of nanomaterials create a need for research and development of techniques to identify possible toxicity. Through a cooperative effort between NIOSH and IBM to address possible occupational exposures, silicon-based nanowires (SiNW), synthesized by the vapor-liquid-solid method, used in bio-sensors, gas sensors, and field effect transistors, were obtained for our study. SiNW are anisotropic filamentary crystals of silicon. Reactive oxygen species (ROS) can be generated when organisms are exposed to a material causing cellular damage such as lipid peroxidation, H2O2 production and DNA damage. SiNW were assessed using three different in vitro models (chemical, RAW 264.7 cells and rat alveolar macrophages) for ROS generation and possible toxicity. We used electron spin resonance, analysis of lipid peroxidation, measurement of H2O2 production and the comet assay to assess generation of ROS from SiNW and define possible mechanisms. Our results demonstrate that SiNW do not appear to be a significant generator of free radicals. Keywords: Reactive Oxygen Species; Nanomaterials; Nanotoxicology; Free Radicals; Silicon
311 Nanoparticle Size, Shape, Coat, and Charge Alter the Bioactivity of Nanosilver

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The antimicrobial activity of nanosilver (AgNP) has made it the largest and fastest growing class of nanomaterial used in consumer products. Studies on the effects of AgNP exposure in mammals are limited however, and new methods for rapid and efficient safety evaluation of nanomaterials are urgently required. This makes small, easily cultured model organisms with short life cycles such as Caenorhabditis elegans attractive in vivo alternatives to mammalian models for toxicity testing. Previously, we demonstrated that C. elegans larval growth assays can predict mammalian toxicity ranking, and that AgNP, while toxic to C. elegans at high concentration, is less toxic than ionic silver which also correlates with data from in vivo rodent studies. Here we assess the effects of AgNP size, shape, and coat on bioactivity in C. elegans. AgNP species used in this study include nanoparticles, which can be “tuned’ to resonate at specific frequencies, and spheres with diameters ranging from 10 to 110 nm, coated in negatively charged citrate, polyvinylpyrrolidone (PVP), neutral polyethylene glycol (PEG), or positively charged BPEI (a highly aminated, organic moiety). Citrate coated 20 nm silver spheres induced an innate immune response that increased over 24 hours of exposure, while regulation of genes involved in metal metabolism peaked at 4 hours and subsequently decreased. For both AgNP spheres and plates, smaller size correlated with higher toxicity, as indicated by reduced larval growth. Smaller AgNP size also correlated with increased tissue uptake of silver for nanospheres, but not nanoparticles. For silver 20 nm spheres, coating altered bioactivity, with a toxicity ranking of PEG > PVP > BPEI > citrate, but a silver uptake ranking of PEG > PVP > citrate > BPEI. These results indicate that the physiochemical properties of AgNP affect toxicity and uptake profiles, and highlight the importance of safety testing for each AgNP species.

312 Uterine Intravital Microscopy 24-Hours After Nanosized Titanium Dioxide Exposure

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With the wide use of engineered nanomaterials, exposure can no longer be confined to healthy male models of occupational exposures. While cardiovascular and endothelial cell dysfunction has been established using in vitro techniques, translation to intact in vivo models may be limited as systemic compensatory mechanisms are unclear. Intravital microscopy has been used extensively to understand microvascular physiology while maintaining in situ neurogenic, humoral, and myogenic control. Therefore, female SD rats were exposed to nano-TiO2 aerosols (176 ± 5 BPM, 85 ± 2 mm Hg), suggesting an enhanced sympathetic nervous system influence. In the spinotrapezius muscle microcirculation, α-adrenergic receptor blockade significantly reduced arteriolar tone and responsiveness to endogenous sympathetic neurotransmitters, suggesting nanoparticle exposure increased sympathetic tone and shifted from a balanced α-adrenergic/neuropeptide Y-mediated contraction to one dominated by an α-adrenergic mechanism, respectively. These results were confirmed by western analysis of isolated spinotrapezius microvessels. Following inhalation exposure, normalized (GAPDH) arteriolar expression of α1-adrenergic receptors was slightly but significantly increased (1.1 fold, p<0.05). Furthermore, nanoparticle exposure significantly decreased neuropeptide Y receptor expression by 2-fold (p<0.01). These data suggest that nanomaterial particles are an adrenergically-dominated mechanism of sympathetic vascular constriction. Furthermore, the data are consistent from multiple perspectives that sympathetic nervous system activity and influence are altered after nanoparticle exposure. Hence, nanomaterial exposure may alter peripheral vascular function by sensitizing vessels to adrenergic stimuli. (Funding NIH ES015022, TRN)

314 Single Intratracheal Instillation of 20 nm Citrate Capped Nanosilver-Elevated Circulating Cytokines and Expanded Cardiac Ischemia/Reperfusion Injury in Male Sprague-Dawley Rats

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The potential uses of engineered nanomaterials have rapidly expanded in the fields of biomedical technology and consumer manufacturing. Silver nanoparticles (AgNP) have garnered much interest due to their innate antimicrobial properties, becoming one of the most utilized nano scale materials. However, potential cardiovascular toxicity associated with exposure has not been thoroughly investigated. We have demonstrated expansion of myocardial infarction after intratracheal (IT) installation of various nanoparticles but the underlying mechanism remains elusive. We hypothesized that pulmonary exposure to Ag core AgNP induces persistent increase in circulating cytokines, and expansion of cardiac ischemia-reperfusion (I/R) injury. To test this hypothesis, we exposed male SD rats to IT instillation of 200 μg of 20 nm citrate capped Ag core AgNP, or a citrate vehicle. Serum samples collected 24 and 168 hours following IT instillation were analyzed for concentrations of selected cytokines. 24 or 168 hours after exposure, cardiac ischemia was induced by coronary artery ligation for 20 minutes, followed by 2 hours of reperfusion. AgNP instillation resulted in expansion of I/R injury and elevated serum cytokines: G-CSF, MIP-1α, IL-1β, IL-2, IL-6, IL-13, IL-10, IL-18, IL-17, TNFα, and RANTES 24 hours post IT compared to vehicle treated rats. Expansion of I/R injury persisted 168 hours post IT instillation and was associated with persistent elevation in cytokines: IL-2, IL-13, and TNFα. Based on these data, IT instillation of AgNP increases circulating levels of several cytokines, which may contribute to persistent expansion of I/R injury. This study is funded in part by NIEHS U19 ES091525 and ECU.

316 Effect of Silver Nanoparticles (AgNP) on Surface Marker Expression of Human Bone Marrow Stromal Cells (hBMSCs)

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Stem cells are present in all adult tissues and are critical for tissue health, maintenance, and response to injury and disease. Stem cells have an ability to self-renew, differentiate, and exhibit co-expression of specific cell surface markers. Human stem cells model are finding uses in toxicology evaluations as an alternative to conventional animal models which are not always predictive of human responses. Exposure to nanomaterials, including particles released from medical devices, may alter the expression of stem cell surface markers, potentially changing their unique stem cell niche. The aim of this study was to 1) determine non-cytotoxic AgNP concentrations for hBMSCs, 2) assess the base level of CD105, CD44, CD73 and CD90 cell surface marker expression in hBMSCs, and 3) evaluate changes in expression of these surface markers under different exposure conditions with varying AgNP concentrations. hBMSCs monolayers were exposed to 10nm AgNP for 24 hr; non-cytotoxic AgNP concentrations were determined to be ≤ 10 μg/ml using...
the MITT cytotoxicity assay. The base level of cell surface marker expression was assessed using fluorescently-labeled antibodies and quantitated using flow cytometry (FC). hBMSCs not exposed to AgNP co-expressed CD44, CD90, CD105, CD73 surface markers and were negative for hematopoietic markers indicating near-pure population. To evaluate AgNP-induced changes in stem cell surface marker expression, monolayers of hMSC were treated with varying non-cytotoxic concentrations (0, 1, 5, and 10 μg/ml) of 10 nm AgNPs. Cells were exposed to AgNP for up to 21 days (1, 7, 14 and 21 d) using three different exposure conditions: one-time exposure, once a week exposure, and continual exposure. Cell surface marker expression was assessed for each exposure time (1, 7, 14 and 21 d). FC data showed that silver nanoparticles did not alter expression of normal stem cell surface markers, indicating no apparent changes in stem cell function for all exposure scenarios.

317 Chronic Exposure to Particulate Hexavalent Chromium Disrupts Sister Chromatid Cohesion in Human Lung Cells

C. Falant and J. P. Wine

Chromosomal instability (CIN) is a hallmark of cancer and can be caused by spindle assembly checkpoint disruption or chromosome mis segregation during mitosis. Hexavalent chromium (Cr(VI)) is a well-known human lung carcinogen, and has shown to induce numerical CIN, however its mechanisms for inducing aneuploidy remain unknown. In this study we are investigating whether Cr(VI) affects key cohesion proteins, Shugoshin 1 (Sgo1) and Rad21. Sgo1 maintains and protects centromeric cohesion in G2 and continues to maintain proper sister-chromatid cohesion during mitosis; while Rad21 is a key component of the cohesin complex. Disruption of these two critical cohesion proteins has been shown to lead to CIN.

We have found that chronic exposure to particulate Cr(VI) disrupts the localization of Sgo1 in G2 cells. Specifically, after a 24 h exposure to 0.1, 0.2, and 0.3 μg/cm² lead chromate, 0.2 μg/cm² zinc chromate, respectively we did not observe any changes in the percent of G2 cells with Sgo1 and Rad21 localization at the kinetochores. However, a 120 h exposure to the same concentrations showed a concentration-dependent decrease in the percent of G2 cells with Sgo1 and Rad21 localization at the kinetochores. In addition, we found that after a 120 h exposure to Cr(VI) decreased Sgo1 but not Rad21 protein expression. Our findings suggest that particulate Cr(VI)-induced CIN is mediated through disrupting Sgo1 localization and ultimately aneuploidy through premature cleavage of Rad21 in G2. This work was supported by NIEHS grant ES016893 (J.P.W.).

318 Assessing the Effects of Hexavalent Chromium in Two Reptilian Species: Implications for Metal Impacts of Global Warming

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Hexavalent chromium (Cr(VI)) is an environmental problem worldwide, recent studies show it is a global marine pollutant. With increased concern over global warming the potential for additional exposure to marine and aquatic species is of concern as metals currently bound in sediments can be released with ocean acidification. Using sea turtles and alligators as models of these environments we have begun to assess the impact of Cr(VI) on these species. We found that particulate lead chromate induced similar amounts of cytotoxicity and genotoxicity in hawksbill and alligator cells. For example, concentrations of 0.5, 1 and 5 μg/cm² lead chromate induced 73, 63, and 15 percent relative survival in alligator cells and 79, 54 and 7 percent relative survival in sea turtle cells, respectively. These same concentrations induced 20, 31, and 39 percent of damaged metaphases in alligator cells and 15, 26, and 36 percent of damaged metaphases in sea turtle cells, respectively. These cells also had similar levels of chromium ion uptake. The effects for soluble chromium were different. Specifically, concentrations of 1, 2.5, and 5 μM sodium chromate induced 78, 60 and 25 percent relative survival in alligator cells and 46, 25 and 5 percent relative survival in sea turtle cells, respectively. These same concentrations induced 18, 28, and 45 percent of damaged metaphases in alligator cells and 16, 26, and 39 percent of damaged metaphases in sea turtle cells, respectively. Sea turtle cells also had higher levels of intracellular Cr ion concentrations than alligator cells. These data suggest that release of Cr(VI) from sediments could be a concern for aquatic species.
repairs DSBs in an error-free manner. Our previous data suggest that prolonged Cr(VI) exposure inhibits HR repair through the mis-regulation of RAD51 and that loss of HR leads to Cr(VI)-induced chromosome instability. RAD51 nuclear transport must be tightly regulated to protect against genomic instability. However, no studies have investigated how a chemical carcinogen affects the transport of RAD51 or its transport partners, RAD51C and BRCA2. In this study, we investigated the effect of particulate Cr(VI) exposure on subcellular localization of RAD51, RAD51C and BRCA2. We exposed human lung fibroblasts to zinc chromate for 24-120 h. Using affinity grid capture visualized with TEM, we found that prolonged Cr(VI) exposure inhibits RAD51 filament formation. Specifically, after 24 h and 120 h exposure to 0.2 ug/cm² zinc chromate, the number of RAD51 filaments decreased from 104 to 7, respectively. In addition, we show an increase in RAD51C and BRCA2 foci formation after 24 h Cr(VI) exposure, which corresponds with no increase in RAD51 cytoplasmic accumulation. However, prolonged Cr(VI) exposure inhibits RAD51C foci formation, corresponding with an increase in RAD51 cytoplasmic accumulation. BRCA2 foci formation was not inhibited after prolonged Cr(VI) exposure. Using RAD51C knock-down cells, we demonstrate that RAD51C depletion can induce the cytoplasmic accumulation of RAD51. These results suggest that prolonged Cr(VI) exposure inhibits RAD51 by inhibiting its transport partner, RAD51C. This work was supported by NIEHS grant ES016893 (J.P.W.).

322 Centriole Defects in Chemical Carcinogenesis: Particulate Cr(VI) Causes Premature Centriole Disengagement through Plk1 and Separase Activation

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Lung cancer is the leading cause of cancer death in the USA. About 9-15% of cases can be attributed to environmental and occupational exposure. Hexavalent chromium (Cr(VI)) is a metal widely used in industry and a common environmental pollutant. Particulate Cr(VI) is also a lung carcinogen. Studies from our laboratory show that chronic exposure to particulate Cr(VI) causes supernumerary centrosomes and centrosome abnormalities. Centrosome defects are common in solid tumors and correlate with chromosome instability. However, the mechanisms involved in particulate Cr(VI)-induced centrosome defects are unknown. We investigated centriole disengagement, the licensing step in centrosome duplication. We exposed human lung cells to zinc chromate for 24-120 hours and evaluated centriole disengagement by immunofluorescence. Western blots were used to assess protein levels of active and inactive Plk1, separase and securin. Our data show that chronic exposure to particulate Cr(VI) causes premature centriole disengagement in S and G2 phase cells. Moreover, Cr(VI) increases the ratio of active vs. inactive Plk1 and protein levels of active separase, while decreasing securin protein levels. Our data suggest that chronic exposure to particulate Cr(VI) causes premature centriole disengagement by activating Plk1 which triggers securin degradation and separase activation. Future work aims at reversing centriole disengagement by manipulating separase activity. This work was supported by NIEHS grant ES016893 (J.P.W.) and the Maine Center for Toxicology and Environmental Health.

323 PI3K/Akt/DAF-16 Activity Is Protective during Excess Iron Exposure in C. elegans


Iron and other essential metals have long been associated with neurologic disease. Evidence suggests a complex relationship between iron and neurotrophic and neuroprotective signaling pathways such as PI3K/Akt/FOXO, although the precise nature of this relationship and its contribution to neuronal health has been elusive. This study used the Caenorhabditis elegans (C. elegans) model to examine interactions between PI3K/Akt/FOXO, exposure to excess iron, and dopamine. Synchronous first stage larvae were placed on nematode growth media plates inoculated with OP-50 E. coli and containing 0 or 24mM ferric ammonium citrate (FAC). Body length, brood size, lifespan, mitochondrial structure, and dopamine-mediated basal slowing behavior were observed in developing and adult animals. Strains used in this study included Bristol N2 (wild type); CF1038 (daf-16 mutant, C. elegans FOXO homologue); TJ1052 (age-1 mutant, C. elegans PI3K p110 subunit which inhibits DAF-16 activity); and CJ112 (cat-2 mutant, tyrosine kinase), 24mM FAC had no effect on brood size, body length, or mitochondrial morphology in any strain examined. Relative to control, 24mM FAC decreased N2 lifespan (10 vs. 12d, p<0.001) although it did not alter basal slowing. Likewise, FAC reduced lifespan of daf-16 mutants (10 vs. 11d, p<0.001); however, FAC also significantly reduced basal slowing in daf-16 animals (p<0.05). FAC exposure did not affect lifespan in age-1 mutants with increased DAF-16 activity (p<ns). Interestingly, excess iron did not reduce the lifespan of dopamine deficient cat-2 mutants (p<ns). These observations are consistent with a protective role for DAF-16 in the context of excess iron exposure, and suggest that dopamine-dependent behavior is at risk in animals with reduced DAF-16. Furthermore, the absence of FAC induced lifespan reduction in dopamine deficient worms indicates that dopamine may contribute to the damaging effects of excess iron and may reflect an interaction between levels of dopamine, iron, and PI3K/Akt/DAF-16 activity.

324 Iron Incorporation to MnSOD Leads to the Formation of a Peroxidase: Possible Implications for Nutrition-Dependent Iron Toxicity


Mn-Fe SOD is a family of metalloenzymes which occurs in organisms from bacteria to humans. These enzymes, when bound to their classical metals, are known to catalyze the dismutation of the free radical superoxide anion to H₂O₂ and oxygen. The Mn-dependent superoxide dismutases are essential for organisms to be able to cope with oxygen, since the deletion of the gene for these enzymes from the genome of all organisms results in oxygen lethality. In the bacteria E. coli, two distinct Fe-Mn SODs are present: SOD B contains iron and SOD A contains manganese. In eukaryotes, active SOD2 contains manganese and has a high homology to the ancestral SOD A of bacteria. We show for the first time that the bacterial Mn-dependent SOD A bound to iron (FeSOD A) has peroxidase activity. The reaction of FeSOD A with H₂O₂ led to oxidation of substrate (Ampex red), generation of protein radicals and release of iron, all of which were H₂O₂ dose-dependent. Interestingly, active FeSOD B did not have a catalytic peroxidase activity, which indicates that iron per se is not responsible for the enzymatic peroxidase activity detected. We also studied the metalation of Mn-dependent SODs in vivo. With high levels of iron in the media the metalation of SOD A with manganese was decreased, probably because the absorption and assimilation of iron and manganese compete in vivo. The in vivo formation of the peroxidase FeSOD A was increased when media had higher levels of iron because of a decreased manganese utilization. With media containing baby formula (regularly fortified with iron), the amount of iron complexly iron and peroxidase activity, while in human milk media, SOD A incorporated manganese and had normal superoxide dismutase activity without peroxidase activity. The biological occurrence of this fundamental antioxidant enzyme in an alternative iron-dependent state can represent a potential important source of free radical formation in vivo.

325 An Exposure and Health Risk Assessment of Lead (Pb) in Lipstick

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The lead (Pb) content in lipstick and other consumer products has become an increasing concern over the past several years. In 2007, the Campaign for Safe Cosmetics published the test results of 33 lipstick samples and found that 61% contained Pb, with a maximum Pb concentration of 0.65 ppm. In a 2010 response, the United States Food and Drug Administration tested 400 lipstick samples and found a median Pb concentration of 0.9 ppm and a maximum Pb concentration of 7.19 ppm. To assess the safety of these lipsticks in adults that chronically apply lipstick as well as instances where children might intentionally or incidentally ingest lipstick products, the US EPA ALM and IEUBK models were used to determine the blood Pb concentrations of adults and children (aged 0 – 7) ingesting varying amounts of lipstick of different Pb concentrations. Modeled blood Pb concentrations were then compared to the United States Consumer Product Safety Commission’s and California’s No Significant Risk Level blood Pb concentration guideline of 15 µg Pb/day (orally) and to the Centers for Disease Control and the US EPA’s actionable blood Pb levels of 5 µg/dL and 10 µg/dL, respectively. In this analysis, background Pb exposure was the primary contributor to estimated blood Pb levels (BLLs) in children and adults, and Pb exposure from lipstick did not significantly increase estimated BLLs. To raise the BLL of an adult with average background exposure to the CDC BLL of interest, 5.0 µg/dL, an adult would need to apply lipstick ~695 times per day. To raise BLLs to the CDC and EPA standards, a child with average background exposure would need to consume 247 and 897 tubules/year, respectively. These results suggest that the safety of consumer products and cosmetics should be assessed not only by the presence and amounts of hazardous contents, but also in conjunction with an assessment of estimated background exposures and comparison to health based standards.
Low levels of lead exposure among adults in the general population may increase blood pressure and enhance the risk of hypertension. The goal of this study was to examine the association of blood lead levels (BLL) with blood pressure and hypertension in a population-based study in a city in Southern Brazil. A total of 948 adults, aged 40 years or older, were randomly selected. Information on socioeconomic status, dietary, lifestyle and occupational background was obtained by orally administered household interviews. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) was measured according to the guidelines VI Brazilian Guidelines on Hypertension. BLL were measured by inductively coupled plasma mass spectrometry technique (ICP-MS). Hypertension was defined as SBP of 140mmHg or higher, or DBP of 90mmHg or higher, or current antihypertensive treatment. The geometric mean of BLL was 1.97 µg/dl (95%CI: 1.90-2.04 µg/dl). Multiple logistic regression was used to examine associations of BLL with hypertension status and with elevated SBP and DBP. Hypertension was adjusted for sex, age, economic status, education, smoking, alcohol intake, body mass index, total cholesterol, triglycerides and glycemia. Adults in the highest blood lead quartile had increased odds of hypertension (OR: 1.81; CI 95%: 1.17-2.80) compared to those in the lowest quartile, with a significant trend across quartiles (p=0.011). After adjustment, the OR for SBP was not significant, while the OR for DBP was significantly higher (OR: 2.57; 95%CI: 1.51-4.39). Despite the low lead levels found in this population, BLL were positively associated with DBP and with hypertension among adults.

**BLOOD LEAD AND HYPERTENSION IN AN ADULT POPULATION IN SOUTHERN BRAZIL**

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**Dose-Dependent Effect of Lead (Pb) on Repetitive and Social Behavior in Male and Female Mice**

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Lead (Pb) is a toxicant that has no benefit to the body. It has been recognized that Pb exposure is implicated in increased risks of autism spectrum disorder (ASD), a neurodevelopmental disorder. Some of the most notable symptoms of ASD are reduced social behavior and increased repetitive behavior. While ASD is more prevalent in males in a ratio of 4:1 compared with females, there are few studies about the sex effect on the dose-response relationship between Pb exposure and autistic behavior in mice. To evaluate the influence of sex on behavioral toxicity caused by Pb exposure, weaning female and male C57BL/6 mice were exposed to 0.2 or 1 mg/mL of Pb acetate trihydrate (PbAc; 0.13 and 0.64 mg as Pb) by drinking water for 4 weeks, after which animals underwent a set of behavior tests: nestlet-shredding, three-chambered sociability, and elevated plus maze. Exposure to Pb continued during the behavior tests. Pb-treated male mice displayed increased repetitive behavior as determined by the nestlet-shredding: 5.9 ± 1.9% (water), 34.4 ± 9.4% (PbAc; 1 mg/mL). Female mice did not show any difference among the three groups. In the sociability test, Pb-exposed male mice demonstrated reduced time spent around the wire cage with the animal: 197 ± 18 sec (water), 159 ± 29 (0.2 mg/mL) and 139 ± 10 (1 mg/mL), while female mice did not show Pb effect. The elevated plus maze test revealed increased activity of the male mice assessed by the distance traveled: 8.3 ± 1.0 meters (water), 10.9 ± 1.5 (0.2 mg/mL) and 11.0 ± 1.1 (1 mg/mL), with no difference in females. Our results demonstrate that these behavioral alterations by Pb exposure occur preferentially in males by a dose-dependent manner, which could be due to a difference in Pb transport between female and male in the brain. Future studies are warranted to explore the possible sex difference in Pb kinetics as well as the downstream signaling pathway of Pb-associated toxicity.

**DOXIM - DETRMINANTS OF BLOOD LEAD LEVELS AMONG CHILDREN IN ULAANBAATAR, MONGOLIA**

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Despite dramatic declines in children’s blood lead levels (BLL) worldwide, significant exposure remains in many developing countries. We conducted a study to determine BLL among children living in Ulaanbaatar, one of the fastest growing cities in the world that has experienced rapid environmental changes, and identify its potential risk factors. A total of 153 children aged 6-8 years from four elementary schools located in different areas of Ulaanbaatar city were enrolled in the study. In 2014, Participants completed a lifestyle and residential environment questionnaire, and their physical and blood lead levels were assessed. LeadCare II analyzer was used to assess BLLs. We detected an average BLL of 5.92 µg/dl (SD=2.86), which is higher than the current WHO standard, with a significant gender difference: BLL of 5.06 µg/dl for girls and 6.99 µg/dl for boys (p<0.01). In addition to gender, we found that BLL increases by 1.12 µg/dl with each year among the study subjects and is higher for those who live in traditional ger dwellings with coal-burning stoves and whose fathers are employed in certain at-risk occupations. Our data indicates that lead exposure among children, especially boys, is a pressing public health issue in Mongolia.

**PRIOR PHOSPHATE TREATMENT OF INTESTINALLY INOCULATED SOIL**


Ingested soil can be an important route of lead (Pb) exposure, particularly in children. Adding phosphate-containing compounds to soils reduces Pb bioavailability by forming insoluble Pb-phosphate complexes. Here, we examined effects of treatment of NIST SRM 2710a Montana soil with three phosphate compounds (sodium phosphate, LockUpLead™, apatite) on Pb distribution and excretion in adult female C57BL/6 mice that receivedAIN-93G rodent diet amended with untreated or phosphate-treated soils. Tissues were collected after an 8-day exposure to one of these soils. Phosphate treatments lowered Pb concentration in soils and diets containing treated soils and significantly reduced (P<0.05) cumulative Pb intake in mice that ingested diets with treated soils. Soil treatment did not significantly increase fecal Pb concentrations; however, cumulative fecal Pb excretion was increased in mice that ingested diets with treated soils. Bone Pb concentrations were reduced in mice receiving diets with treated soils; this effect was significant (P<0.05) only with apatite-treated soil. Liver Pb concentrations were lower in mice that received any treated-soil diet. This difference was significant (P<0.05) for phosphate- or apatite-treated soils. Cumulative liver Pb burdens were decreased in mice that received any treated-soil diet. Soil treatment with agents that reduced Pb's solubility in vivo bioavailability assays was associated with changes in Pb distribution and excretion in mice that received diets containing treated soils. Increased fecal Pb excretion in mice that ingested treated soils was associated with decreased Pb concentrations and burdens of Pb in skeleton and liver, two important depots for this metal. The mouse model may be used to evaluate efficacies of soil treatments and validated in vivo measures of Pb bioavailability. (This abstract does not reflect EPA policy).

**INTRANASAL MANGANESE (Mn) EXPOSURE LEADS TO A SIGNIFICANT ACCUMULATION OF Mn IN BONE**

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Chronic exposure to Mn among welders, smelters and other Mn production and application industries can lead to a condition called manganism that displays analogous but distinct symptoms to idiopathic Parkinson’s disease. The primary route of exposure to Mn in these occupations is by inhalation. Recent studies from this group have established that Mn can accumulate in bone with a half-life equivalent to 8.5 years in humans. However, the question as to whether Mn inhalation exposure may result in Mn accumulation in bone remained elusive. This study was designed to test the hypothesis that intranasal instillation (IN) of Mn led to a significant Mn accumulation in bone, to the extent that was comparable to oral Mn exposure. Adult male SD rats received IN of 0.8 mg Mn/kg as MnCl2 daily for 2 weeks or oral gavage (PO) of 50 mg/kg 5 days/week for 6 weeks; control animals received an equivalent volume of saline. Rats were necropsied 24hrs after the last dose to collect femur and brain samples for quantification of Mn levels. AAS analysis revealed that IN exposure increased Mn levels in bone (MnBn) of exposed animals (2.235 ± 0.354 µg/g) by 7-fold more than that of controls (0.314 ± 0.034 µg/g) (n=5-9; p < 0.001), whereas PO exposure resulted in a 3.5-fold increase of MnBn in exposed animals (2.607 ± 0.499 µg/g) vs. controls (0.749 ± 0.098 µg/g) (n=5; p < 0.001). Mn concentrations in control striatum (STR) and hippocampus (HP) between two routes of exposure were comparable (0.53-0.65 µg/g). Exposure to Mn by both IN and PO routes significantly increased Mn levels in STR and HP (p<0.05). Moreover, Mn levels in STR and HP of exposed groups were significantly higher in IN animals than PO animals (p<0.01). Noticeably, the cumulative dose administered was much lower in IN than PO. This study provides evidence...
that Mn accumulates significantly in bone following intranasal exposure. The data also suggest that IN exposure can lead to significantly more accumulation of Mn in bone and key brain regions implicated in Mn intoxication than oral exposure. (Supported in part by NIH/NIEHS ES008146)

331 Dietary Exposure Assessment of the Trace Elements Se and Mn in Meat Products Consumed in the United States


The Food Safety and Inspection Service (FSIS) is the public health agency in the US Department of Agriculture responsible for ensuring that the nation’s commercial supply of meat, poultry, and egg products is safe, wholesome, and correctly labeled and packaged. For many years, FSIS has monitored these products through the National Residue Program. Along with lead and cadmium, which have been quantified for many years, FSIS began testing for other trace elements, including Se and Mn. Se and Mn are essential dietary micro-nutrients; however, excessive dietary intake of these metals could pose a public health risk. Concentrations of Se measured in swine and Mn in bovine muscle tissues collected in 2013 were used to conduct exposure assessments for both metals. Consumption data obtained from the What We Eat in America database for beef and pork and average body weights (BW) for various age groups estimated by the US Environmental Protection Agency (EPA) were used to estimate exposures for infants, children, and adults. The assessment examined three different estimated means for Se concentrations (162.17, 304.67, and 447.17 μg/kg), the upper and lower 95th percentile confidence limit for each mean (Mean±2 SD), and the maximum concentration found in pork muscle tissue (772.31 μg/kg). The Se health-based guidance value (HBGV) via oral ingestion recommended by the EPA and the Agency for Toxic Substances and Disease Registry is 5 μg/kg-BW/d. Our estimates suggest that only individuals consuming large amounts of pork products (95 percentile) with Se concentrations above the estimated mean of 304.67 μg/kg might be at risk of exposure to levels above the HBGV. Conversely, the Mn exposure assessment in beef estimated a minimal public health risk to populations exposed to the product. FSIS continues to perform these dietary assessments on trace elements in the regulated commod-

332 Polymorphisms in the Solute Carrier SLC30A10 Determine Blood Manganese Concentrations—Evidence from Three Human Populations


Manganese is critical and essential for cell function, however, overexposure is known to be neurotoxic and causes "manganism", a parkinson-like movement disorder. The solute carrier SLC30A10 was recently identified as the affected gene in an inherited type of hypermanganemia, where patients accumulate manganese even without elevated environmental exposure. We hypothesised that part of the variation in blood manganese concentrations in healthy individuals is due to genetic variation in the SLC30A10 transporter. We therefore genotyped two single nucleotide polymorphisms (SNP) in the SLC30A10 gene in three different populations: i) elderly men and women in Brescia, Italy (n=238); ii) pregnant women in rural Bangladesh (n=404); and iii) men and women in the Argentinean Andes mountains (n=272). Manganese concentrations in blood were determined by atomic absorption spectroscopy or by inductively coupled plasma mass spectrometry. We found that the variant allele of both SNPs significantly modified the manganese concentrations in blood, with one SNP being associated with lower and the other with higher blood manganese levels. The direction and sizes of effects of the variant alleles were similar between cohorts, and the effects of the SNPs remained when taking sex and iron status into account. Blood manganese concentrations were also weakly positively correlated to SLC30A10 expression in blood cells. Further, we found that one SNP significantly modified concentrations of the liver enzyme alanine aminotransferase and nonsignificantly motor performance. We have here identified genetic variants that world-wide determine manganese homeostasis and our results give further evidence that SLC30A10 is crucial for regulation of manganese in the body.

333 Pharmacokinetic Evaluation of the Equivalency of Oral Routes of Manganese Exposure in F344 Rats

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Neurotoxic effects occur in people following high dose manganese (Mn) exposure. Several physiologically-based pharmacokinetic (PBPK) models useful for Mn risk assessment have been developed; however these models primarily focus on inhalation and dietary exposure. This study addresses PBPK model data gaps concerning the pharmacokinetics of ingested manganese (Mn) following non-dietary oral exposure. Adult male rats were allocated to control diet (10 ppm Mn), high Mn diet (200 ppm Mn), Mn-supplemented drinking water, and Mn gavage treatment groups. Animals in the drinking water and gavage groups were given the 10 ppm Mn diet and supplemented with MnCl₂ in drinking water or once-daily gavage to provide a daily Mn intake equivalent to that seen in the high Mn diet group. Mean dietary and body weight data were used to adjust weekly Mn intake rates for these groups. Mean body weight changes seen between groups suggest that high-dose oral Mn exposure slows body weight gain. Rats were anesthetized with ketamine and xylazine following 7 and 60 exposure days and samples of bile and blood were collected. Rats were then euthanized and striatum, olfactory bulb, frontal cortex, cerebellum, liver, spleen, and femur samples collected for chemical analysis. Hemato crit was unaffected by Mn exposure. Tissue Mn concentration was determined using inductively coupled plasma mass spectrometry (ICP-MS). Tissue Mn concentrations in multiple sites including liver, femur, striatum, and olfactory bulb were elevated following some Mn exposures (when compared to the control group). Gavage was associated with the highest increases in tissue Mn concentrations, suggesting that dose rate is an important factor in the pharmacokinetics of oral manganese.

334 Zinc Rescue of DNA Repair Inhibition by Uranium

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Numerous metals, including uranium, are cytotoxic, mutagenic, and carcinogenic. Uranium has radiological and non-radiological impact within biological systems and there is increasing evidence for DNA damaging and carcinogenic properties attributable to depleted uranium through its heavy metal properties. In this study we report that low concentration of uranium (as uranyl acetate, predominantly U<sub>2</sub>₃₅; <10 μM) significantly inhibits the zinc finger DNA repair protein poly(ADP-ribose)polymerase (PARP)-1. Exposure to uranyl acetate caused a dose-dependent loss of zinc from PARP-1 and xeroderma pigmentosum, complementation group A (XPA). In keeping with the observed inhibition of PARP activity, exposure to uranyl acetate caused retention of DNA damage. Co-incubation with zinc largely overcame the impact of uranium on PARP activity and DNA damage. These findings present evidence that low concentration of uranium can inhibit DNA repair through disruption of zinc finger domains of DNA repair proteins. The evidence of short term (24 h) zinc reversing the observed DNA damage retention has led to the question of whether adapting keratinocytes to concentrations of zinc higher than that typically found in culture medium leads to further protection. As normal keratinocytes have relatively few doublings before senescence, an immortalized keratinocyte cell line is required. The NIKS cell line was selected for these zinc adaptation experiments and preliminary characterization of responses to metals with the parental and zinc adapted (2 & 10.7 μM) cells are shown demonstrating their suitability for future studies.

335 Proximity to Uranium Mine Waste on the Navajo Nation Is Associated with Elevated ANA and IL-17

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From 1948 to 1986, hundreds of uranium mining and milling operations left more than 1,000 un-remediated and abandoned uranium mines and associated waste sites on Navajo lands, creating a legacy of potential mining waste exposure through drinking water and soil contamination. Adverse health outcomes directly attributable to chronic environmental exposure to legacy mine waste are not known. A survey on Navajo (N=1304; average age 55) on water use, self-reported health
conditions, and self-reported exposures to uranium (U) resulting from a range of activities including occupational history, cultural, demographic and socioeconomic factors showed that in the areas with the highest number of mines (41), 20% of participants reported autoimmune disease, while only 0 to 2% reported autoim-

mune disease in regions with less than 4 mines, indicating a significant increase in the likelihood of autoimmune disease (self-report of clinical diagnosis) in people living in proximity to large mining waste sources. Strikingly, 48% of individuals in this cohort were positive for serum levels of anti-nuclear antibody (ANA), a biomarker of autoimmune disease and a frequent precursor to clinical disease. ANA prevalence is reported at 13.8% for the US population as a whole. ANA detec-
tion was predicted by both a geographical proximity variable and the participants’ urine arsenic levels (p = 0.0073), using linear regression models and Bayesian model averaging approaches. IL17-producing Th17 cells have been associated with the pathogenesis of autoimmune diseases and other inflammatory conditions. Serum cytokine analysis demonstrated a positive and significant association between envi-

ronmental uranium and legacy waste exposure and increased production of IL-17A (β = 1.77 and p = 0.014) which supports the hypothesis that exposure to a low, chronic level of mining waste can modify immune responses, potentially toward induction of autoimmune disease.

336 Depleted Uranium Inhibition of Metal-Induced Metallothionein Expression Profiles

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Metallothioneins (MTs) are proteins that have a critical role in heavy metal ho-

meostasis serving as biomarkers of metal exposure and toxicity. In humans, fifteen metallothionein genes encompass four groupings, MT1, MT2A, MT3, and MT4. Expression regulation of each MT gene is unique, where MT expression profiling facilitates a more complete understanding of responses to metal exposure. MT responses to depleted uranium, an environmental contaminant, have not been evaluated extensively. The rationale of this study was to provide a more compre-

hensive understanding of MT gene responses to depleted uranium in combination with other metals. In these studies, LNCaP, 22Rv1 and HEK293 cells in culture were used with qPCR to measure MT mRNA levels. In HEK293 cells, varied MT1 responses were observed after exposure with 1 μM Mn, Cu, Cd, and Hg for 24 h. Neither Mn nor Hg exposure altered MT1 expression, whereas Cu produced a 4.5-fold increase in MT1 gene expression and Cd produced a 42-fold increase, illustrating the diversity of MT1 responses were metal-dependent. Further, ura-

nium, as uranyl acetate (UAc), was investigated. LNCaP, 22Rv1, and HEK293 cells were treated with doses of UAc up to 100 μM for 48 h with no effects on cell viability. In LNCaP cells, UAc surprisingly decreased MT1 expression. In contrast, UAc did not change MT1, MT2A, or MT3 expression in 22Rv1 cells, whereas 1 μM Cd increased their expression 17-, 10-, and 9-fold, respectively. In HEK293 cells, UAc did not alter MT1, whereas 1 μM As, as arsenic trioxide, was found to increase MT1 levels 2.1-fold. Concurrent exposure with UAc and As produced MT1 mRNA at control levels. Intriguingly, whereas 1 μM Cd increased MT1 levels 28.5-fold in HEK293 cells, co-administration of UAc and Cd resulted in a reduced 13-fold MT1 increase, significantly decreased compared to Cd alone. Thus, uranium inhibited MT1 expression stimulated by metals that up-regulated MT1, introducing a novel paradigm of MT response to concurrent metal exposure.

337 Early Evidence of Organ Injury after Acute Oral Exposure to Nickel in Rats

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U.S. Service members are at risk of exposure to a variety of environmental health hazards throughout their normal duty activities; including deployments, training exercises, and homeland defense situations. Nickel (Ni) is a substantial industrial hazard due to its wide usage, environmental release, and persistence in biological systems. While the toxic effects of Ni have been widely studied, the exact mech-

danisms of toxicity remain unclear. Rats were exposed to three concentrations of

338 Characterization of Aqueous Formulations of Tetra- and Pentavalent Forms of Vanadium at Various pH to Aid in the Test Article Selection for Toxicology Studies

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Tetrapotent (V⁴⁺) and pentavalent (V⁵⁺) forms of vanadium were nominated to the National Toxicology Program for toxicity testing due to its presence in drinking water and dietary supplements. One tetrapotent (vanadyl sulfate) and two pentava-

lent (ortho- and metavanadate) compounds were selected to characterize the chemis-

try in drinking water formulations (125 to 2000 mg/L). The pH of orthovanadate in tap water increased from 9.5 to 11.5 with increasing concentration, whereas for metavanadate pH was between 7 and 8. Solutions of vanadyl sulfate in tap water were basic (pH ~3.4). Solutions were prepared for all compounds in tap water at an acidic (pH ~3.5), neutral (pH ~7) and basic (pH ~11) and analyzed by ultraviolet/ visible (UV/VIS) spectroscopy, mass spectrometry (MS) and ⁵¹V nuclear magnetic resonance (NMR) spectroscopy (V⁵⁺ forms only). Comparison of UV/VIS spectra of ortho- and metavanadate solutions showed no visible difference. Spectation for polyoxovanadates by NMR and MS showed that in both ortho- and metavanadate solutions the dominant species were monovanadate and decavanadate at pH 11 and pH 3.5, respectively. A mixture of mono-, di-, tri-, tetra-, and pentavantphosphate species were present in both ortho- and metavanadate solutions at pH 7, while decavanadate was observed only in orthovanadate. In vanadyl sulfate solutions at pH 3.5, com-

mon polyoxovanadate ions were not detected indicating absence of oxidation to V⁵⁺; however, polyoxovanadate ions were present at higher pH indicating oxidation of V⁴⁺ to V⁵⁺. In conclusion, these data support that both ortho and metavanadate form similar vanadate species at an acidic, neutral and basic pH and exist mainly in V⁵⁺ form. Vanadyl sulfate aqueous solutions (pH approximately 3.5) exist mainly in V⁴⁺ form. These findings will aid in the selection of test articles and pH for rodent drinking water toxicity studies.

339 Global Assessment of Copper and Zinc Concentrations in Free-Ranging Sperm Whales (Physeter macrocephalus)

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Copper (Cu) and zinc (Zn) are naturally occurring metals, essential for health and subject to homeostatic regulation mechanisms; however, can reach tissue concentra-

tions that pose a health risk due to increasing anthropogenic emissions. The sperm whale (Physeter macrocephalus) has a global distribution and high trophic level and is an indicator species of oceanic health. The aim was to establish Cu and Zn concentrations in free-ranging sperm whales. Skin biopsies (n = 342) were collected during the voyage of the Odyssey (2000-2005) from 17 regions worldwide and analyzed for Cu and Zn concentrations via inductively coupled plasma-mass spectrometry. Cu was detectable in all samples with a global mean of 15.2 μg/g wet weight (ww) ranging from 0.2 to 1.856 μg/g ww. Previous work in toothed whale skin reported Cu concentrations ranging from 24.8 to 1,050 μg/g ww. Previous work in toothed whale skin reported Zn concentrations ranging from 21.3 to 347 μg/g ww. Cu was positively correlated to Zn in sperm whale skin in this study and reported to be positively correlated

cell lines using DNA microarrays. Enriched pathways, networks, and biological functions were determined using a variety of bioinformatic tools. Differentially expressed genes enriched for biological processes such as energy metabolism, re-

sponse to oxidative stress, and Hif1α pathways were evident. This study provides further insight into the mechanism of Ni toxicity and provides a basis for bio-

marker identification. This research was supported in part by an appointment to the Postgraduate Research Participation Program at the USACEHR adminis-

tered by the ORISE through an interagency agreement between the US DOE and USACEHR. Research was conducted in compliance with the Animal Welfare Act, and all other Federal requirements. The views expressed are those of the authors and do not constitute endorsement by the U.S. Army.
in cetacean tissue in other studies. Overall, this work finds higher Cu and Zn concentrations in toothed whale skin compared to other studies and presents a baseline of Cu and Zn concentrations in an oceanic indicator species.

340 Health Risk Assessment of Heavy Metals for Population via Consumption of Seafoods from Ogoniland, Rivers State, Nigeria: A Case Study of Kaa, B-Dere and Bodo City


This study was designed to evaluate the potential human health risk through consumption of seafoods from contaminated sites in Kaa, B-Dere and Bodo City all in Ogoniland, Rivers State, Nigeria. The potential non-carcinogenic health risk assessment for consumers were investigated by estimating the daily intake (EDI) and target hazard quotients (THQs) for Cr, Cd, Zn, Pb, Mn and Fe while carcinogenic health effect from Cr, Cd and Pb in seafoods was also estimated by calculating the carcinogenic risk (CR). The EDI from seafood consumption were below the threshold values for Cr, Mn, Zn while they exceeded the threshold for Cd, Pb and Fe. The THQ for Cr, Zn and Mn were below 1, ranging from 0.002-0.011, 0.12-0.26 and 0.11-0.75 respectively. These indicated minimal health risk from these elements for consumers of seafoods from study sites. However, THQ values for Cd, Pb and Fe were greater than 1, suggesting health risks from long-term consumption of these seafoods. Furthermore, estimation of carcinogenic risk for Cr for Cd in all samples under study exceeded the accepted risk level of 10-4. On the other hand, C. pali (12E-3) collected from B-Dere together with L. falcipins and C. pali (5E-3 and 11E-3 respectively) collected from Bodo City also exceeded the accepted risk level of 10-4 for Cd. However, estimation of carcinogenic risk for Pb in samples from all the contaminated seafoods. Furthermore, the acceptable range of total hardness, 1.0 ± 0.6 for phosphate, 60.0 ± 130 for nitrate and 245 ± 140 for COD, 0.7 ± 0.3 and 0.5 ± 0.2 for Zn, 1.5 ± 1.0 and 0.5 ± 1.1 for Cu, 2.5 ± 0.3, 0.0 ± 10.03 for Cr and correspondingly THQ for Cd was 10 ± 0.1, 5.5 ± 1.2 respectively. Effluents from foods and beverages, basic metal, paint, conglomerate, and mixed/collective had lower levels of TDS, alkalinity, phosphate. Analysis of variance (p<0.05) indicates significant difference among the types of industry for pH, TDS, alkalinity, total hardness, nitrate, DO, Pb, Cd, Zn, Cu, Cr and Ni. The study further indicated that metals of varying concentration. The results showed textile and pharmaceutical industries having higher pollution load in pH, Biochemical Oxygen Demand and Chemical Oxygen Demand.

343 Heavy Metal Levels in Leachates from Two Electronic Waste Dumpsites in South West Nigeria


The levels of some selected heavy metals (Pb, Cu, Cd, Fe, Cr, and Hg) were investigated in raw leachates from electronic waste dumpsites found in two major electronic sales markets (Alaba and Agegunle) in Lagos and Osun states, Southwest Nigeria. Leachate samples collected from the dumpsites were digested and analyzed quarterly for heavy metals using Pye Unicam model 969 atomic absorption spectrophotometer. Mercury was extracted from samples using the cold vapour method and the metal levels determined by ICP AAS. Some heavy metals (Fe, Pb, Cr, Cd, Hg) from Alaba leachates were found to be higher than the levels in Agegunle except for that of Cu (94.50±81.96 mg/l) respectively. This could be attributed to the volume and composition of the waste stream. Generally higher levels above the USEPA and Nigerian Environmental Standard and Regulation Agency (NESREA) environmental health and safety standards were recorded for all the metals. The results of the analysis are as follows: Fe 188/220 mg/l, Cu 94.5/81.96 mg/l, Cr 0.05/0.07 mg/l, Pb 0.048/0.068 mg/l, Hg 0.04/0.055 mg/l. Cd 0.03/0.04 in both dumpsites. The environmental significance of the toxic heavy metal levels from electronic waste dumpsites in a developing country poses a severe risk to public health particularly given the use of this water for drinking and bathing.

341 Metallothionein Isoform 3 Expression in Human Skin, Related Cancers, and Human Skin-Derived Cell Cultures

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Human skin is a well known target site of inorganic arsenic with effects ranging from hyperkeratosis to dermal malignancies. The current study characterizes the expression of a protein known to bind inorganic arsenic, metallothionein 3 (MT-3). Expression of this protein was assessed immunohistochemically with a specific MT-3 antibody on human formalin-fixed, paraffin-embedded biopsy specimens in normal skin, squamous cell carcinoma (SCC), basal cell carcinoma (BCC) and melanoma. Assessment in normal skin using nine normal specimens showed moderate to intense MT-3 staining in epidermal keratinocytes with staining extending into the basal cells and moderate to intense staining in melanocytes of nevi. MT-3 immunoreactivity was shown to be moderate to intense in 12 of 13 of SCC, low to moderate in 8 of 10 BCC, and moderate to intense staining in melanocytes of nevi. MT-3 expression in cell culture models (normal human epidermal keratinocytes, normal human melanocytes, and HaCaT cells) showed only trace expression of MT-3, while exposures to the histone deacetylase inhibitor, MS-275, partially restored expression levels. These results indicate that the epidermis of human skin and resulting malignancies express high level of MT-3 and potentially impact on the known association of arsenic exposure and the development of skin disorders and related cancers.

344 Toxic Effects of Water-Soluble Fraction of Nail Metal Dust in Rat

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Humans are exposed to heavy metals which are mainly discharged into the environment through industrial pollution. In this study, toxicity of water soluble fraction of nail metal dust (WSFN) was evaluated in rat. X-ray fluorescence analysis of the nail-metal dust was carried out. Twenty-five rats were randomly distributed into five groups. Group 1 was the control group administered distilled water only while groups 2, 3, 4 and 5 were exposed to 25, 50, 75 and 100 ppm of WSFN in drinking water for 4 weeks. The animals were sacrificed and the liver, kidney and brain of rats were removed. The results revealed that nail metal dust contained Ti, Cr, Mn, Fe, Ni, Cu and Zn. The liver-body weight ratio of rats administered 75 and 100 ppm of WSFN were increased significantly (p<0.05) compared to controls. There was a significant decrease (p<0.05) in the white blood cell count, red blood cell count, haemoglobin concentration, packed cell volume and mean corpuscular hemoglobin concentration but a significant increase (p<0.05) in platelet count in the rats at 50, 75 and 100 ppm of WSFN. The same rats also exhibited a significant increase (p<0.05) in their serum total cholesterol, triglyceride, LDL-cholesterol and VLDL concentrations with a significant reduction (p<0.05) in serum HDL-cholesterol concentration compared to controls. WSFN also caused a significant reduction (p<0.05) in liver and kidney ALP activities but a significant increase (p<0.05) in serum ALP activity at doses higher than 25ppm compared to control. A significant decrease (p<0.05) in the tissues CAT and SOD activities were observed in all treated groups. A significant increase (p<0.05) MDA values in the tissues of the WSFN-treated rats at higher doses was observed. Results of this study suggest that water soluble fraction of nail metal dust at high doses may predispose subjects to hepatoxicity, nephrotoxicity and neurotoxicity over time.
Recent animal studies and human evidence indicate that metals in the body are not inert and have the potential to cause both local and systemic health effects. As a result, the Department of Veterans Affairs (VA) established a medical surveillance program for injured Veterans who have embedded fragments. A total of 579 Veterans have submitted an exposure questionnaire and 24-hour urine sample as part of this effort. The majority (82.7%) of these Veterans sustained a blast or explosion injury, 7.4% a bullet injury, and 9.8% sustained both types of injuries. Concentrations of 14 metals (Al, As, Cd, Cr, Co, Cu, Fe, Mn, Mo, Ni, Pb, U, W and Zn), frequently found in analyzed fragments and/or of toxicological concern, were measured in urine samples using high-resolution ICP-MS. More than half of the Veterans had no elevated metal concentrations in their urine. The 3 most common metal elevations observed were Zn (12.3%), W (11.6%) and Co (9.2%). Veterans with a bullet only injury were more likely to have an elevated Pb concentration compared to those who report only a blast or explosion injury (p=0.04). Given the use of Pb in bullets, this finding suggests that elevated urine metal concentrations can be related to mobilization of materials from retained fragments; however, other potential sources of metal exposure such as occupational and medical implants need to be considered. For example, the presence of a joint implant (hip, shoulder, or knee) was associated with having an elevated Co concentration (p=0.04). Other known exposure sources were not significantly associated with metal concentrations, suggesting the fragment as a likely source. These data provide evidence that over time metals released from fragments can enter the systemic circulation, thus warranting long-term biomonitoring, surveillance and medical management of Veterans with known embedded fragments. Supported by the VA and approved by the VA's VAMC's Office of Research & Development and UMD's School of Medicine IRB.

Rationale: Exposure to ambient metals has been associated with respiratory outcomes. However, the effect of airborne manganese (Mn) and nickel (Ni) on respiratory health remains understudied. We hypothesize that exposure to ambient levels of Ni and Mn will be associated with increased respiratory symptoms. Methods: As part of a NIH study in the industrial province of Brescia, Italy, adolescents aged 11-14 years were recruited. Participants were enrolled from 3 different communities with varying Mn levels: Bagnolo Mella which has an active ferroalloy plant, Valcamonica, which had three ferroalloy plants operating for a century until 2001 and Garda Lake which has no history of ferroalloy plant activity. Air Ni and Mn levels are higher than expected in individuals living in Butte drinking from well water. ICP-MS analysis of metals in the blood samples are on-going as are the 96-well gene plates. These preliminary data illustrate the need for additional study and data collection of samples from individuals living downwind of an active open-pit copper mine.

Background: Despite its safety in humans, the release of amalgam into the publically-owned wastewater system (POWS) is an environmental concern due to the potential for chemical and biological transformation. To reduce the burden of Hg release into POWS, chairside amalgam separators (CAS) have been designed to capture amalgam waste prior to entry into POWS.

Objective: To determine the longevity of DD2011 chairside amalgam separators (DD2011-CAS) and characterize the contents and acute toxicity of filtered effluents. Materials/Methods: DD2011-CAS were installed on 10 dental chairs in a high volume dental treatment facility. Effluents were collected downstream of DD2011-CAS after 30, 45, 60, 75, and 90 working days. Hg levels in acid digested effluents were measured using cold-vapor atomic absorption spectrometry. Ag, Cu, and Zn levels were assessed by flame atomic absorption spectrometry. Acute toxicity of effluents was tested using the Microtox assay (n=3). Linear regression analyses were performed to assess metal content and acute toxicity of effluents. Results: Extractable Hg, Ag, Cu and Zn fluctuated between collections and did not accum...
Cobalt use is increasing particularly due to its use as one of the primary metals in cobalt-chromium-molybdenum (CoCrMo) metal-on-metal prosthetics. CoCrMo is a high strength, wear resistant alloy with reduced risk for prosthetic loosening and device fracture. More than 500,000 people receive hip implants each year in the United States which puts them at potential risk for exposure to metal ions and particles released by the prosthetic implants. Data show cobalt ions released from prosthetics reach the bloodstream and accumulate in the bladder. As patients with failed hip implants show increased urinary and blood cobalt levels no studies have considered the effects of cobalt on human urothelial cells. Accordingly, we investigated the cytotoxic and genotoxic effects of particulate and soluble cobalt ions in urothelial cells. Exposure to both particulate and soluble cobalt resulted in a concentration-dependent increase in cytotoxicity, genotoxicity, and intracellular cobalt ions. Based on intracellular cobalt ion levels, we found, when compared to particulate cobalt, soluble cobalt was more cytotoxic, but induced similar levels of genotoxicity. Interestingly, soluble cobalt induced cell cycle arrest indicated by the lack of metaphases at lower intracellular cobalt ion concentrations which was not observed after particulate cobalt treatment. These data indicate that cobalt compounds are cytotoxic and genotoxic to human urothelial cells and solubility may play a key role in cobalt-induced toxicity. This work was supported by AR0 Grant #W31-19-P-0002 (J.P.W.), and the Maine Center for Toxicology and Environmental Health.

Iron deficiency is the most common nutritional deficiency in the world, with an estimated 4-5 billion affected persons, which can lead to debilitating fatigue, altered immune function, decreased work capacity and anaemia. Similarly, iron overload is a significant health concern. Iron overload targets the liver, heart, and pancreas, and can lead to multiple complications including liver disease, cardiomyopathy, and diabetes mellitus. The great diversity in genetic disorders of iron metabolism in modern man and other vertebrates suggest that these loci contribute to the susceptibility of iron deficiency and severity of iron overload. Several individual studies have shown genetic differences in iron homeostasis between inbred strains of mice. We hypothesize that this wide genetic variation underlies the differences seen in iron metabolism between inbred mice. Here we show variation in tissue iron levels from six inbred mouse strains and selected RI strains of these mice (male and female), fed low (12ppm), high (251/404ppm), and sufficient (35ppm) iron diets. Variation in elemental tissue content, hematological markers of iron status, as well as body weight and obesity markers are seen in this subset of the hybrid mouse diversity panel. This study highlights the population diversity in the ability to handle iron stress (deficiency or overload), and indicates the genetic variation in this population plays an important role.

Tellurium (Te) is a metalloid, with no known physiologic role in humans. Increasing use of Te compounds in optical blue ray discs and photographic materials suggests that environmental exposure will increase in the future. Neurotoxicity of Te compounds has been documented, particularly in animal studies however, little has been reported regarding the gastrointestinal toxicity of Te, despite the fact that ingestion represents a major exposure route. Occupational exposure may occur through food and air, and water will likely to increase. Previous studies in our laboratory demonstrated that tellurium tetrachloride (TeCl4) causes necrosis and diphenyl thiocarbazide (DPDT) causes apoptosis via the intrinsic pathway (Vij and Hardej, 2012). The purpose of this study was to evaluate the potential of tellurium compounds TeCl4 and DPDT to induce oxidative stress by examining the protein level of glutathione peroxidase (GPx) and protein expression of Metallotrichonin (MT-3), as upregulation of the antioxidant genes such as GPx and MT-3 were noted in Real-time qPCR (RT-qPCR). The main biological role of GPx is to protect the organism from oxidative damage. The induction of GPx is one of the important events in cellular response to pro-oxidative insults. Metallotrichonin (MT) may provide protection against metal toxicity and oxidative damage. Its synthesis may increase by several folds during oxidative stress to protect the cells from cytotoxicity. Upon exposure to concentrations ranging from 62.5 mM to 1000 mM of TeCl4 and DPDT in HT-29 colon cancer cells, oxidative stress was confirmed by a significant increase in the GPx activity at the concentrations ranging from 62.5 mM to 1000 mM in HT-29 cells with DPDT and from 250 mM to 1000 mM with TeCl4 treatment when compared to the control group. Preliminary Western blot analysis also demonstrated a concentration dependent increase of MT-3 protein expression. It is concluded that DPDT and TeCl4 exposure increases the GPx activity in transformed cells.
replacements, ethylene-bis-tetra bromophthalimide (EBTP), tetrabromophosphinophenol A (TBPPA) on thyroid-mediated metamorphosis. Tier 1 EDSP amphibian meta- morphosis 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 or TBPPA from stage 51 to 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 histopathology. EBTBP or TBPPA exposure did not alter the rate of metamorphosis or normal histology of the thyroid gland. Plasma 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 plasma from DE-71 relative to controls, whereas no effect was observed in the EBTBP- or TBPPA-treated larvae. None of the flame retardants significantly altered thyroid gland T4 level, nor altered the expression of TRβ. Thus, although DE-71 slowed metamorphosis by altering peripheral mechanisms of thyroid hor- mone homeostasis, neither EBTBP nor TBPPA altered thyroid-mediated meta- morphosis in X. laevis at environmentally relevant concentrations.

355 Reducing Fish Use in Bioconcentration Studies for General Chemicals

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Fish bioconcentration studies assist in determining the potential for substances to bioaccumulate. The resulting bioconcentration factor (BCF) values are used as part of Pesticide, Bioaccumulation and Toxicity (PBT) and secondary poisoning assessments. Bioconcentration tests are time-consuming, expensive, and use large numbers of animals (≥108 per study). Alternative methods that replace, reduce or refine the use of fish for BCF testing would therefore be of value in improving efficiency, reducing costs and supporting animal welfare considerations. Fish bioconcentration test guidelines generally require that BCFs are determined at two exposure concentrations. However, recent revisions to the OECD test guideline for bioconcentration testing (TG 305) provide the option to use only one exposure concentration, when justification is provided, although two concentrations may still be required for some regulatory purposes. This justification has been demonstrated for plant protection product active ingredients. To determine whether this justification has a broader validity, the BCF values determined following two exposure concentrations were compared for 236 general chemicals. The results demonstrate that BCF values do not significantly differ between the two test con- centrations. This relationship is particularly strong for BCFs ≥1000 L/kg, which is useful since only chemicals with BCFs >2000 L/kg may require regulatory action. This analysis therefore provides a data-driven rationale for using the one test con- centration approach for general chemical substances and thus could contribute to a substantial reduction in the use of fish for BCF assessment of general chemicals.

356 Copper Pyrithione Induces Abnormal Muscle and Notochord Architecture in Developing Zebrafish Embryos

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Copper pyrithione (CuPT), an active component in antifouling biocides predomi- nantly found in marine paints, has been shown to cause anomalies during early de- velopment of the mummichog, Fundulus heteroclitus. Since the strict regulatory use of organotins as antifouling agents has been imposed, a frequent substitute within the Japanese market has been the use of metal pyrithiones, principally zinc and copper. Zebrafish, Danio rerio, embryos were exposed within the first hour after fer- tilization to 12 and 64 μg/L of CuPT for 24 hours. Morphological abnormalities of the notochord and muscle architecture were observed at 96 hours post fertilization (hpf). The distortions of the notochord began in the tail at the lower concentra- tions and proceeded rostrally as the dose increased. Animals exposed to 64 μg/L of CuPT resulted in a severe twisting of the notochord and a curved body axis. Edema was observed in the cardiac and yolk sac regions in embryos exposed to 12 μg/L of CuPT. Microscopy showed edema within and between the myotomes resulting in disorganization of muscle fibers, disruption and distortion of the transverse myoseptum and vacuolization of the myocyte. Acidine orange staining resulted in an increase in apoptosis particularly in the brain, heart and tail regions of both treated groups. Total antioxidant capacity was significantly decreased in embryos exposed to 12 and 64 μg/L of CuPT. However, lipid peroxidation products were only significantly increased in animals exposed to 64 μg/L of CuPT. These results demonstrate that apoptosis plays a role in toxicity and that oxidative stress may also play a role in this injury. The abnormalities and deformities observed in fish larvae would greatly decrease survival in a polluted aqua-system and question the use of this product as an antifouling agent.

357 Tributyltin Chloride Induces Penis and Vas Deferens Development in Females of the Purple Snail (Plicopurpura panusa)

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Tributyltin (TBT) and its derivatives are widely used as antifouling paints, resulting in their being released into the marine environment. Aquatic invertebrates, par- ticularly marine gastropods, are extremely sensitive to TBT and undergo changes in the imposition of male secondary sex characteristics in response to exposure. This study aimed to evaluate the development of imposex and the expression of the retinoid X receptor (RXR) in tissues of Plicopurpurapanae(males and females) exposed to tributyltin chloride (TBTCl). The histological results showed a penis-like structure in imposed females and a undeveloped vas deferens that lacked circular muscular layers. TBTCl treatment increased the messenger RNA (mRNA) of RXR in females with imposex. The highest level of mRNA RXR was found in the digestive gland and penis-forming area in females under in vivo exposure com- pared with control females. These results indicate that TBTCl modulates mRNA levels of RXR in females. mRNAXXR in imposex females and females exposed to TBTCl only was similar to that of males, indicating that RXR might contribute to the development of imposex. To our knowledge, this study is the first to show that TBTCl induces imposex and biphallia in this snail species, and that this effect is accompanied by an increase in RXR expression.

358 Chronic Fish Toxicity Data for Identification and Risk Assessment of Endocrine Disruptors

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Publicly available chronic fish toxicity data were examined to determine their util- ity in both identification and risk assessment of endocrine disruptors (EDs). We focused on the following regulatory laboratory fish tests: early life stage test (ELS), fish sexual development test (FSDT), fish short term reproduction assays (FSTR), fish partial-life cycle test (FPLT), fish full-life cycle test (FLLT) and fish multi-generation test (FMGT). Test species were fathead minnow (Pimephales promelas), medaka (Oryzias latipes) and zebrafish (Danio rerio). Vitellogenin levels, secondary sex characteristics, and sex ratio from 143 studies were shown to be indicative for chemicals interfering with EAS (estrogen, androgen and steroidogenesis) path- ways. These biomarkers, however, can also be modulated by chemicals without directly targeting the EAS pathways, suggesting both in vitro and in vivo tests are needed to increase evidence for the identification of EDs in regulatory frameworks. Comparison of LOECs (lowest observed effect concentration) of 21 FMGT studies showed that biomarker data for the F2 generation may be redundant because no significant differences were observed between the F1 and F2. In contrast, effects on fecundity and fertility have only been observed in the F2 for estrone in medaka. The preliminary conclusion is that the F2 may be needed for risk assessment of some EDs. We have collected more than 200 studies on ELS, FSDT, FSTR, FPLT, FLLT and FMGT. By analysing LOEC values for various endpoints in these stud- ies, we will evaluate the sensitivity and the utility of these fish chronic tests for identification and risk assessment purposes. By integrating our overall results we will propose a fish testing strategy for EDs which is important for minimizing ver- tebrate testing and economic costs.

359 Effects of Oxadiazon on Nutrient Uitilization and Growth of Catfish (Clarias gariepinus)

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The effects of herbicides and its bioaccumulation in ecosystem and its subsequent propagation through trophic chain cannot be over-emphasised. Therefore, there is the need to carry out the study on the effects of Oxadiazon, a herbicide used in rice fields, on the growth and nutrient utilization of the fish, Clarias gariepinus (Cg) bearing in mind that the practice of polyculture is now being encouraged in Nigeria and the fish is the most cultured due to its hardy nature. Three hundred Cg with mean weight 262.17±72.80g were acclimatized for 2 weeks. Experimental fish were fed twice daily at 5% body weight for 96 hours. Fish were then kept 24 hours to the commencement of the bioassay and during the exposure period which lasted for 96 hours. Range finding and acute toxicity test (96hLC50) were done according to standard methods. The LC50 was determined by Probit analysis method. Test fish were
subjected to the sub-lethal concentration of 1/2 and 1/5 of the LC50 of Osadiazon and the concentration was continuously renewed every two days for 6weeks. Weight gain was determined weekly. Total weight gain, %body weight gain and specific growth rate was best in Cg juvenile exposed to 0.008ml/l concentration. The mean feed intake exposed to 0.008ml/l and 0.02ml/l concentrations were significantly lower than the control which had no exposure. The feed conversion ratio of 0.02ml/l concentration was significantly lower than the control and 0.008ml/l concentration. The protein efficiency ratio of control, 0.008ml/l and 0.02ml/l concentrations were not significantly different. The decrease observed in weight gain, percentage body weight gain and specific growth rate at 0.02ml/l might have been related to the high concentration of Osadiazon. To guarantee minimal negative side effects on rice field ecosystems, herbicides should be used with caution. Improved formulations is recommended to reduce off-target deposition and improve retention on target species.

360 Movement of Mercury and Selenium from Soil to Earthworms to Zebrafish
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Earthworms, Lumbricus terrestris, were used as a vector to investigate the movement of mercury (Hg) and selenium (Se) ions from contaminated soils into an aquatic food chain. Earthworms were grown in plastic terrariums each containing 1.0 kg soil under three experimental conditions: control, control treated with 100 mg mercuric ions, and control treated with 100 mg mercuric ions plus 50 mg selenium as selenate ions. After sixteen days, worms were harvested from each terrarium, euthanized, and dissected to remove gut content. Worm tissues were freeze-dried and ground into small particles. Wild type zebra fish, Danio rerio, were maintained in three 4-liter aquariums at a density of eleven fish per aquarium. In the control tank, fish were fed only freeze-dried earthworms from the control group. These worms had a mean Hg concentration of 0.41 +/- 0.06 micrograms Hg/g dried tissue, and a selenium concentration of 4.61 +/- 5.16 micrograms/g. Fish in the Hg treatment tank were fed only earthworms from the Hg soil treatment. These worms had a mean Hg concentration of 120.41 +/- 21.93 micrograms/g and a Se concentration of 2.20 +/- 1.90 micrograms/g. Fish in the Hg + Se tank were fed only earthworms from from the Hg + Se soil treatment. These worms had a mean Hg concentration of 365.79 +/- 33.90 micrograms/g and a mean Se concentration of 100.44 +/- 20.60 micrograms/g. Fish were maintained on the worm diet for twelve days and were then euthanized and freeze-dried. Fish tissues were analyzed for Hg and Se. Neither Hg nor Se was detected in fish tissues from the control tank. Fish from the Hg only tank had mean Hg levels of 26.63 +/- 4.38 micrograms/g and no detectable Se. Fish from the Hg + Se tank had a mean Hg level of 26.23 +/- 3.29 micrograms/g and a mean Se level of 4.78 +/- 0.60. The data indicate that the earthworms accumulated Hg and Se, and could serve as a means for these metals to enter an aquatic food chain.

361 Stress Hormone Response in Frogs after Pesticide Exposure

Amphibian populations are in decline around the globe due to a variety of factors ranging from habitat destruction to pesticide use. With respect to pesticides, amphibians are nontarget organisms that may experience dermal exposure from contaminated soil, water and vegetation. Homeowners and farmers most commonly purchase pesticide formulations that consist of an active ingredient as well as inactive ingredients. The chemical compounds that constitute the inactive ingredient fraction of the pesticide formulation vary widely and may themselves cause adverse effects among amphibians. The purpose of our study was to compare body burdens and stress response in frogs after exposure to atrazine (ATZ), one of the most commonly used pesticides, and one of its formulated products, St. Augustine. Southern leopard frog (Lithobates sphenocephala) juveniles were exposed to ATZ or St. Augustine for a period of eight hours by dermal contact with contaminated soil. A subset of frogs was then assessed for pesticide body burden using an amphibian specific extraction protocol followed by GC-MS analysis. The remaining frogs were evaluated for levels of corticosterone (CORT), a hormone released in response to stress, by using a non-lethal method for hormone collection from water. Corticosterone samples were extracted and analyzed using Caymen Chemical EIA kits. Preliminary results show that although body burdens were not significantly different between the ATZ and St. Augustine groups, CORT levels were higher among frogs exposed to the formulation, St. Augustine. These results indicate that inactive ingredients in formulated pesticide products have the potential to induce higher levels of stress among exposed amphibians, although they may not necessarily facilitate greater uptake and accumulation of the pesticide through dermal contact. Although the long-term effects of pesticide exposure are not known, short-term effects such as stress may alter reproductive output and survival among amphibians.

362 Identification of a Developmental Window of Susceptibility to Selenomethionine and Hypersaline Toxicity in the Japanese Medaka (Oryzias latipes)
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Activities that perturb naturally seleniferous soils, such as mining and agriculture, can release the selenium into waterways, which can have a profound effect on fish populations. Although selenium is an essential micronutrient, it has demonstrated embryotoxicity to fish. Selenomethionine (SeMet) is an organic form of selenium often passed to developing embryos through maternal transfer. As climate change worsens, the salinity of important spawning grounds in certain estuaries is increasing. Hypersalinity may not have direct lethal effects on adult fish, but osmotic stress may alter detoxification strategies of developing organisms. We have previously demonstrated the ability of salinity to potentiate SeMet toxicity in Japanese medaka by decreasing percent hatch and increasing deformities. In this study, we sought to identify a window of susceptibility for deformities and mortality caused by SeMet and hypersalinity in Japanese medaka embryos. Embryos were treated for 24hours periods with 0.5μM, 5μM and 50μM SeMet at six different developmental stages: 9: early gastrula, 17: early neurula, 25: early liver development, 29: onset of cartilage development, 34: skeletal mineralization, and 38: late organogenesis. Treatments were performed in both freshwater and 13ppth sulfate-based saltwater. Following treatments, survival, hatching, deformities and days to hatch were quantified. SeMet treatment at 5μM and 50μM resulted in significantly decreased survival and hatch. SeMet also resulted in increased deformities of the spine, face, heart and swim bladder. Furthermore, SeMet treatments in saltwater resulted in greater mortality and deformities than in freshwater. Stages 17 and 25 were identified as the most sensitive stages to SeMet embryo toxicity. These results provide insight into determining SeMet’s mode of action for developmental toxicity in order to inform risk assessment about selenium contamination in multi-stress ecosystems.

363 Prostacyclin Inhibits Precarcinogenic Edema in Developing Zebrafish Exposed to 2, 3, 7, 8-Tetrachlorodibenzo-p-dioxin

Knowledge of the mechanism of dioxin toxicity after activation of aryl hydrocarbon receptor (AHR) and its partner molecule, AHR nuclear translocator (ARNT) is still limited. Precarcinogenic edema, a typical of developmental toxicity by dioxin and many chemicals in zebrafish, is a model organism. Recently, we reported that cyclooxygenase type 2b (COX2b) - thromboxane (TBX) pathway is involved in edema by decreasing percent hatch and increasing deformities. In this study, we report the preventive effect of prostacyclin on TCDD-induced precarcinogenic edema. TCDD-induced precarcinogenic edema was markedly inhibited by beraprost, a prostacyclin receptor (IP) agonist. This preventive effect was reduced by CAY10441, an IP antagonist or IP knockdown with morpholino antisense oligo (MO). Knockdown of IP with higher concentration of MO or of prostacyclin synthase (PGTIS) caused edema by themselves. On the other hand, short exposure of U46619, a thromboxane receptor (TP) agonist, caused precarcinogenic edema by itself from 48 hpf. Edema by U46619 was inhibited by ICI-192,605, TP antagonist and beraprost. The same concentration of U46619 changed the shape of yolk sac in the case of earlier exposure from 36 hpf, affecting the measurement of precarcinogenic edema. PGTIS expression was increased from fertilization and reached a pleatue by 48 hpf and kept by at least 96 hpf. These results suggest the preventive effect of prostacyclin-IP pathway in TCDD-induced precarcinogenic edema in developing zebrafish.
Disposal of industrial effluent is one of the major ecological challenges. The presence of toxic and persistent chemicals causes adverse effects on soil, water bodies, agriculture, flora and fauna. Therefore, environmental pollution has become a global problem and maintaining ecosystem health is a serious issue. The samples were collected from three different locations (near Sachin, Gujarat). The samples were assessed for toxicity in zebrafish embryos. Zebrafish (Danio rerio) embryos are widely used as an experimental model for testing of industrial effluent. The study was performed in semi-static conditions where media was renewed at 24 h intervals. Fertilized 1 hour-post-fertilization (hpf) zebrafish embryos were exposed to range of dilutions (Sample A: 0.78 - 12.5%, Sample B: 10.66 - 100% and Sample C: 0.72 - 6.0%) in 24 well culture plates along with controls. Different morphological changes were observed including coagulation of embryos, non-detachment of tail, lower frequency of heartbeat, curved body, pericardial oedema and yolksac oedema as compared to control. The acute toxicity (LC50) of effluents on zebrafish embryo for 96 h were 3.44%, 50.64% and 2.60% for Sample A, B and C, respectively. The acute toxicity (LC50) of effluents on zebrafish embryo as compared to control. The acute toxicity (LC50) of effluents on zebrafish embryo for 96 h were 3.44%, 50.64% and 2.60% for Sample A, B and C, respectively. Based on the result of the present study, it can be concluded that industrial effluents have shown adverse effects to zebrafish embryos even at very high dilutions. Exposure of industrial effluent to zebrafish embryo shows that it is a highly sensitive organism to the pollutants present in industrial effluent.
sporatory capacity, including both state 3 and maximally uncoupled respiration, relative to fish sampled from the minimally-contaminated reference site. Similarly, mitochondria from sculpin collected at one of the impacted sites exhibited a decline in ETS respiratory capacity, including significant reductions in state 3 (32%) and maximally uncoupled respiration (25%), relative to reference site animals. Laboratory experiments of mitochondrial function using the Seahorse XF24 Extracellular Flux Analyzer and a salmonid cell line dose with CEC were used to validate our field results. These results support the use of analyzing mitochondrial function as a novel biomarker of sublethal effects of CEC exposures in fish. Supported by WA Department of Ecology Grant G1389, NIEHS Superfund (ES 04696), WA Sea Grant (ROCEH-5), and NIH R2C AG06606.

369 Occurrence of Microbiological Contaminants in a Watershed Impacted by a Wastewater Treatment Facility

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Exposure to contamination of environmental waters may cause various adverse health outcomes such as gastrointestinal and respiratory illnesses, skin, ear, eye, and neurological infections. Treated sewage is usually released to the environment upon disinfection by either chlorine or UV. One major public health concern is release of antibiotic resistant bacteria (ARB) after the treatment process. The ARBs in the environment can cause dramatic changes in the ecosystem, often leading to more adverse impacts on human health upon exposure. In the study, we examined the presence of fecal indicator bacteria (FIB); total coliform, fecal coliform and Escherichia coli throughout the wastewater treatment process. Samples were taken once a month from November 2013 to October 2014 from five different sampling points (influent, secondary treatment, UV disinfection, upstream river and downstream). Serial diluted water samples were analyzed for total coliform (TC) and E. coli by using a rapid US EPA approved method (Defined Substrate Technology by Colilert®). The results showed that E. coli in the influent was 2.51 X 106 CFU/100ml after secondary treatment, followed by 1.34 X 101 CFU/100ml after UV disinfection. Mean log removal of E. coli post-UV disinfection was 5.5±0.46. The lowest reduction was detected in January (4.8) and the greatest reduction was detected in April (6.1). Among all fecal indicator bacteria, TC had the highest concentrations. The relation between TC and E. coli was significant (R2=0.928, p=0.000). Our results showed that UV disinfection applied in this setting provided better pathogen removal than other WWTP that used chlorine disinfection in other studies. WWTP effluents created a dilution effect on river; indicating that the effluents had lower bacteria concentrations than the stream. Ongoing investigation by using molecular methods to detect the antibiotic resistance gene occurrence in this area is expected to help understand the impact of wastewater effluents on the environmental water quality.

370 Screening-Level Ecological Risk Assessment Approach for Quantifying Lead Ammunition Ingestion and Adverse Effects in Upland and Wetland Birds


The deleterious effects to wildlife from ingestion of lead (Pb) shot or bullet fragments have been well documented over many years and in numerous scientific studies. Despite this fact, many ecological risk assessments submitted for regulatory review in the State of California do not typically consider direct ingestion of Pb shot from soil as an exposure pathway. This is of particular concern when assessing former or abandoned sket and firing ranges. We propose a probabilistic model for examining the relationship between Pb particle contamination in soil and the incidental or purposeful (grit) ingestion by birds. Our approach is an extension of Peddick and LaKind (2000). It relies on measurements of the abundance of Pb particles in the preferred grit size range relative to the natural particles found in soil. We evaluated four bird species that select grit within the 0.5 to 2.8 mm ingestible range. The model quantifies how smaller birds with longer lifespans are more likely to consume more Pb particles (per body weight) than large birds with shorter lifespan. The number of particles ingested also increases with the density of Pb shot in soil and the implied particle ingestion rate of the species of interest. When establishing Pb shot cleanup levels, considerations should include: level of protectiveness, species and resources at risk, cost of cleanup, and the total amount of Pb particles that would remain in soil and degrade over time.

371 Impact of Illegal Mining Activities on Water Quality and Cost of Water Treatment in the Central Region of Ghana

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Water availability in Ghana is decreasing owing to variability in rainfall patterns, rapid growth of population and increased illegal mining activities (IMAs) along river and stream channels. In this study, the investigative team examined the impact of IMAs on the physico-chemical properties of surface water, the cost of treating the water polluted by IMAs, and the potentially associated toxicological and public health implications in Ghana. Approximately 3 years of data (October 2009 – December 2012) from two water treatment plants - Sekyere-Heman (SH, with IMA upstream) and Baafikrom (BK, without IMA upstream) - which abstract raw water from River Pra and River Ochi, respectively, were subjected to comparative analysis. Results indicated that the levels of each of the following parameters were significantly different between SH and BK: pH (p<0.028), color (p<0.000), turbidity (p<0.000), aluminum (p<0.000), iron (p<0.001), manganese (p<0.000) and sulfates (p<0.003). However, zinc (p=0.134) and nitrates (p=0.260) levels were not significantly different between the two treatment plants. Elemental levels of Fe, Al and Mg showed significantly high correlation with the cost of raw water treatment at SH compared to those of BK (p = 0.000). Additionally, the cost of treating the raw water at the SH plant was 34% higher compared to that of BK and these were statistically significant (p = 0.000). Also, color and turbidity increases correlated with cost of treatment at the SH plant which was 23 times higher compared to that of BK. These high correlations for most of the physico-chemical parameters at SH are indicative of the impact of IMAs on water quality and the cost of raw water treatment. Thus, IMAs occurring upstream of a water treatment plant negatively affect the quality of water and cost incurred in treating the water - making it wholesome for human consumption.

372 Temperature Effects on Lithobates pipiens Chronically Exposed to PCB and PBDE

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A major feature of climate change is global temperature increase, which can affect anthropogenic toxicant exposure and toxic effects in wildlife. To investigate how different temperatures affect the acute toxicity of toxicants. However, there is a paucity of information for ectothermic vertebrates on how temperature affects the chronic toxicity of environmental contaminants. In the following studies, northern leopard frogs (Lithobates pipiens) tadpoles were chronically exposed to either polybrominated diphenyl ethers (PBDE) or polychlorinated biphenyls (PCB) at two different temperatures (23°C and 28°C). Growth (snout-vent length and mass), development (days to reach metamorphic climax), sexual differentiation and immune function data was collected to assess the effects of temperature when L. pipiens are exposed to PBDE or PCB. For the PBDE study, the dietary treatments were: 0, 50, 500 ng/g PCB-70 and 0, 5, 10 ng/g PCB-126. For animals exposed to PCB-70, there was an interaction (P<0.05). For the PCB study, the dietary treatments were: 0, 50, 500 ng/g PCB-70 and 0, 5, 10 ng/g PCB-126. For animals exposed to PCB-70, there was a significant temperature and toxicant exposure effect on development (P<0.001). Toxicant exposure also had significant effects on growth (P<0.001) and development (P<0.001). In particular, temperature and toxicant exposure had a significant interaction (P<0.05). For the PCB study, the dietary treatments were: 0, 50, 500 ng/g PCB-70 and 0, 5, 10 ng/g PCB-126. For animals exposed to PCB-70, there was a significant temperature and toxicant exposure effect on development (P<0.001). Similarly, temperature and toxicant exposure effect on development (P<0.001) and toxicant exposure on the survival of tadpoles (P<0.001). There was also an interaction effect (P<0.05) between temperature and toxicant exposure in development. For animals exposed to PCB-126, there was a significant temperature and toxicant exposure effect on body length (P<0.01) and also exhibited an interaction between temperature and toxicant exposure (P<0.01). From our study, interaction between temperature and toxicant exposure effects are found in multiple cases, indicating that temperature should be carefully considered when we try to understand how the warming climate affects toxicology research.

373 Early-Life Stage Toxicities of Atlantic Killfish Exposed to Treated “Fracking” Water


In recent years, natural gas extraction from unconventional resources has proliferated throughout the United States. This has been made possible through the use of high-volume horizontal hydraulic fracturing (“fracking”). Unfortunately, the toxic effects of these fluids on human health and ecosystem function are largely
unknown. To investigate the effects of early-life-stage exposure to treated fracking water, Atlantic killifish (Fundulus heteroclitus) embryos were exposed to graded doses of fracking fluid effluent from a treatment plant on the Allegheny River, Pennsylvania. Fertilized embryos were exposed to five graded doses (undiluted, 10-1, 10-2, 10-3, 10-4) of fracking fluid or to water collected from a site upstream of the treatment plant outfall (n=35 embryos/treatment). Embryos were exposed to fresh doses of fracking water or upstream water (25mL), and mortality and hatching were recorded, every other day until hatching (14-28d). Exposures were carried out at ambient temperatures and in triplicate. As dose increased, survival to hatching decreased, and average time to hatch increased. Exposure to undiluted fracking fluid had a 100% mortality. Average embryonic mortality was 46%, 52%, 59%, 61%, and 50% for the upstream water, 10-4, 10-3, 10-2, and 10-1 dilutions, respectively. Average hatch time was 17.6, 19.95, 20.2, 20.9, and 20.4 days-post-fertilization for the upstream water, 10-4, 10-3, 10-2, and 10-1 dilutions, respectively. This study demonstrates that exposure to treated fracking fluid results in harmful developmental effects in Atlantic killifish embryos. When considering this fish model as a sentinel for human health, further study of the potential toxicity of fracking fluids may be warranted. Pilot study funding from NIEHS Center Grant ES02620.

374 Investigating the Influence of Environmental Factors on Pesticide Exposure in Amphibians

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375 The Effects of Fluoxetine on the Startle Response of Adult Zebrfish (Danio rerio)

K. Fulda and M. A. Connaughton. Biology, Washington College, Chestertown, MD. Sponsor: M. Reynolds. Pharmaceuticals and their metabolites contaminate waterways near effluent point sources. The most commonly prescribed antidepressants, selective serotonin re-uptake inhibitors (SSRIs) such as fluoxetine, have been found at concentrations up to 0.1 μg/L. Fluoxetine bioaccumulates in many fish creating potentially harmful side effects. Zebrfish (Danio rerio) were used to determine the effect of chronic exposure to fluoxetine on the overall anxiety and the startle response of adult zebrfish. Zebrfish were grouped according to exposure to 0.1 μg/L fluoxetine (1X maximum environmental concentration), 1.0 μg/L fluoxetine (10X), and 10.0 μg/L fluoxetine (100X) for 14 days. Anxiety levels were measured from video recordings of 10 minute trials in a standard novel dive tank; 5 minute pre- and 5 minute post-startle stimulus. The acoustic/vibrational stimulus was created by snapping a mouse trap attached to the side of the tank. The fish were tracked utilizing Ethovision (Noldus, ver. 9), a video tracking software, and their behavior was analyzed. During pre-startle and post-startle periods, fish treated with fluoxetine spent significantly more time in the top half of the dive tank and transitioned to the top of the tank with significantly greater frequency. These effects were dose dependent, increasing at higher dosages. A similar but non-significant trend was seen in latency to the top of the tank. There were no significant differences in total distance traveled, mean velocity or total time spent in erratic (rapid velocity) swimming between groups. Increased swimming velocity during the period 10 sec after the startle stimulus (compare to the 10 sec pre-stimulus) was noted in the control groups. This increase was not seen in the fluoxetine treated fish, suggesting that fluoxetine decreased the th10, erratic swimming exhibited in response to the startle stimulus. A decrease in responsiveness to startle stimuli or other danger cues by fish exposed to fluoxetine in the field might lead to greater likelihood of predation.

376 Can Coexposure with Ellagic Acid Mitigate the Adverse Effects of Aflatoxin B1 on the Visual System of Developing Zebrafish Larvae

J. Rogers and M. A. Connaughton. Biology, Washington College, Chestertown, MD. Sponsor: M. Reynolds. Aflatoxin B1 (AFB1) is produced by the mold Aspergillus flavus which grows on foodstuffs after harvest. It is a powerful mutagen and carcinogen that produces reactive oxygen species (ROS) and can enter the human body through ingestion. Ellagic acid (EA), a natural antioxidant found in foods such as walnuts and raspberries, might mitigate the harmful effects of AFB1. Previous studies have found that embryonic zebrfish, when exposed to AFB1, express disrupted eye development and decreased visual ability. This study investigated the possible mitigating effects of EA on AFB1-induced decreases in visual acuity. Zebrfish visual ability was examined behaviorally by the phototaxis assay and immunohistochemistry to determine the expression and function of RyR related pathways in NBH killifish relative to killifish. Atlantic killifish (Fundulus heteroclitus) thrive in heavily polluted locations including New Bedford Harbor (NBH), MA, contaminated with polychlorinated biphenyls (PCBs). Non-dioxin like PCBs (NDL PCBs), predominiate in NBH yet to date studies regarding the mechanism of PCB tolerance have focused on dioxin-like PCBs that target the arylhydrocarbon receptor (AhR). NDL PCBs do not act toward the AhR but rather enhance the activity of the ryanodine receptor (OKR); the tracking of a rotating striped drum by the eyes of larvae. Larvae were co-exposed to AFB1 (0.14 μg/mL) and low (1.7 μg/mL), medium (3.4 μg/mL), or high (6.8 μg/mL) dosages of EA for two 48-hour periods overlapping with development in the retina: 0-48 or 24-72 hours post fertilization (hpf). Larval OKR responses were quantified during 1 minute trials on day 5 post fertilization. Zebrfish exposed to medium and high dosages of EA had significantly higher OKR scores than zebrfish solely exposed to AFB1. These effects were greater during the 24-72 hpf exposure than the 0-48 hpf exposure suggesting that cell types and synapse connections that occur later in eye development might be more sensitive to both the effects of AFB1 and the mitigating effects of EA. It is hoped that this research could be applied to mitigating the effects of AFB1 on exposed human embryos through dietary intake of EA.
Persistent organochlorine contaminants in sediments are bioavailable to aquatic organisms and prone to bioaccumulate at higher trophic levels. Muck farms of the north shore of Lake Apopka, FL are historically contaminated with legacy organochlorine pesticides (OCPs) from heavy agricultural application from 1940-1970. Sediments and soils in the muck farms have high levels of OCPs (e.g., 252 ± 164 and 1720 ± 383 ng/g dry wt for dieldrin and p,p’-DDE, respectively, among other OCPs) which are known to impair the nervous system and reproductive processes in fish in the wild. Although concentrations for emerging contaminants (e.g., modern insecticides and pharmaceutical and personal care products) are below detection limits in the sediment of the north shore area, these emerging and legacy contaminants together are endocrine disruptors. In this study, we performed a fish feeding study to examine the molecular events produced by feeding fish on contaminated food. *Lumbriculus variegatus* (blackworm) were exposed to water spiked with 5 organochlorine chemicals (triclofosan, triclocarban, fipronil, dieldrin, and p,p’-DDE) at a nominal concentration of 10 μg/L for 7 days. These spiked worms were then fed daily to *Pimephales promelas* (fathead minnow) up to 28 days. Fish liver was collected, flash-frozen, and stored at -80°C prior to RNA extraction while carcass was preserved for chemical analysis. Microarray was performed to identify genes altered in the male liver. Body burdens of chemicals in worms and fish were analyzed on LC/MS/MS or GC/MS. Preliminary data show that fish quickly bioaccumulated chemicals within 48 days of feeding. One-way ANOVA and principal component analysis (PCA) of microarray data showed specific changes in gene expression for hsp-3, hsp-6, hsp-16.2, hsp-70, sod-1, sod-4, gpx-4, gpx-6, mtl-2, and the whole cell comet assay that was also accompanied by the early increase (30 min after exposure) of 8OHdG detected by LC/MS/MS, and was partially suppressed by either of peroxisome proliferator activated receptor alpha (PPAR alpha) antagonists, NK886 or GW96471 at 2 micro-moles per ml. A known PPAR alpha agonist, clofibrate induced DNA damage in the whole cell comet assay that was also accompanied by the early increase (30 min after exposure) of 8OHdG after 4 hrs. expression at 1mg/ml. The PFOA-induced DNA damage in the whole cell comet assay was not observed in the sample after a trypsin treatment. PFOA neither induced single strand breaks of DNA nor produced 8OHdG in the pure DNA extracted from TK6 cells. Clofibrate did not induce DNA damage in the acellular comet assay. These results suggest that PFOA may induce DNA damage in comet assay by PPAR alpha mediated oxidative stress, and interaction between PFOA and protein.

**Bioactivity of Legacy and Emerging Contaminants in Fish via Feeding Study**

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that decreased locomotion and fertility were located downstream oil refinery and urban-industrial areas, generating lethality values up to 22% and decreasing locomotion up to 87% when employing undiluted extracts. In short, worst discharges received by the Magdalena River, mostly from industrial activities, incorporate xenobiotics in sediments that produce toxic responses that can be followed using *C. elegans*, allowing the generation of the first toxicity map for this waterbody.

**Perfluorooctanoic Acid (PFOA)-Induced DNA Damage in Comet Assay by Two Distinct Mechanisms**

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Genotoxicity of Perfluorooctanoic acid (PFOA) is still controversial. In this study, we detected DNA damage in both the whole cell and the acellular comet assays using human lymphoblastoid TK6 cells after 2 hrs. expression of 0.25 to 1mg/ml PFOA. The PFOA-induced DNA damage in the whole cell comet assay was accompanied by an early increase (30 min after exposure) of 8OHdG detected by LC/MS/MS, and was partially suppressed by either of peroxisome proliferator activated receptor alpha (PPAR alpha) antagonists, NK886 or GW96471 at 2 micro-moles per ml. A known PPAR alpha agonist, clofibrate induced DNA damage in the whole cell comet assay that was also accompanied by the early increase (30 min after exposure) of 8OHdG after 4 hrs. expression at 1mg/ml. The PFOA-induced DNA damage detected in the acellular comet assay was not observed in the sample after a trypsin treatment. PFOA neither induced single strand breaks of DNA nor produced 8OHdG in the pure DNA extracted from TK6 cells. Clofibrate did not induce DNA damage in the acellular comet assay. These results suggest that PFOA may induce DNA damage in comet assay by PPAR alpha mediated oxidative stress, and interaction between PFOA and protein.

**Oxidative Stress Responses of Euglena agilis to Nanoparticles**

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There have been growing concerns about the potential risks posed by engineered nanomaterials due to their extensive application in industry, medicine and consumer products. *Euglena* is a fresh water unicellular flagellate which has both animals and plants characteristics. *Euglena* has been used as a test-organism to screen the toxicity of various chemicals including heavy metals. To evaluate the ecological toxic effects of nanomaterials, the acute toxicity test was conducted for 4 nanoparticles (Ag, TiO2, SiO2, ZnO) using a *Euglena agilis* (E. agilis). With the same organisms, gluthathione peroxidase (Gpx), gluthathione reductase (Grx) activities and formation of reactive oxygen species (ROS) were assessed. Treatment of nanoparticles for 4 days increased the cell motility and decreased the growth rate of *E. agilis* in a dose-dependent manner. Gpx activity and ROS formation were also altered by nanoparticles treatment. Results obtained from this study demonstrate that oxidative stress associated mechanisms might be related with the toxic effects of nanoparticles to *E. agilis*.

**Nematodes As Biosensors of Toxicity from Magdalena River Sediments**

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The Magdalena River is the main freshwater ecosystem of Colombia, with almost 50% of the population using environmental services from it. Unfortunately, the river receives contaminated effluents along its course. The aim of this work was to evaluate the toxicity profile of Magdalena river sediments using Caenorhabditis elegans as biological model. Sediment samples were taken at 20 different points along the river, freeze dried, and aqueous extracts obtained with ultrapure water. N2 worms in L4 larval stage were exposed to these extracts and lethality and locomotion assessed after 24 h, whereas fertility based on the brood size was evaluated after 72 h. GFP transgenic strains were employed to determine changes in gene expression for hsp-3, hsp-6, hsp-16.2, hsp-70, sod-1, sod-4, gpx-4, gpx-6, mtf-2, mtf-1, and gst-1, utilizing ultrapure water as vehicle control. There were significant differences between samples and control in terms of lethality, locomotion and fertility for the different stations. Significant alterations in gene expression occurred for mtf-2, sod-4, gst-1 and cyp34A9. Sediments from sites that caused lethality increased expression of stress response and metallothionein genes. Samples
duced white adipose tissue gene expression in mice. Moreover, PFOS effect on insulin-stimulated glucose uptake was also evaluated. PFOS increased hormone-induced 3T3-L1 fibroblast differentiation to adipocytes, induced Pparγ, CCAAT/ enhancer-binding protein α, Fatty acid-binding protein 4, and Lipoprotein lipase expression. Additionally, insulin-stimulated glucose uptake, enhanced Glut4 and Insulin receptor substrate-1 expression was increased after PFOS treatment. PFOS increased Nuclear factor E2-related factor 2, as well as, NAD(P)H:quinone oxidoreductase 1 and glutamate-cysteine ligase catalytic subunit expression along with ARE activity in mouse embryonic fibroblasts isolated from ARE-hPAP transgenic mice. These data suggests that PFOS increased ARE activity and induced Nrf2 signaling activation during the process of adipocyte differentiation. Finally, PFOS administration (0.1 mg/kg) induced adipogenic gene expression and Nrf2 signaling in mouse white adipose tissue. Overall, it is hypothesized that PFOS increase ARE binding activity, which resulted in induction of Nrf2 signaling to synergistically induce Pparγ expression and further induce adipogenic gene expression. This study points to the potential role of PFOS in perturbing adipogenesis and adipocyte biology.

384 Thyroid Hormone-Disrupting Potentials of Bisphenol A and Its Analogues: A Comparison Study

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Bisphenol A (BPA) has been used in various industrial and commercial applications such as food containers, toys, paper currences, and thermographic and pressure sensitive papers. Due to growing concerns on adverse health effects of BPA, the use of BPA analogues such as bisphenol AF (BP4F), bisphenol F (BPF), bisphenol S (BPS) etc. has increased. However, toxicity of the BPA analogues has not been well understood. The aim of this study is to screen thyroid hormone disrupting effect of BPA analogues using a rat pituitary cell line, GH3. Pituitary gland plays a crucial role in regulating the level of thyroid hormones. Six BPA analogues, i.e., BP4F, BPAP, BPF, BPS, and BPS, along with BPA were chosen as study chemicals. Following 48 h exposure, GH3 cell was measured for gene transcription related to thyroid hormone systems (TSHα, TSHβ, TRα, Dio1 and Dio2) using quantitative real-time PCR. T3 was used as a positive control. After the exposure to BPA, deiodinase 1 (Dio1) gene was up-regulated significantly. Up-regulation of Dio1 gene was also observed following exposure to BPAP, BPF, and BPS even at the lower molar concentrations. BPAP, BPF, BPS significantly up-regulated TSHβ gene at 0.01 μM. Similar to T3 which was used as a positive chemical, BPF down-regulated TSHβ, TRα, and TRβ genes. BPS also down-regulated TRα gene significantly. All the BPA analogues tested in this study showed significant transcriptional changes in genes related to thyroid hormone regulation in GH3 cell. The results of this study show several BPA analogues exert even greater thyroid disrupting potential than BPA in GH3 cell. Further studies are warranted to confirm this observation in vivo and to understand the consequences of thyroid disruption by BPA alternatives.

385 A Major Source of Dietary Exposure to Long-Chain Perfluorinated Carboxylic Acids

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Perfluorochemicals such as perfluoroalkyl carboxylic acids (PFCAs) are environmental contaminants of public health concern because of their persistence in the environment. This study investigates the levels of PFCAs (C8–C14) from Pacific cods to assess the impact of sea fish consumption for PFCA exposure in the Japanese population. Gas chromatography/mass spectrometry (GC/MS) was coupled with electron capture negative ionization (ECNI) for PFCAs analysis. Benzyl alcohol was used as a primary internal standard and deuterated PFCAs were used as deuterated internal standards. The levels of perfluorooctanoic acids (C8), perfluorononanoic acids (C9), perfluorodecanoic acids (C10), perfluoroundecanoic acids (C11), perfluorododecanoic acids (C12), perfluorotridecanoic acids (C13), and perfluorotetradecanoic acid (C14) were chosen as study chemicals. Levels of PFCAs were determined in 100 U/mL of serum and whether serum concentrations of OH-PBDEs in Japanese women (n=60) and whether the OH-PBDEs detected are of natural origin or metabolites of PBDEs. The serum concentration of 6-OH-BDE47 ranged from 56 to 525 mg/L and other PBDEs, which are of the other PBDEs, which may be de-methylated metabolites of natural MeO-PBDEs which are abundantly distributed in algae as well as fish from the Asia-Pacific. The accumulation mechanism is considered to be binding to serum proteins that may cause the adverse effects such as endocrine disruption.

386 Human Exposure to Hydroxylated PBDE Seaweeds As Dietary Source

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Of brominated flame retardants, polybrominated diphenyl ethers (PBDEs) are known to be transported to their hydroxylated metabolites (OH-PBDEs), some of which are retained in the blood of human. The present study investigated the serum concentrations of OH-PBDEs in Japanese women (n=60) and whether the OH-PBDEs detected are of natural origin or metabolites of PBDEs. The serum concentration of 6-OH-BDE47 ranged from 56 to 525 mg/L, about twenty times higher than that of BDE-47, whereas 2′-OH-BDE68 and 2,2′-dihydroxy-BB80 were also detected at similar levels to BDE-47. The serum profiles of these hydroxy-PBDEs in Japan were quite different from those in the other American and European countries. The concentrations of 6-OH-BDE47 were two orders of magnitude higher in Japan than those in US and Canada, while the me-thoxylated analogs (MeO-PBDEs) and PBDE levels were comparable. The current levels were not correlated to age (20-50 years old). The exposure source may be seafood, such as algae (Hijikia sp) which are frequently consumed by Japanese, but not from metabolites of anthropogenic PBDEs. Another possibility of the source may be de-methylated metabolites of natural MeO-PBDEs which are abundantly distributed in algae as well as fish from the Asia-Pacific. The accumulation mechanism is considered to be binding to serum proteins that may cause the adverse effects such as endocrine disruption.

387 The Induction of Autophagy by Rapamycin-Protected HepG2 Cells from the Toxicity of BDE-47

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PBDEs, a subgroup of brominated flame retardants, are persistent and bioaccumulative industrial chemicals that cause numerous problems including endocrine disruption and cancer. Autophagy is a physiological cellular process involved in the degradation and recycling of damaged components of cytosol and cell organelles, to maintain cellular homeostasis in adverse conditions such as nutrient deprivation, presence of pathogens and exposure to chemical compounds. The main objective of this work is to verify whether the autophagic process protects HepG2 cells from cytotoxicity caused by exposure to BDE-47 (0.1 – 25 μM). Briefly, HepG2 cells in 6-well plates were exposed to BDE-47 for periods of 1, 24 and 48 hours. The cytotoxic effect was investigated by testing of cell viability by the MTT assay and cell proliferation assessed with Sulforhodamine B. The results showed that over the 3 hours of exposure, there was no significant cytotoxicity induced by BDE-47. However, at 24 and 48 hours of exposure, cells treated with BDE-47 + rapamycin showed a reduction of protein content and cell viability at 25 μM. However, when the experiment was performed without the induction of the autophagic process, significant toxic effects were observed already from 5 μM at 24 and 48 hours of exposure to BDE-47. These results suggest that rapamycin induction of autophagy protected HepG2 cells from the toxic effects generated by BDE-47 at 5 and 10 μM. These results suggest that autophagy acted as a primary mechanism of cell protection, in order to reduce the toxic effects caused by BDE-47. Supported by: FAPESP - Proc. 2012/15220-3

388 Analysis of the Serum Perfluoroalkyl Carboxylic Acids (PFCAs) Levels for Repeated-Dose Toxicity Studies Conducted for Long-Chain PFCAs in Rats

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Perfluoralkyl carboxylic acids (PFCAs) are environmental persistent pollutants that have received attention due to their possible accumulation and health effects on wildlife and human. We conducted combined repeated dose toxicity studies with reproduction/developmental screening tests for four PFCAs including perfluorooctanoic acids (C12), perfluorotetradecanoic acid (C14), perfluorohexadeca-
Polychlorinated biphenyls (PCBs), industrial chemicals and persistent environmental pollutants, are found in rural and urban settings. Rodent studies have shown that exposure to PCB126, a dioxin-like PCB, causes a significant disruption of hepatic metal homeostasis and an increase in metallothionein (MT), an antioxidant and metal carrier. The current study investigates this phenomenon in a MT knockout mouse strain in order to assess the role of metallothionein in this disruption. Twenty-four 129S5 male mice were obtained from Jackson labs (12 wild type (WT) and 12 MT knockout (KO)) and placed on a purified diet (AIN-93G) for 3 weeks to achieve hepatic metal equilibrium. Mice were then given a single injection, IP, of either soy oil or 150 µmol/kg PCB126 in soy oil. The animals were sacrificed 2 weeks later and organs processed for analysis. The expression of MT, MITI, and classic AhR regulated genes were investigated by qRT-PCR and western blotting and hepatic metals status determined by inductively coupled plasma mass spectrometry (ICP-MS). Also intracellular metals status was investigated using energy dispersive spectroscopy transmission electron microscopy (EDS-TEM). Liver tissue was also analyzed histologically. Liver weights increased with PCB126 exposure, typically considered a hallmark of PCB126 exposure, however, no differences were seen between WT and KO. Metallothionein was seen to increase with PCB126 exposure as expected and was not seen in the KO. Hepatic metals status (Ca, Zn, Mn and Se) were investigated and their distribution follows a similar pattern of modulation as has been seen before, both within the cell (EDS-TEM) and within the organ (ICP-MS). Histologically, the liver shows signs of steatosis in PCB126 treated animals. Given its role in metal homeostasis, metallothionein has been shown to modulate metal status by its induction; this research suggests that MT may not be the sole cause of the metal disruption caused by PCB126 exposure. (P42 ES013661)
Non-Aroclor PCBs in Human Serum from Populations Living in Northwestern Indiana and Rural Iowa

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Polychlorinated biphenyls (PCBs) are persistent and bioaccumulating toxic pollutants which pose health risk to humans. They are known to be carcinogenic and have been identified as human endocrine disruptors and neurotoxicants. Since their manufacturing, Aroclor mixtures PCB congeners have been well-investigated in numerous matrices such as air, sediments, soils, biota and human. However, there are still many unknowns and uncertainty about the presence of non-Aroclor PCBs in the environment and in human. We suggest that non-Aroclor PCBs are actually emerging contaminants because they are so poorly understood yet clearly contributing to environmental exposure. We have examined human blood serum collected from participants living in East Chicago, Indiana and Columbus Junction, Iowa in 2010 and 2011 as part of Airborne Exposure Semi-Volatile Organic Pollutants (AESOP) Study. The serum samples were extracted and detected for 209 PCB and 64 OH-PCB congeners using GC/MS/MS. Surrogate standard injections in each sample, evaluation of sera standard reference materials, and background correction from method blanks were included as our quality control evaluation. Our results showed the presence of non-Aroclor PCB congeners in our study participants. To our knowledge, we have determined for the first time the prevalence of non-Aroclor in human serum. Non-Aroclor PCBs have found to dominate an average of 10% of sum PCBs in human serum. Adolescents have significantly lower levels of sum non-Aroclor PCBs than their mothers (p<0.05). Furthermore, we found significantly different in sum non-Aroclor PCBs between participants living in East Chicago, IN and those in Columbus Junction, IA (p<0.05). Our study also indicates the need for further studies on assessing the potential health effects and risk of non-Aroclor PCBs to the general population and children in particular.

Relative Effect Potencies of 2, 3, 7-Tribromodibenzo-p-dioxin and 1, 2, 3, 4, 7-Pentabromodibenzo-p-dioxin in Female Rat after a Single Oral Dose

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In the marine environment a number of polybrominated dibenzo-p-dioxins (PBDDs) are formed that can eventually end up in the human food chain via e.g. fish and shellfish. The PBDDs formed include a number of congeners for which there is very limited information available with respect to risks for human health. Currently, the use of similar interim TEFs for brominated and chlorinated contaminants is recommended for human risk assessment, pending more toxicity data concerning brominated congeners. The aim of this study was to determine effect potencies of two important brominated congeners which are formed in the Baltic sea, namely 2,3,7-tribromodibenzo-p-dioxin (237-TriBDD) and 1,2,3,4,7-pentabromodibenzo-p-dioxin (12347-PeBDD) relative to the reference compound 2,3,7,8-tetrabromo-dibenzo-p-dioxin (2378-TBDD) in female rats after a single oral dose. Three days after exposure, relative effect potencies (REPs) were calculated for hepatic ethoxyresorufin-O-deethylase (EROD) activity using the dose needed for a congener to reach 20% of the 2,3,7,8-TBDD response. Preliminary results show a REP of -0.00001 for 2,3,7-TriBDD. This value is relatively low compared to the range of REPs of 2,3,7-TriBDD from -0.0001 to 0.85 reported in literature. Due to difficulties in synthesizing 1,2,3,4,7-PeBDD, no REPs have yet been published for this congener. In this study, preliminary results show a REP of -0.0001 for 1,2,3,4,7-PeBDD. As a next step, systemic REPs based on plasma, adipose tissue and liver concentration will be determined, as metabolism may play an essential role in the eventual toxicity of these congeners. All together these data can help to better understand the toxicity of PBDDs and to determine their impact on (human) risk assessment.

Preparation and Evaluation of Fluorescent Moleularly Imprinted Polymers Capable of Detecting Polyaromatic Hydrocarbons

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Detection of Polyaromatic hydrocarbons has so far been a challenge because current methods used have not been so effective. The aims of this study was to evaluate if a prepared fluorescent MIP(Molecularly Imprinted Polymers) could be able to detect the presence of a standard Polyaromatic hydrocarbon(Pyrene) in a test solution by a change in the existing fluorescence of the MIP and also whether this translates to a true imprinting effect. The fluorescence emission was also characterized for a monomeric emission and also a possible excimer formation due to Pyrene- Pyrene interaction. This work also considers how the variations in the preparation of the fluorescent (MIP) affects its recognition properties. Fluorescent MIPs were prepared by the semi-covalent approach using a standard Polyaromatic hydrocarbon (Pyrene) as template, 1-pyrenylmethylmethacrylate as functional monomer, Ethylene glycol Dimethacrylate as the cross linker and toluene as the Porogen. The template (Pyrene) was then extracted before exposure to test solutions which contained a fluorescent template (Pyrene) in chloroform and toluene (3mg/ml) respectively. MIP polymers were then prepared with different strengths of functional monomers and templates Corresponding control polymers (NIP) that did not contain a template were also prepared and also exposed to similar test solutions to validate a true imprinting effect. MIP and NIP Polymers were then evaluated under fluorescence. At 395 Nm(maximum emission wavelength) a true imprinting effect was observed for all the molecularly imprinted polymer exposed to both test solutions containing the standard Polyaromatic hydrocarbon (Pyrene). The MIP(molecularly imprinted polymer) which contained 0.075mmol functional monomer and 0.025 mmol template had the highest imprinting effect for both test solutions. There was no visible Excimer formation for all prepared molecularly imprinted polymers. Fluorescent molecularly imprinted polymers successfully detected a standard Polyaromatic hydrocarbon (Pyrene).
In all parts of the world pesticides have been found in the aquatic ecosystem and scientific evidence has also shown that they can enter the food chain. Diazinon is an organophosphate pesticide, widely used in agriculture to control a wide variety of sucking and leaf eating insects and recently in fish culture to suppress some parasitic diseases; nevertheless, there is little study on its adverse effect on fish. In this study, seventy-two (72) each apparently healthy catfish comprising adult and juvenile of both sexes were used to set up triplicate experimental groups of those exposed to culture water alone (negative control group), fish exposed to pre-determined no-effect concentration (0.405ppm) of Diazinon (test group). The fish were exposed for 28 days, sacrificed and organs harvested on days 21 and 28 to determine the effect of long-term exposure. Histological changes observed in diazinon-exposed catfish were hyperplasia and fusion of the gill epithelium, hyperplasia of mucoid producing cells, and aggregation of melanin pigment in the skin. Histological lesions were also seen observed in other organs, including severe diffuse cellular swelling and fatty degeneration of the liver, interstitial congestion of the kidney, carbon deposit on the wall of the heart and multifocal haemorrhage. The water quality of the control was not significantly different from that of the test group throughout the experiment. The lesions detected in cells, tissue, or organs represent an integration of cumulative effects of physiological and biochemical stressors. The histological alterations observed in vital organ of fish shows that exposure to “no-effect” concentration of diazinon induced structural damage in fish organs and is likely to affect the functionality of the organs. For example, the adverse effect on the gill might disrupt its feeding and oxygen uptake.

Using Human-Induced Pluripotent Stem Cells (iPSC) in High-Content Screening (HCS) to Prioritize Chemicals for Developmental Neurotoxicity Testing

The developing brain is vulnerable to chemical-induced injury yet many chemicals remain inadequately tested for developmental neurotoxicity (DNT). In an effort to develop and characterize an in vitro model system for DNT screening, we exposed human iPSC-derived neurons to a diverse set of 80 chemicals (e.g., neurotoxicants, drugs, pesticides, flame retardants (FRs), polycyclic aromatic hydrocarbons (PAHs)) across a 6-point concentration range (~0.3 to 100 µM) in 384-well plates. Using HCS imaging, effects on neurite outgrowth parameters (total outgrowth, processes, branching) and cell viability were monitored after 72 h of exposure. Also, mitochondrial membrane potential (MMP) was evaluated at 1 h to assess the potential contribution of MMP to altered neurite outgrowth. The assay-specific noise threshold was calculated based on DMSC control variability and concentration-response profiles were evaluated using a Hill model to derive benchmark concentrations (BMC) point-of-departure values. Following assay validation with controls and test replicates, chemicals were ranked by toxicity and selectivity (i.e., effects on neurite outgrowth parameters independent of cytotoxicity). Neurite total outgrowth and branching were the most sensitive endpoints; 15 chemicals (19%) had an effect on neurite outgrowth independent of cytotoxicity. The pesticide rotenone was the most selective, while rotenone was the most selective, while1 FR (triphenyl phosphate) and 3 PAHs had an effect on neurite outgrowth independent of cytotoxicity. The pesticide rotenone was the most selective, while1 FR (triphenyl phosphate) and 3 PAHs had an effect on neurite outgrowth independent of cytotoxicity. The pesticide rotenone was the most selective, while1 FR (triphenyl phosphate) and 3 PAHs had an effect on neurite outgrowth independent of cytotoxicity. The pesticide rotenone was the most selective, while1 FR (triphenyl phosphate) and 3 PAHs had an effect on neurite outgrowth independent of cytotoxicity. The pesticide rotenone was the most selective, while1 FR (triphenyl phosphate) and 3 PAHs had an effect on neurite outgrowth independent of cytotoxicity. The pesticide rotenone was the most selective, while1 FR (triphenyl phosphate) and 3 PAHs had an effect on neurite outgrowth independent of cytotoxicity. The pesticide rotenone was the most selective, while1 FR (triphenyl phosphate) and 3 PAHs had an effect on neurite outgrowth independent of cytotoxicity. The pesticide rotenone was the most selective, while1 FR (triphenyl phosphate) and 3 PAHs had an effect on neurite outgrowth independent of cytotoxicity.
hematopoietic stem and progenitor cells (HSPCs) cultured in either methylcellu-
lose or collagen-based media differentiate into mature cells of different lineages. The CFU assay is time-consuming and not amenable to high-throughput formats, thus limiting the number of compounds that can be efficiently screened. We have developed a new 96-well liquid media assay for HSPC toxicity screening. CD34+ cells enriched from human cord blood and BM were cultured in the presence of varying concentrations of small molecule drugs from three classes with known marrow toxicity (Topotecan, Imetotecan, Sunlitinib, Imatinib, 5-Fluouracil (5-
FU), Cisplatin) in StemSpan22 SFEM medium supplemented with cytokines that selectively engage either early or megakaryocyte lineage cells. The cells were cultured for 7 and 10 days in the erythroid and megakaryocyte-specific cultures, respectively, and then enumerated and phenotyped by flow cytometry. The 96-well assay was reproducible, producing similar 50% inhibitory concentra-
tions (IC50) in repeated experiments with cells from the same and different donors (e.g. 5-FU IC50 range = 0.91 - 2.84 μM; 4 donors over 7 experiments). IC50 values for each drug were also similar to those observed in standard CFU assay. Most drugs showed similar toxicity for both lineages, except Sunlitinib, which was 10-
fold more toxic in erythroid than in megakaryocyte cultures (mean IC50 = 0.02 vs. 0.59 μM, n=3). This was also observed in CFU assays. These results demonstrate that the new 96-well liquid assay is predictive of results of standard CFU assays and is useful for toxicity screening of drugs during early development.

**402 An Ex Vivo Megakaryocyte Platform to Evaluate the Effects of Compounds on Different Stages of Platelet Development**
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Thrombocytopenia may be caused by drug effects at various stages of platelet de-
velopment, including toxicity to platelet progenitors in the bone marrow, alteration or arrest of lineage commitment, or inhibition of megakaryocyte maturation and formation of platelets. We developed an ex vivo platform that can assess drug ef-
fects at these specific developmental stages. Four distinct compound classes, some with known thrombocytopenic effects, were assessed: an antineoplastic (5-FU), a HDAC inhibitor (abexinostat), a thalidomide derivative (lenolidomide), and a PDE inhibitor (anagrelide). All 4 drugs were tested on both human and NHP cells over a broad concentration range, and evaluated for effects on megakaryo-
cytic progenitors (CFU-Mk), proliferation and differentiation of megakaryocytes (FACS analyses of CD41 and platelet formation) (platelet counts in 1% ammo-
niun oxalate). 5-FU significantly inhibited CFU-Mk, with an IC50 of 0.1 μg/mL. Anagrelide had less of an effect on this primitive cell population, with an IC50 value of 70 μM. Neither abexinostat (100 - 1 μM) nor lenolidomide (576 - 1 μM) affected CFU-Mk number. However, both abexinostat and lenolidomide inhibited the proliferative and differential potential of CD34+ cells towards megakaryocytes in a liquid culture system in both human and NHP models. Abexinostat decreased the early megakaryocyte maturation whereas lenolidomide appeared to have a greater effect on the late megakaryocytes. Platelet counts increased from 1 x 10^6 /mL to 20 x 10^6 /mL over the 14 days in control liquid cultures initiated with 10^6/mL thrombocytopenic effects. At these specific developmental stages, four distinct compound classes, some with known thrombocytopenic effects, were assessed: an antineoplastic (5-FU), an HDAC inhibitor (abexinostat), a thalidomide derivative (lenolidomide), and a PDE inhibitor (anagrelide). All 4 drugs were tested on both human and NHP cells over a broad concentration range, and evaluated for effects on megakaryo-
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**404 Cadmium Alters Gene Expression Patterns of Stem Cell Transcription Factors Essential for Embryonic Development and Differentiation in Mouse Embryonic Stem Cells**
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According to the fetal basis of adult disease hypothesis, prenatal exposure to en-
vironmental pollutants, such as cadmium (Cd), are responsible for the etiology of diverse adulthood diseases. Using undiffereniated pluripotent mouse embry-
onic stem (mES) cells as a model of early embryogenesis, we evaluated the effect of Cd on cell division and transcriptional processes essential for embryonic development and differ-
entiation. Previously we reported that exposure of mES cells to CdCl2 for 24 hours in the absence of LIF (leukemia inhibitory factor) resulted in inhibitory concentration 50% (IC50) and 25% (IC25) of 40 and 20 μM, respectively. We then examined the effect of Cd (minus LIF) on gene expression of key embryonic stem cell transcription factors using real-time PCR. Exposure to CdCl2 (40 and 20 μM) resulted in a statistically significant 2-fold or greater up-regulation of genes important in embryonic development and differentiation (Dlk1, Dkk2, Egr3, Ert1, Hexa7, Hexa9, Hexb1, Hexb3, Hexb5, Hexb8, Hexc10, Hxrc5, Hxrc7, Hxrc8, Paxc1, Pax5, Pax9, Sisx2, Tn5, and Tcf7). A statistically significant 2-fold or greater down-regulation was observed in genes including Lmx2b, Lmx2h, Stat1, and Tert. No significant changes were observed in gene expression patterns of pluripotency markers (Pou5f1, Nanog, and Spp3) except for a decrease in Sex2 expression (4.5-
fold) at 40 μM CdCl2. Using flow cytometry, DNA cell cycle analysis revealed that exposure of mES cells to CdCl2 (40 and 20 μM; minus LIF) caused an increased shift towards G2/M phases of the cell cycle (i.e. G2/M accumulation) in Cd-treated mES cells as compared to control gated subpopulations. The results suggest that Cd affects important downstream signaling processes that are essential for proper embryonic development.

**405 Cumulative Toxicity of Low-Dose Cadmium Exposure in Mouse Embryonic Stem Cells Is Characterized by Slowed Mitotic Progression**
D. O’Brien and F. A. Barile, St. John’s University, Queens, NY.

Mouse embryonic stem cells (mES cells) proliferate rapidly, having a cell cycle of 12-14 hours. Environmental exposures affecting cell division have the poten-
tial to disrupt normal development. Exposure to cadmium, a poorly mutagenic carcinogen, induces toxicity via epigenetic mechanisms. In order to elucidate the mechanism of low dose Cd in mES cells, we measured cell viability, cell cycle progression and mitotic index following 24-hr exposure to Cd, with and without a 24-hr recovery period. Based on the IC50 determinations from MTT, we con-
cluded experiments at 1/2 and 1/4 of the extrapolated concentration—thus, 20μM and 10μM. Following 20μM Cd exposure, cell cycle data reveal a significant ac-
cumulation in the G2/M phase with (+10.9%) and without recovery (+12.1%), as
compared to respective controls. At 10μM Cd, the % cells increased in the G0/
G1 (+1.53) and G2/M (+3.15) phases, but when allotted a 24-hr recovery period, the % cells in G0/G1 declines to control levels. We also observed a heightened accumulation in the G2/M phase (+6.01%). Additionally, this was substantiated by a dose-dependent decrease in the % cells in S-phase. Since propidium iodide is a 24-hr recovery period. Based on the IC50 determinations from MTT, we con-
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Inorganic arsenic (iAs) is an environmental toxicant commonly found in drinking water that has been linked to a variety of cancers, including prostate cancer. While the mechanism(s) underlying the carcinogenic potential of iAs are not fully understood, little work has been done to elucidate the role that iAs exposure has on the tumor microenvironment (TME). We hypothesize that exposure to biologically relevant levels of iAs can alter TME-mediated cell-cell signaling, creating a favorable environment for tumor progression. To demonstrate this, human adipose-derived mesenchymal stem/stromal cells (hASC), which have been shown to be an enriched population of the TME in prostate cancer patients, were exposed to 1, 10, and 75 ppb iAs (sodium arsenite) for one week in vitro. These doses reflect sub-EPA, EPA, and biologically relevant super-EPA levels of iAs. After exposure, mass spectrometry analysis revealed several proteins of interest that were statistically changed in both biological donors. Two proteins of interest, Heme Oxygenase-1 (HMOX1) and Thrombospondin-1 (THBS1) were altered in a dose dependent manner, HMOX1 and THBS1 protein levels were orthogonally validated by western blot, showing an average fold change of -1.2 (1 ppb), 4.3 (10 ppb), and 9.6 (75 ppb) for HMOX1 and -1.9 (1 ppb), -2.1 (10 ppb), and -4.4 (75 ppb) for THBS1. Ingenuity Pathway Analysis (IPA) was utilized to elucidate potential mechanisms of iAs-mediated TME alteration in hASC. IPA revealed a direct link between increased HMOX1 and the decrease in THBS1, which has been shown to be an activator of the TGF-β signaling pathway. Loss of TGF-β signaling induced by iAs may involve modulation of the HMOX1/THBS1 signaling axis and may play a vital role in enhancing tumor cell proliferation. These results demonstrate a novel mechanism behind the carcinogenic potential of iAs as a modulator of TME-epithelial interactions.
Confronting and Overcoming the Barriers to Sharing Toxicological Research Data for Risk Assessment in the 21st Century

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Research Triangle Park, NC, Emory, Atlanta, GA, NIH, Bethesda, MD, ACC, Washington, DC, BYU, Provo, UT, NLM, Bethesda, MD, ECU, Greenville, NC, and NIEHS, Research Triangle Park, NC.

The need for better data-sharing opportunities is a highlight of the NAS document "Toxicity Testing in the 21st Century: A Vision and a Strategy", which included recommendations to develop the data management infrastructure "to enable broad data-sharing across academic, government, industry, and NGO sectors and institutions." This applies equally to high-throughput assay results, in vivo animal studies, and human clinical and epidemiological studies. The need is critical in quantitative analysis, where raw data from individual subjects or groups provides greater power in an analysis than will summarized results. Substantial barriers block access to these toxicological research data. An effective strategy to identify and propose remedies to those barriers has yet to be formulated. The issues are multifaceted; the need to protect personally identifiable information versus protection of public health, intellectual property rights versus public access to data developed using public funds, publication of research findings by the originator of the data versus allowing more powerful analysis using data from multiple studies, and many others. The primary intent of this roundtable discussion is to begin a dialogue to define a proper balance between these competing needs and stakeholder perspectives, while at the same time enhance the science of toxicology, protect public health, and ensure scientific credibility. Panelists have been selected to represent one or more stakeholder groups. Open dialogue with the audience will be encouraged to include the perspectives of the larger SOToM community. The views expressed here are those of the authors and do not necessarily reflect the views or policies of the US Environmental Protection Agency.

Toxicological Application of Studies Funded by California Stem Cell Research and Cures Act (Prop 71)

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In 2004, Proposition 71 was passed to support stem cell research in California. The California Institute for Regenerative Medicine (CIRM) was created to allocate funds to establish stem cell research in the state. This session highlights research funded through this initiative that is translatable to toxicology. The introduction will provide a brief overview of the goals and implementation of the various California-based stem cell research initiatives funded through CIRM. The ability to create induced pluripotent cells from adult somatic cells has revolutionized cell biology. The first presentation will focus on the quality manufacturing aspects of iPSC and highlight their application in a number of California/CIRM-funded projects that are translatable to toxicological research. The second presentation will feature the function of the Cormill Institute for Medical Research. This institute was funded by CIRM to establish a centralized resource of well-characterized iPSCs. Tissue samples from 3,000 subjects enrolled through seven California-based research groups serve as starting material for deriving iPSCs. Samples are collected from healthy controls and patients with Alzheimer’s disease, autism spectrum disorders, liver, cardiovascular, eye, and respiratory diseases. The Cormill-CIRM Biobank, residing at the Buck Institute for Research on Aging, ensures exceptional storage and distribution of high-quality iPSCs, which can provide a useful model for toxicological testing of environmental and pharmaceutical agents. The last presentation will cover the development of stem cells in a toxicology study with emphasis on how live cell imaging in conjunction with video bioinformatics software tools can be used to assess the effects of environmental chemicals on cells that model stages of prenatal development.

Adaptive Leadership: Anticipating, Initiating, and Responding to Change

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The magnitude of change in organizations has grown tremendously over the past two decades. A hallmark of successful organizations and individuals is their ability to anticipate and respond to change or even initiate change to meet the demand of the moment. Our current workplace environment must address changes in organizational structure, economic factors, and increase in global competitiveness. As individuals, we encounter changes in family structures, personal expectations, career pathways, and trajectories to dynamically sense and respond with actions that are focused, fast, and flexible. A successful leader, team, or organization will evolve through purposeful strategies that influence and respond effectively to unpredictable and shifting demands and world events. This session will be composed of four presentations that will focus on the changes currently facing the industry, government, contract research organization, and academic sectors. Within each presentation, organizational changes, leadership challenges, and the impact on the individual will be addressed. Each speaker will emphasize their sector-specific changes and future adaptations to change. The speakers will thus provide practical advice and concrete examples on adaptive leadership that demonstrate a leader’s ability at all levels to effectively accomplish the initiatives every day.

Development of an In Vitro Washing Protocol in Reconstructed Human Epidermal Tissue to Evaluate the Efficacy of Skin Cleaning Methods

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The 3M Strategic Toxicology Laboratory (STL) is an internal corporate resource providing support to 3M businesses that emphasizes the use of in vitro methodology. The STL routinely uses EpiDermTM, a three dimensional skin model currently validated for classification of chemicals for dermal irritation in OECD 439 and dermal corrosion in OECD 431. In addition to the standard assays, the STL develops custom protocols to examine specific issues of interest to 3M businesses, including a method to examine the efficacy of skin washing agents for providing occupational health recommendations for workers. Investigations have focused on mixtures containing potentially corrosive chemicals formulated into industrial or professional use products that may be difficult to remove from the skin due to the mixture properties, during cases of inadvertent exposure. Alteration of several aspects of the standard irritation/corrosion protocols was necessary, including modification of exposure/post-exposure incubation times and investigation of different mechanical scrubbing techniques. Irritation/corrosion was assessed using MTT based viability measurements, with the timing of the assays being critical because suitable post-exposure time was necessary to allow differentiation in tissue damage between washed/unwashed tissues. Comparison with washing agent only controls was also critical, as treatment with commercially available soaps/detergents combined with mechanical scrubbing decreased cell viability up to 40%. Effective washing agents produced approximately three to eight fold improvements in tissue viability, compared to no washing, with differences shown between washing agents and the use of mechanical scrubbing. In summary, these methods have been very useful for providing a basis for occupational health recommendations and illustrate how customized in vitro studies can provide practical guidance without animal use.

Evaluation of the CADRE-SS™ In Silico Model for Predicting Dermal Sensitization Potential

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Dermal sensitization leading to allergic contact dermatitis is one of the most common health issues encountered in the workplace. For hazard identification purposes, there exists a need for tools capable of providing rapid and accurate assessments of both dermal sensitization potential and potency. Given the desire to comply with the 3R principle (reduce, refine, replace), these tools should rely on in vitro or in silico methods rather than in vivo animal testing. In this context, we evaluated the performance of a novel in silico model, Computer Aided Discovery and REdesign – Skin Sensitization (CADRE-SS™), using Bristol-Myers Squibb (BMS) pharmaceutical intermediates and active pharmaceutical ingredients, and...
compared its performance against data compiled from in vivo local lymph node assays (LLNAs). CADRE-SSTM is a tiered system evaluating dermal sensitization as a function of skin permeability, metabolism, and haptenation with skin proteins, using a combination of quantum-mechanical calculations, expert rules, molecular simulations, and multivariate statistics. It is capable of providing both dichotomous and potency predictions based on established ECETOC categories. The structures of up to 363 BMS compounds possessing diverse physical-chemical properties and a broad range of LLNA sensitization potencies were analyzed. The accuracy, precision, sensitivity, and specificity for this test set as determined by CADRE-SSTM were 95%, 95%, 95%, and 95%, respectively. Additionally, CADRE-SSTM correctly classified the potency for 90% of the compounds tested. These parameters were also determined via other commonly used in silico models, including Derek Nexus, and were found to be substantially less predictive than CADRE-SSTM. Overall, CADRE-SSTM was a sound in silico screen for predicting dermal sensitization potential and potency, and is being further validated in order to identify how to best integrate these evaluations into current BMS hazard identification practices.

416 Differences on Protocols for Skin Permeation and Stratum Corneum Diffusion/Partition Coefficients

The Cosmetics Europe Skin Bioavailability and Metabolism Task Force aims to improve the measurement and prediction of the bioavailability of dermally-exposed compounds. The designs of skin penetration and stratum corneum partition (Ksc) diffusion (Dsc) coefficient studies were addressed using caffeine, resorcinol and 7-ethoxycoumarin (7-EC). Penetration studies with human (acquired with donor consent) and pig (a waste by-product of the meat industry) skin were conducted according to the OECD 428 guideline. The disposition in skin was measured 24h after application of finite doses. The distribution of each chemical was similar in human and pig ear skin and was equivalent using cold and radiolabelled chemicals. The penetration profiles of the chemicals in pig skin correlated between 2 labs. Two Ksc and Dsc protocols were compared: 1) Determination of the concentration-depth profile by tape-stripping SC after applying an infinite dose for 30min; 2) Ksc by incubation of compounds with dried SC for 24h and Dsc by measuring diffusion through isolated SC after applying an infinite dose for 24h. Both methods produced similar Ksc values for caffeine and resorcinol but not 7 EC, suggesting further refinement of protocol 1 is needed. Dsc values were most comparable for resorcinol. In conclusion, the penetration protocol was reproducible across labs and both protocols for Ksc and Dsc generated comparable data. Protocol 2 can also be used for K and D in the epidermis and dermis and will therefore be used in the main studies. For both assays, if human skin is a limiting factor, pig skin may be considered as an alternative. If radiolabelled chemicals are not available, cold chemicals can be used, providing the influence of chemical stability, reactivity or metabolism on the experimental design and the relevance of the data obtained is considered.

417 A Tiered In Vitro Irritation/Corrosion Testing Strategy for GHS Classification of Pharmaceutical Compounds
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Irritation reactions are a frequently reported occupational health hazard. To reduce animal testing, BMS and IVS have developed a testing strategy using three in vitro assays to assess the irritation/corrosive potential of pharmaceutical compounds (PC) for worker safety. The strategy allows for GHS classification by utilizing the Corrositex® assay for corrosivity (OECD TG 435), the Bovine Corneal Opacity and Permeability (BCOP) assay for ocular irritation (OECD TG 437), and the EpiDerm™ skin irritation test (SIT) for dermal irritation (OECD TG 439). Twenty-five solid PCs were evaluated in this tiered testing strategy. First the pH of each substance was determined. If the pH was ≥11 or ≤4, a Corrositex® assay was conducted. If the compound was negative in the Corrositex® assay or the pH was between 2 - 11, a BCOP assay was performed followed by a SIT assay. Based on their extreme pH, 4 compounds were tested in the Corrositex® assay, which resulted in corrosive predictions (pH ≥11 or ≤4) and thus no further testing was needed. Twenty-two compounds were evaluated in the BCOP assay (both neat and as a 20% dilution), with the higher response used for classification. The results were 5 Category 1 (score>55), and 8 non-irritants (score<3). There were 9 compounds with scores between 3 - 25, which were described as mild irritants on internal BMS hazard communications. Twenty-five compounds were evaluated using the SIT assay and were classified as non-irritants to skin. This is consistent with the BMS historical animal model results showing very low number of PCs as skin irritants. The comparison also confirmed 50% viability as an acceptable cut off for GHS dermal irritation classification. This tiered testing strategy, which replaces the use of animal studies, represents a rational platform that can be utilized for the prediction of ocular and dermal irritation/corrosive potential of PCs.

418 A Human Keratinocyte Tissue Model Differentiates between Phototoxicity and Direct Skin Toxicity
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A coumarin-type compound displayed phototoxic effects in vitro based on its properties to absorb UVA. In monkeys, the compound produced parakeratosis, keratinocyte dyskeratosis and apoptosis on the skin without UV exposure. To judge possible effect on human skin, we investigated the effects of the compound in the human skin model epiCSS® on keratinocytes with and without artificial sunlight. The epiCSS® tissue consists of normal, human-derived epithelial keratinocytes cultured to form a multilayered differentiated model of the human epidermis. Treatment with the compound was carried out on a daily basis for 7/14 days. Cultures were irradiated for 60 minutes with 5.94 J/cm2. Cytotoxicity (MTT); cytochrome release (IL-1α, IL-6, IL-8, TNF-α) and histopathological evaluation followed. Distinct cytotoxic effects of the compound were observed for the irradiated as well as for the non-irradiated cultures in the MTT assay. The cytotoxic effect was more severe in irradiated samples indicating phototoxicity. Treatment with the compound triggered the release of IL-1, IL-8 and TNFα, which was generally more severe in irradiated as compared to non-irradiated samples confirming a phototoxic effect. Histopathological assessment could distinguish a phototoxic effect from a direct toxic effect of the test item with presence of pyknotic nuclei only being present only in irradiated samples and inter-cellular edema occurring at lower doses in irradiated versus non-irradiated samples. Overall histopathological toxicity scores correlated well with MTT results in non-irradiated samples after 7 and 14 days of treatment and irradiated samples after 14 days of treatment. In conclusion, this in vitro model of human skin was able to differentiate between the two types of toxicity of the test compound, its direct effects on skin as well as its effects upon UVA absorption. Further investigations into molecular understanding of the skin toxicity of the test item indicate that the effect may be associated with post-transcriptional control of gene expression.

419 Intra- and Interlaboratory Validation of LuSens: A Reporter Gene-Cell Line to Detect Keratinocyte Activation by Skin Sensitizers
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Several in vitro methods address the steps leading to skin sensitization as defined by the adverse outcome pathway. KeratinoSensTM has been validated in the EU to address the cellular event of keratinocyte activation. Herein, we report on the me-too validation of the LuSens assay, a simple bioassay that uses a human keratinocyte cell line harboring a reporter gene construct composed of the rat antioxidant response element (ARE) of the gene of the NADPH:quinone oxidoreductase 1 and the luciferase gene. In-house validation with 74 substances showed predictivity of 82% in comparison to human data. LuSens is, however, intended to be used in a battery of in vitro methods which result in even higher predictivities. To meet European validation criteria, a study was conducted with 5 partners from US, Germany and Switzerland. The study was divided into two phases, to assess 1) transferability of the method and 2) reproducibility and reliability. Phase I showed a good transferability to naïve labs and within laboratory reproducibility, leading to correct prediction of 80%. Phase II was performed with 2 coded test substances (current performance standards of the ARE OECD Draft TG), and is under evaluation. Preliminary data show a remarkable reproducibility within the testing labs and a good concordance of the data towards the human in vivo data. The study demonstrates the transferability and reliability of LuSens for detecting skin sensitizers.
420 Partition Coefficient Determinations for Benzoic Acid, Resorcinol, and Methyl Paraben in Isolated Human Skin Layers for In Silico Dermal Pen Modeling


The development of a precise in silico skin penetration model is needed to enable moving away from a 100% systemic bioavailability assumption to a more realistic one and to confidently assess the actual fraction of a compound systemically absorbed for finite dose scenarios. We are replacing empirical relationships (with limited applicability domains) by mechanistically based simulations to characterize trans-dermal transport. To this end, diffusion and particularly partition coefficient determinations are being conducted for several radiolabelled compounds on isolated human skin layers, which include measurements in whole skin and isolated dermis, whole epidermis (stratum corneum (SC) plus viable epidermis), SC and delipidized SC and SC lipids where appropriate. The first 3 compounds to be tested are 14C-benzoic acid, 14C-resorcinol and 14C-methyl paraben. Partition coefficient determinations were made after overnight incubations of the isolated tissue layers or lipid component in buffer solutions spiked with the radiolabelled compound, and by subsequent direct measurements of the radioactivity in the tissue layers/lipid component vs. buffer samples. Partition coefficient values were extrapolated for the viable epidermis, since that layer was not able to be isolated in an intact form. The extrapolation utilized a mathematically derived relationship between the whole epidermis and the SC and physical characteristics of each. For each of the tested compounds the partition coefficients for the whole skin, dermis, whole epidermis, viable epidermis, fully hydrated SC and fully hydrated delipidised SC were all very similar; ranging from about 1 for benzoic acid to about 2-3 for methyl paraben to about 2-4 for resorcinol. The partition coefficient values were always about 3-4 fold higher in partially hydrated SC, partially hydrated delipidized SC and in SC lipids; ranging from about 3 for benzoic acid to about 6-9 for methyl paraben to about 9-19 for resorcinol.

421 ICCVAM Integrated Decision Strategy for Skin Sensitization


The development of an integrated decision strategy for skin sensitization that includes the Toolbox protocol had a lower false positive rate, it will be evaluated as part of an integrated decision strategy for skin sensitization that includes in vitro data and physicochemical parameters. This project was funded in whole or in part with Federal funds from the NIEHS, NIH under Contract No. HHSN27320140003C.

422 In Silico Predictions of Skin Sensitization Using OECD QSAR Toolbox

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This project was funded in whole or in part with Federal funds from the NIEHS, NIH under Contract No. HHSN27320140003C.

423 The GARD Assay for Potency Assessing Skin Sensitizing Chemicals

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The development of a precise in silico skin penetration model is needed to enable moving away from a 100% systemic bioavailability assumption to a more realistic one and to confidently assess the actual fraction of a compound systemically absorbed for finite dose scenarios. We are replacing empirical relationships (with limited applicability domains) by mechanistically based simulations to characterize trans-dermal transport. To this end, diffusion and particularly partition coefficient determinations are being conducted for several radiolabelled compounds on isolated human skin layers, which include measurements in whole skin and isolated dermis, whole epidermis (stratum corneum (SC) plus viable epidermis), SC and delipidized SC and SC lipids where appropriate. The first 3 compounds to be tested are 14C-benzoic acid, 14C-resorcinol and 14C-methyl paraben. Partition coefficient determinations were made after overnight incubations of the isolated tissue layers or lipid component in buffer solutions spiked with the radiolabelled compound, and by subsequent direct measurements of the radioactivity in the tissue layers/lipid component vs. buffer samples. Partition coefficient values were extrapolated for the viable epidermis, since that layer was not able to be isolated in an intact form. The extrapolation utilized a mathematically derived relationship between the whole epidermis and the SC and physical characteristics of each. For each of the tested compounds the partition coefficients for the whole skin, dermis, whole epidermis, viable epidermis, fully hydrated SC and fully hydrated delipidised SC were all very similar; ranging from about 1 for benzoic acid to about 2-3 for methyl paraben to about 2-4 for resorcinol. The partition coefficient values were always about 3-4 fold higher in partially hydrated SC, partially hydrated delipidized SC and in SC lipids; ranging from about 3 for benzoic acid to about 6-9 for methyl paraben to about 9-19 for resorcinol.

424 Predicting Skin-Sensitizing Potency Based on In Vitro Data from Keratinosens™ and Kinetic Peptide Binding: Global vs. Domain-Based Assessment

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ICCVAM Toolbox for making read-across skin sensitization predictions using murine local lymph node assay (LLNA) outcomes as reference data. The Toolbox protocol identified analogs for 120 target substances (87 sensitizers and 33 nonsensitizers) using mechanism of protein binding and chemical structure schemes in the Toolbox. In vivo protein binding alerts were not identified in a substance, auto-oxidation and skin metabolism products were predicted; a representative product with a protein binding alert was used in the evaluation. If neither parent nor products had protein binding alerts, the substance was classified as a nonsensitizer. For parent or products with protein binding alerts, in vitro skin sensitization data for analogs were used to predict the sensitization potential. Accuracy of the Toolbox protocol was 77% (92/120) with sensitivity = 77% (67/87) and specificity = 76% (25/33). Using only protein binding alerts in the parent compound to predict sensitization potential yielded accuracy = 69% (83/120), sensitivity = 66% (57/87), and specificity = 79% (26/33). Using only protein binding alerts in the parent or product to classify substances as sensitizers improved accuracy (82% [98/120] and sensitivity (91% [79/87]) compared to the Toolbox protocol (98% [95/98] and specificity (58% [19/33]). Thus, potential skin sensitizers may be predicted with similar accuracy using either the Toolbox protocol or only protein binding alerts. Because the Toolbox protocol had a lower false positive rate, it will be evaluated as part of an integrated decision strategy for skin sensitization that includes in vitro data and physicochemical parameters. This project was funded in whole or in part with Federal funds from the NIEHS, NIH under Contract No. HHSN27320140003C.

Allergic contact dermatitis is caused by an adverse immune response towards chemical hapten. The disease affects a significant proportion of the population, and is especially problematic for certain occupational groups. EU legislation concerning registration and use of chemicals within chemical and cosmetic industries requires the replacement of animal-based test methods by alternative, high-throughput tests for the prediction of skin sensitization capacity and potency of known and new substances. We have developed a human cell-based assay for the prediction of sensitizing chemicals, called Genomic Allergen Rapid Detection, GARD. By analyzing RNA extracted from cells of the MUTZ-3 cell line treated with a panel of chemicals we have identified a genomic biomarker signature with potent discriminatory ability. In order to implement human potency prediction, we have recently investigated regulated cellular pathways, and our data show that sensitizers rather down- and up-regulate genes. Metabolic and cell-cycle-associated pathways seem to correlate most with sensitizing potency. Now we continue to expand the data set among other chemicals belonging to the same chemical domain correlating GARD decision values and human potency. We will focus on the identification of genes or pathways that correlate with potency, such as the Nrfl-2 pathway, in order to obtain further insights into the biological mechanisms involved.

Three in vitro endpoints to predict the skin sensitization hazard have finalized validation. However, predicting sensitizer potency is now a key additional requirement for risk assessment. Here we report analysis of a database of 712 chemicals tested in the Keratinosens™ assay and for kinetic peptide binding. These continuous quantitative data were used in multiple regression analysis against potency in the local lymph node assay (LLNA). The data set covers the majority of chemicals from the validation of the LLNA to predict human potency and this subset was further analyzed for prediction of human sensitization potency by in vitro data. Global analysis yields a regression of in vitro data to LLNA EC3 with an r2 of 60% predicting LLNA EC3 with a mean error of 3.5-fold. The highest weight in the global regression has the reaction rate with peptides, followed by Nrf2-induction and cytotoxicity in Keratinosens™. The correlation of chemicals tested positive in vitro with human data has an r2 of 49%, which is similar to the correlation between LLNA and human data. Chemicals were then grouped into mechanistic domains based on the TIMES SS software and experimentally observed peptide-adduct formation. Predictions within these domains made with a leave-one-out approach were more accurate. For several mechanistic domains, LLNA EC3 was predicted with an error
of around 2-3 fold. However, not all chemicals fall into data-rich domains. Thus, this analysis indicates that combining global and domain-based models to assess sensitizer potency may be a practical way forward.

**425 Evaluation of Two Methods of Skin Integrity Check in an In Vitro Dermal Absorption Study with Benzoic Acid Using Rat Split-Thickness Skin**

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Skin integrity evaluation is an essential part of the in vitro method. Therefore, present study was undertaken to evaluate the two skin integrity check methods (permeability coefficient (Kp) of tritiated water and measuring electrical resistance (ER) across the skin membrane) in an *in vitro* dermal absorption study using benzoic acid as reference chemical through rat split-thickness skin. Presented approach in this study allows for rapid selection of rat skin membranes for use on *in vitro* dermal regulatory studies. Dose group included eight replicates from four donors (2 replicates/donor) in this study. Split-thickness skin membranes (thickness between 346 to 367 μm) were placed in flow-through diffusion cells with 0.64-cm2 exposure area and exposed at 32°C at 16-cm head, at ambient humidity. All the skin membranes after skin integrity evaluation by both methods were exposed to benzoic acid (4 mg/mL). The exposure time was 8h with post-exposure sampling for 16h and sampling up to 24h. Mass balance analysis was conducted for samples of receptor fluid, the residues remaining in/on the skin and in the stratum corneum (at 24h) by using Liquid Scintillation Counter. Our results show that rat skin membrane 3H2O-Kp values were between 0.391 x 10^-3 cm/h to 1.16 x 10^-3 cm/h. The corresponding electrical resistance measurements were between 5 - 12 kΩ/cm for rat skin membranes. The mean cumulative absorption of benzoic acid into the receptor fluid after 24 h was 17.65 μg/cm², i.e. 43.32% of the applied dose and the mean total recovery of benzoic acid was 101.39±3.35%. The mean maximal flux was 2.418 μg/cm²/h. In summary, both the methods of skin integrity check gave the comparable results; therefore use of electrical resistance (ER) for skin integrity check could provide simpler, quicker, cost effective and appropriate alternative to Kp method.

**426 Functionalized Electrospun Nanofibers for the Development of a Three-Dimensional Skin Model**

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An appropriate simulation of the basement membrane (BM) is essential to build up a skin model. BM separate the epithelium from the stroma of any given tissue and play an important role in regulation of cell behavior. Modification of electrospun fibers with a functional, amphiphilic macromolecule based on a star-shaped poly(ethylene oxide) derivate transforms hydrophobic fibers into hydrophilic fibers and makes binding of cell-adhesion mediating peptides possible. These functionalized fibers provide a promising environment for the generation of 3D *in vitro* systems and complex tissue models. Electrospinning was performed with a solution of NCO-P(EO-stat-PO) and PLGA RG 504 in a mixture of acetone, Dimethylsulfoxide and acidic water. Fibers were modified with binding motifs of Fibrinectin, collagen type IV and laminin. Coculture experiments with HaCaT and primary human fibroblasts revealed that it is possible to create skin equivalents with these functionalized scaffolds. The HaCaT cells grew in several layers and expressed epithelial differentiation markers such as cytokeratin 10 in the supra basal cell layers and cytokeratin 14 in the basal layer. Vimentin staining of the fibroblasts showed that the cells infiltrated into the electrospun membrane. It could also be confirmed that cells produce laminin, collagen and fibrinectin, which illustrates matrix remodelling. The establishment of a method which specifically modifies surfaces of electrospun fibers offers great opportunities to generate nanofoam with appropriate physical, mechanical and biological properties essential for cell growth, proliferation, migration and differentiation. Adding cells to the artificial membranes enables the formation of biomimetic *in vitro* system. The development of a simple, reproducible and easy to use 3D-model is a promising test system in order to replace animal studies.

**427 Investigation of Novel In Vitro Methods for Predicting the Dermal Sensitization Potential of Synthetic Process Intermediates**

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Skin Sensitization is a critical endpoint in the evaluation of synthetic process intermediates used in the manufacture of active pharmaceutical ingredients. Although the *in vitro* Local Lymph node assay (LLNA) has traditionally been used to qualitatively and quantitatively assess dermal sensitization, many novel alternative assays have reached an advanced stage of pre-validation and official testing guidelines are expected within the year. In this study we sought to evaluate two alternative assays, the KeratinoSens assay and the Direct Peptide Reactivity Assay (DPRA), for the prediction of dermal sensitization of synthetic process intermediates. The KeratinoSens assay is a cell-based reporter gene assay which identifies skin sensitizers by measuring the induction of a luciferase under the control of the antioxidant response element (ARE) derived from the human AKR1C2 gene. Luciferase induction indicates activation of ARE-dependent genes which are involved in the dermal sensitization mechanism. The DPRA is an *in chemico* assay which identifies dermal sensitizers based on their reactivity with model peptides. Test substances are incubated with cysteine/lysine containing peptides and the depletion of each peptide is then used to determine the degree of reactivity and dermal sensitization potential. Seven process intermediates were evaluated in the KeratinoSens assay and DPRA and the results were compared to existing LLNA data on these test substances. Six of the seven test substances were identified correctly by the KeratinoSens assay; four of the seven test substances were identified correctly by DPRA. Continued evaluation of these alternative assays for assessment of the dermal sensitization potential of synthetic process intermediates will be beneficial in establishing testing strategies to reduce animal testing while providing a useful tool for risk assessments.

**428 Skin Sensitization Assessment of Various Cosmetic Ingredients towards Ultimate Replacement of Animal Testing**


Test batteries combining DPRA, KeratinoSens™, and h-CLAT have been proposed in order to reproduce complex sensitization mechanisms towards ultimately replacing animal testing. These test batteries have the high predictivity to animal tests evaluating purified and single chemical substances, but are not sufficiently designed in consideration of the applicability of mixtures consisting of substances with unknown molecular weights and the low water soluble substances. In this study, we attempted to develop a testing strategy to predict sensitizing potential of such complex mixtures and low water soluble substances like those often used as cosmetic ingredients. We first focused on the combination of h-CLAT and KeratinoSens™ where complex mixtures can be applied. The DPRA is not suitable for mixtures with unknown molecular weights. The results of the test battery for over 100 substances were compared to data of the LLNA and human data. The test battery exhibited good predictiveness when compared with LLNA and human data, and comparable predictivities when compared with test batteries previously reported. Next, we developed a reconstructed human epidermis-based assay, EpisensA, as new technology that can reproduce correctly a series of key events (i.e., skin penetration, protein binding, keratinocyte responses) in sensitization, in order to consider potential interactions. Regarding the keratinocyte responses, while KeratinoSens™ reflects only the cyto-protective responses such as antioxidative reactions, EpisensA focuses on the expression of four genes relevant to either the inflammatory responses or cyto-protective responses. When tested using EpisensA, sensitizing potential of many low water soluble substances was detected with high predictivity to LLNA. Our study results suggested that, in addition to a test battery combining two or more *in vitro* methods reflecting different key events in sensitization, a method that reproduces two or more key events in one assay would be useful for skin sensitization evaluation of various cosmetic ingredients without using animal testing.
429 Potency Ranking of Dermal Sensitizing Chemicals Using the IVSA and epiCS® Skin Tissues
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Human 3D reconstructed skin epidermal equivalents have been shown to release IL-18 in response to a wide range of dermal sensitizing chemicals. The concentration of these chemicals that produce greater than a threshold positive response (Stimulation Index, SI ≥ 2.0) is correlated to their potency or strength in an In Vitro Sensitization Assay (IVSA). In our experiments, 4-Nitrobenzyl bromide (NBB) and DNCB were strong inducers of IL-18 secretion into the culture medium (EC2.0 = 0.0288 and 0.03%, respectively), Isoeugenol (IE) and Cinnamaldehyde (CA) were moderate sensitizers, while Resorcinol (RES, EC2.0 = 2.5%) and Hexylcinnamaldehyde (HCA, EC2.0 = 2%) were weak sensitizers. Sensitizer potency ranked as follows: NBB > DNCB > PPD, IE = CA > RES > HCA, with NBB, DNCB and PPD classified as strong, IE and CA as moderate, and RES and HCA classified as weak sensitizers. Of the total of 20 chemicals tested, seven were irritants and two were non-sensitizers (Glycerol and Isopropanol); of these, only Chlorobenzene (50%) was incorrectly predicted as a very weak sensitizer. When including all chemicals where viability of epiCS® tissues was between 10% and 100% (via MT1 assay), employing a cutoff SI ≥ 1.8 gave an Accuracy of 95% and Sensitivity of 100%, and all other Cooper Statistics (Specificity, Negative and Positive Predictivity) values greater than 90%. We also compared using all data regardless of low viability (< 10%), and SI cutoffs ranging from 1.6 to 2.0 in contingency tables with different cutoff SI values. In summation, measuring IL-18 release from 3D tissues allows for highly accurate and sensitive identification of dermal sensitizers. Also, the ability to rank-order potency of these chemicals based on EC1.8 – EC2.0 values of IL-18 secretion is a powerful tool for further classification into potency categories.

430 Potency Classifications for Contact Dermal Sensitization As Determined by the h-CLAT Assay
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Due to societal concerns, as well as new regulations in the EU, animal-based dermal sensitization assays are in disfavor for total validity. We have performed validation studies using the Human Cell Line Activation Test (h-CLAT) protocol under EU guidelines and with minor modifications. We analyzed the standard THP-1 cell line responses to a recommended training set of validation chemicals, as well as some in pharmacology dendritic cells (pDC) in this in vitro sensitization test. Test set chemicals include DNCB, Isoeugenol (IE), Cinnamaldehyde (CA), and the non-sensitizing irritants Lactic Acid (LA) and Salicylic Acid (SA), tested in a dose-response assay. DNCB, a strong sensitizer, induced a 62-fold increase in CD86 expression and a 3.8-fold increase in CD54 expression as measured by flow cytometry, and as compared to LA at up to 2.0%, which failed to produce a positive response (less than 1.5-fold increase). All chemicals were able to be exposed at a low or non-irritating concentration, yielding a CV75 or higher viability. Sensitizer potency was measured by the concentration of test chemical that induced a Stimulation Index (SI) that was a threshold positive response (CD86 > 2.0, CD54 > 1.5). DNCB yielded well above the SI cutoffs with large increases at 0.0004%, a very low concentration. The potency ranking of the chemical test set we analyzed was DNCB > IE = CA > LA > SA, which are correctly ordered as per human and LLNA potency class (strong, moderate, weak sensitizer, non-sensitizer).

431 A Novel Assay for Evaluating Wound Healing in a Full-Thickness In Vitro Human Skin Model
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Cutaneous wound healing involves interactions between dermal fibroblasts and epidermal keratinocytes as well as cell-extracellular matrix interactions. The current study describes wound healing experiments conducted in a full thickness in vitro human skin model (EpiDermFT). This model exhibits stratified epidermal components and a fully developed basement membrane and resembles in vivo skin in regard to both morphology and barrier function. Small epidermal only wounds were induced in the model using a 3mm punch biopsy and subsequently evaluated at various recovery time points by two methods. Historically, EpiDermFT has been used to evaluate re-epithelialization of the wound by: a) manually biassing the tissues through the center of the wound, b) staining with hematoxylin and eosin, and c) quantifying migration from the wound origin. Accurate bisection of the wound is difficult and often leads to variability in assay results. Here we describe a novel method of visualizing wound re-epithelialization in situ simplifying analysis and reducing introduction of variables inherent in tissue processing that can confound data. Following wounding, tissues were fixed and immunostained with markers of epidermal differentiation as well as a marker of fibroblasts allowing simultaneous visualization of migrating keratinocytes (keratin 14), differentiated suprabasal cells (keratin 5 and 14), and dermal fibroblasts (vimentin). We have performed histological and immunohistochemical analysis showed keratinocyte migration at 2 days following wounding. In both methods, wounded tissues cultured without growth factors (2% human serum) had a reduced healing rate in which keratinocytes did not cover the entire wound within a 6 day timeframe. In contrast, wounded tissues cultured with greater factors demonstrated a dramatic increase in healing rate. Histological and immunohistochemical analysis showed keratinocyte migration completely covered the wounded area by day 6. In conclusion, this novel method of evaluating re-epithelialization by utilizing immunohistochemical markers of differentiation is a quicker and more reproducible method of analyzing wound healing.

432 Establishment of a Novel In Vitro Model Based on Retinal Pigment Epithelial Cells to Assess Safety of Ophthalmic Drugs

Ocular diseases leading to visual impairment are a major concern and therefore trigger development of a significant number of new drugs. However, as of today no relevant in vitro models to assess safety of ophthalmology drugs are available. The retinal pigment epithelium (RPE) plays an important role for maintaining retinal function as it is involved in multiple key processes. As such, the RPE is part of the blood-retina barrier, responsible for phagocytosis of photoreceptor outer segments, for recycling of visual pigment as well as for releasing pro- and anti-inflammatory factors. We built an in vitro model to assess drug-induced retinal toxicity based on human ARPE 19 cells and established a defined battery of read outs to monitor key retina functions including barrier integrity measured by impedance analysis, inflammatory response measured as cytokine release and phagocytosis or cellular degeneration assessed by high content imaging. We could demonstrate that ARPE19 cells could be treated by several chemical inhibitors of blood plasma proteins such as Claudin-5, ZO1 and Occludin were expressed. Barrier integrity of ARPE19 cells could be modulated by treating cells with known modulators II beta, TNFalpha or Thrombine which caused a decrease in impedance levels. These effects could be reverted by Anakinra or Remicade, specific antagonists for II beta and TNFalpha. ARPE19 cells did respond to an inflammatory stimulus by LPS by releasing IL6 and IL8. Next, we measured phagocytosis by uptake of Cy3 labelled latex beads as a surrogate readout for photoreceptor outer segment phagocytosis in vivo. By incubation of ARPE19 cells with Chloroquine, a known ocular toxicant, we could recapitulate the in vivo phenotype of disturbed cellular degradation in vitro measured as lysosomal swelling and accumulation of autophagosomes. Taken together we have established a comprehensive RPE-based in vitro model to assess retinal safety of ophthalmology drugs. The in vitro model is capable to monitor important RPE based processes which are crucial to maintain vision.

433 C241-Ceramide May Be a Novel Lipid Biomarker for Eye Irritation in 3D Human Corneal Epithelial Model, MCTT HCE™
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Eye irritation test is mandatory for cosmetics and pharmaceuticals. Drazie rabbit eye irritation test method has been widely used and internationally accepted (OECD Test Guideline 405). However, due to an invasive and painful experimental procedure, alternative in vitro test methods replacing conventional Draize rabbit eye irritation test are being actively pursued. Recently, 3D reconstructed human corneal models receive enormous attention since they are morphologically and physiologically similar to human cornea. These 3D models are employing cell viability as a common endpoint for eye irritant but extra biomarkers are necessary to improve test performance. Here, we explored new lipid biomarkers for eye irritation using human corneal epithelial model (MCTT HCE model). Surfactants were selected as model eye irritants since they are widely used in cosmetics, and pharmaceutical products. Three irritants: sodium lauryl sulfate, benzalkonium and triton X-100 were selected as relevant anionic, cationic and non-ionic surfactants. After treating 3 irritants at different concentrations on MCTT HCE model, we extracted lipid in supernatant using methyl-tert-butyl ether. And we quantified the amount of ceramides and fatty acids, representative lipid components in outer surface of body, using sensitive LC/MS/MS method. It was found that among diverse fatty
acids and ceramides, C24:1Cer was significantly and reproducibly increased by three eye irritants. Moreover, C24:1Cer was increased by 3 irritants in a dose-dependent manner suggesting that it can be a novel lipid biomarker for eye irritants.

Using the Novel NociOcular Assay to Predict the Eye Sting Potential of Shampoos and Sunscreen Products

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Although several in vitro eye irritation models exist, none have demonstrated the ability to predict eye stinging. The NociOcular assay, a novel neuronal in vitro model with high expression of capsaicin-responsive Transient Receptor Potential Vanilloid type 1 (TRPV1) channels, has been shown to distinguish stinging from non-stinging baby bath products. We sought to evaluate the eye stinging potential of additional surfactant-based products and sunscreen formulations. In the assay, SH-SY5Y neuroblastoma cells are cultured in 96-well plates and exposed to serially diluted test substance and TRPV1 channel activation is measured by acute increases in the intracellular free calcium. In separate wells, cells are treated with the TRPV1 antagonist capsazepine to confirm TRPV1-mediated calcium influx. The positive control, an adult shampoo that contains cocamide MEA, a known stinging ingredient, was the most active surfactant-based test substance evaluated in the assay. The negative control, a baby shampoo, was negative in the NociOcular assay and clinical tests. Four shampoo products demonstrated a range of responses between these controls and were classified as either stinging or non-stinging based on the percentage calcium influx as compared to capsaicin over the dose-response. During pilot studies with sunscreen formulations, several technical challenges arose including insolubility in assay buffers and pipetting the subsequent dilutions onto the cells. In order to achieve greater solubility, alternate solvents composed of detergents along with assay buffers were used. These alternate solvents allowed for increased solubility and dilutions were successfully administered onto the cells. Ten sunscreen formulations were evaluated and ranked according to TRPV1 response and compared to available consumer experience reviews for eye stinging. Future research aims to assess the accuracy of the predictions for both the shampoos and sunscreen products through clinical data comparison.

Optimization of an Eye Irritation Assay for Hazard Identification and Labelling of Materials to Address the EU Cosmetic Directive and REACH Legislation

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Implementation of the 7th Amendment to the EU Cosmetic Directive and the EU REACH legislation has heightened the need for predictive in vitro ocular test methods. To address this need, we have developed an eye irritation test (EIT) which utilizes a three dimensional organotypic tissue model based on normal human corneal cells. The test utilizes two separate protocols, one specifically designed for liquid test materials and the other for solids. During the optimization of the assay, several technical challenges came to light, including the need to ensure optimal solubility of the test substance which is crucial for reproducible test results. By changing the exposure for solid materials from 2 to 6 hours, the EIT achieved compliance with the acceptance criteria set by the VMG and indicated the need for improvement of the assay sensitivity for solids. Here we report results of the assay optimization.

Effect of Long-Term Shipping on an Eye Irritation Test for Hazard Identification and Labelling of Materials

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The recently implemented 7th Amendment to the EU Cosmetics Directive and the EU REACH legislation has heightened the need for in vitro ocular test methods. To address this need, we developed an Eye Irritation Test (EIT) that utilizes an organotypic tissue model based on normal human cells. The test discriminates...
between ocular irritants (“I”, GHS Cat 1 and 2) and non-irritants (“NI”, GHS No category) with 95.5/68.2/and 81.8% sensitivity/specitivity/and accuracy (SS&A) for liquids and with 100.0/70.0/and 85.7% SS&A for solids. The assay underwent a formal, multi-laboratory validation study under the auspices of the European Centre for the Validation of Alternative Methods (ECVAM) to assess the relevance and reliability and meet all the acceptance criteria set by the Validation Management Group (VMG) for eye irritation and an OECD draft guideline has been submitted. To meet OECD guidelines, the EIT needs to be valid worldwide. In initial validation work was performed in the US and Europe. Here we present data regarding assay performance at Kurabo Industries (Japan) after extended shipment (4-5 days). The EIT protocol that utilized tissues following shipment for 4 days discriminated between ocular “I” and “NI” with 100.0/63.6/and 81.8% SS&A for liquids, with 100.0/70.0 and 85.7% SS&A for solids, and with 100.0/65.6 and 83.1% overall SS&A. These results were nearly identical to those obtained following 1-2 day shipments in the US and EU and meet all the acceptance criteria set by the VMG. In addition, Negative and Positive Controls (NC and PC) of 24 (and 11) different tissue lots have been tested in Japan with Liquid (and Solid) protocols and all obtained acceptable criteria (OD NC=0.8 and PC Viability=60%). Thus, we conclude that extended shipping (4 days) does not alter the EIT performance.

PS 439 Protocol Considerations for Testing Surfactants and Surfactant-Based Formulations in the Bovine Corneal Opacity and Permeability Assay

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The Bovine Corneal Opacity and Permeability (BCOP) assay is an ex vivo test for predicting ocular irritation. For regulatory classification, OECD Test Guideline (TG) 437 specifies that liquid and solid surfactants may be tested as 10% aqueous dilutions for 10 minutes, although alternate dilutions and exposure times may be conducted with scientific rationale. Guidance Document (GD) No. 160 also presents that solid and concentrated liquid surfactants may be diluted to 10% for testing. GD No. 160 further directs that surfactant-based formulations are usually tested neat, but could be diluted with justifying, imparting some confusion in identifying the most appropriate test methods. Without question, surfactant solids should not be tested using the solid chemical protocol, since over-exposure conditions are likely. In the absence of clear guidance from these regulatory documents, we present our testing of a few common surfactant ingredients (sodium lauryl sulfate, Triton X-100, and benzalkonium chloride), and surfactant-based liquid and solid formulations in BCOP using standard and modified dilutions and exposures to evaluate the impact of these variables. Whereas the opacity values for the non-ionic and anionic surfactants were low, changes in the fluorescein permeability values correlated well to expected surfactant activities in all of the surfactant classes tested. Histopathology was performed to confirm corneal changes. We found that surfactants at very high concentrations may not exhibit dose-related effects, as irritation optima may occur at aqueous concentrations between 10 and 30%. Furthermore, since surfactants induce corneal erosion, we advocate that the fluorescein permeability endpoint in the BCOP assay should be evaluated individually from the In Vitro Irritation score in a hazard assessment. Accordingly, a framework to guide the testing of surfactants and surfactant-based products is presented.

PS 440 Dichloroacetate- and Trichloroacetate-Induced Toxicity and Oxidative Stress in AML 12 Cells

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Dichloroacetate (DCA) and trichloroacetate (TCA) are among the halocacetates formed as by-products during the process of water chlorination. The compounds were found to be hepatotoxic/ hepatocarcinogenic in rodents, and oxidative stress (OS) was found to play a role in that. In an attempt to find an in vitro system to screen the effects of all of the halocacetates and their unlimited number of mixtures, AML 12 cell line was used for this study. The toxic effects of DCA and TCA have been tested initially in this system, since sufficient in vivo toxicity data are available for them that may be used to assess the relevancies of the two systems to each other. Treatment of the cells with DCA and TCA at concentrations ranging from 770 to 4100 ppm for 24 to 72 h resulted in concentration -, and time-dependent reductions in cellular viability, with TCA producing significantly greater effects than DCA. Both compounds were found to induce various levels of superoxide anion and lipid peroxidation, and to result in concentration-dependent induction of superoxide dismutase, after 48 h of incubation. It is concluded that, similar to the in vivo system, OS plays a role in the compounds-induced cellular toxicity, but effective compounds concentrations are 10 fold greater than the in vitro ones. Also, various biomarkers of OS may contribute differently to the toxicities of DCA and TCA, and that if this system is to be used for screening the halocacetate-induced cellular toxicity and OS, more than one biomarker of OS should be assessed.

PS 441 Role of Chemical Structure in the Genotoxic Potential of Alkoxo-Substituted Allylbenzenes

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Alkoxo-substituted allylbenzenes, flavoring agents naturally occurring in herbs in- cluding nutmeg, basil, anise, are established to be cytotoxic and hepatocarcinogenic in rodent assays, causing increased incidences of hepatocellular adenoma and carci- noma and cholangiosarcoma in mice and rats of both genders (NTD 2006; 2008). A current hypothesis is that the carcinogenic potential of alkylalkoxybenzenes is determined by their chemical structure, specifically the position of the double bond in the alkyl side chain and substitution of the alkoxo group by hydroxyl groups. The goal of the present study was to test this hypothesis and to elucidate the mode of action of several representatives of this class using Turkey Egg Genotoxicity Assay (TEGA). Medium white turkey eggs containing 22 to 24 day old fetuses received 3 daily injections with different doses of safrole (0.125, 0.5, 1, 2 ul/egg), estragole (5, 10, 20, 40 mg/egg), methyl eugenol (1, 2, 4 ul/egg), eugenol (1, 2, 5 mg/egg), and anethole (1, 5, 10 mg/egg). Three hours after the last injection, fetal livers were harvested for measurement of two endpoints: DNA strand breaks, using alkaline single cell gel electrophoresis assay, and DNA adducts formation, using 32P-nucleotide postlabeling assay. Safrole and methyl eugenol caused dose-depen- dent increases in the level of DNA adducts. Estragole induced linear increase of DNA strand breaks and, in the highest dose, formation of DNA adducts. In contrast, exposure to eugenol, a compound with a hydroxyl group substitution, and anethole, compound with shifted double bond, under the same conditions did not cause direct DNA damage, consistent with the lack of data regarding carcinogenicity of these compounds. To sum up, our findings, congruent with the data obtained from previous studies, confirm the genotoxic potential of safrole, estragole and methyl eugenol and lack of genotoxicity of eugenol and anethole, thus sup- porting the hypothesis that the genotoxicity of alkylalkoxybenzenes is determined by their chemical structure.

PS 442 Histopathological Changes Induced by 2-Acetylaminofluorene and Diethylnitrosamine in Fetal Chicken Livers

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Previously, two activation-dependent DNA-reactive hepatocarcinogens, 2-acetyl- aminofluorene (AAF) and diethylnitrosamine (DEN) were assessed in the Chicken Egg Genotoxicity Assay (CEGA). Both chemicals produced DNA strand breaks; additionally, AAF formed DNA adducts. The objective of the current study was to analyze the histopathological changes in chicken fetal livers after dosing with these carcinogens. White leghorn chicken eggs were administered 1 and 2 mg DEN/egg and 0.3 and 0.6 mg AAF/egg in 3 daily injections on days 9 - 11 of incubation. One week after exposure was discontinued, fetal livers were collected and qualitative and quantitative microscopical evaluation was performed on liver sections stained with hematoxylin and eosin. Control livers displayed typical trabecular architecture. DEN exposure caused anisocytosis and anisokaryosis of both hepatocytes and cholangiocytes accompanied by dose-related dysplasia with extensive pleomorphism and anaplasia. Observed oval cell proliferation was probably an adaptive response to replace dysplastic hepatocytes and cholangiocytes. Gall bladder agenesis was caused by the higher dose of DEN along with stellate cells activation, resulting in extracellular matrix restructuring, which probably represents an effort to compensate for the dysplasia of biliary epithelium. Dosing with AAF produced dose-related hyperplasty of parenchymal hepatocytes; cholangiocytes showed anisocytosis and anisokaryosis. Gallbladders were present, and hepatocellular trabecular structure was preserved. Also, no oval or stellate cell proliferation was found. Importantly, these changes were observed in the absence of inflammation. Moreover, eggs are aseptic. Accordingly, observed histomorphological alterations are directly related to the effect of exposure to DEN and AAF. Thus, the results of the study provided microscopic evidence of DEN and AAF induced interference with proliferation and re-differentiation in fetal chicken livers.
Liver fibrosis is one of the leading causes of death worldwide. Currently, mostly rodent \textit{in vivo} models are used to study the disease and to test potential anti-fibrotic drugs. A major drawback of these rodent models is that data derived therefrom can only partially be extrapolated to the situation in humans. Furthermore, animal models of fibrosis are labour-intensive and experimental conditions implicate high toxicity and mortality. On a cellular level, it is well known that liver injury induces the conversion of quiescent hepatic stellate cells (HSC) into myofibroblasts. First, the paracrine stimulus from Kupffer cells and injured hepatocytes induces proliferation of HSC, which then secrete pro-inflammatory cytokines and chemokines. Then, proliferative HSC trans-differentiate into myofibroblasts and secrete growth factors which stimulate synthesis of extracellular matrix proteins (ECM). HSC migrate to the damaged area, where they repair scar formation by secretion of ECM. Evolving fibrogenesis finally leads to oxidative damage and massive apoptosis of hepatocytes. Most of the existing cell culture models do not recapitulate these multicellular processes of fibrogenesis since they lack cell-cell and cell-matrix interactions. We therefore induced liver fibrosis in a 3-dimensional (3d) liver co-culture model encompassing primary hepatocytes, HSC, Kupffer and endothelial cells. Fibrosis was induced in human and rat 3d liver models by multiple known pro-fibrotic agents such as ethanol, Concanavalin A or carbon tetrachloride. Our data demonstrate that this model recapitulates the cellular changes upon a pro-fibrotic challenge over an extended period of time and thus could represent a promising approach to study the processes underlying activation of hepatic stellate cells and liver fibrosis development. Furthermore, we show that this model can be used to test anti-fibrotic drug candidates which have the potential to revert the fibrotic phenotype from an efficacy as well as from a safety point of view.

Systemic absorption and metabolism of drugs in the small intestine, as well as metabolism by the liver, are key determinants of efficacy and safety for therapeutic candidates. However, these systemic responses of applied substances are ignored in most \textit{in vitro} tests. In this study, a co-culture of 3D human liver organoids and differentiated primary human small intestine epithelial tissues were combined in a dynamically-perfused Multi-Organ-Chip (MOC) system. An on-chip micro-pump enabled metabolite transport between the 2 organotypic cultures and added physiological shear stress. The liver and primary intestine equivalents were kept viable in a co-culture for 14 days. The small intestine model showed columnar epithelial morphology similar to \textit{in vivo} human small intestine, and showed expression of tight junctions and specific drug transporters. Test chemicals ethanol and troglitazone were applied to the co-culture system daily for 11 days. The effects of apical zone were applied to the co-culture system daily for 11 days. The effects of apical application onto the small intestine were compared to the effects of direct substance application onto the small intestine. In this study, a co-culture of 3D human liver organoids and differentiated primary human small intestine epithelial tissues were combined in a dynamically-perfused Multi-Organ-Chip (MOC) system. An on-chip micro-pump enabled metabolite transport between the 2 organotypic cultures and added physiological shear stress. The liver and primary intestine equivalents were kept viable in a co-culture for 14 days. The small intestine model showed columnar epithelial morphology similar to \textit{in vivo} human small intestine, and showed expression of tight junctions and specific drug transporters. Test chemicals ethanol and troglitazone were applied to the co-culture system daily for 11 days. The effects of apical application onto the small intestine were compared to the effects of direct substance application onto the small intestine. The results revealed that treatment of HepaRG cells with AFB1 and AFB2 at the IC\textsubscript{50} (90% survival) dose caused significant changes in levels of 10 microRNAs relative to control cells; 4 of these (miR-122, miR-134, miR-362-3p, and miR-410) were unique to AFB1 treatment based on levels in AFB2 treated cells. Single microRNA qRT-PCR assays verified that miR-134 and miR-410 were significantly up-regulated by low-dose AFB1 exposure, and miR-122 was significantly down-regulated. We also exposed cells to a higher dose (IC\textsubscript{90}) and found that miR-134, miR-410, and miR-122 levels were also altered in AFB1-treated cells. Further assessment of these microRNAs at both IC\textsubscript{50} and IC\textsubscript{90} doses revealed that miR-122 levels inversely correlate with exposure to AFB1 in a time- and dose-dependent manner. Additional gene expression profiling revealed down-regulation of miR-122 by AFB1 treatment or enforced down-regulation of miR-122 inhibited the expression of CYP2B6, CYP2C9, and CYP2C19. Our data suggest that miR-122 may be a sensitive biomarker for the evaluation of potential hepatotoxins and carcinogens.

HepG2 cell-based monolayer models are widely used for studying the liver toxicity of xenobiotics. Under these conditions, HepG2 cells have low expression of metabolic enzymes and drug transporters, which will compromise the value of safety assessment studies. Our recent findings have shown that HepG2 cells cultured in 3D hydrogels undergo morphological and functional differentiation with a marked upregulation of xenobiotic metabolism enzymes and transporters. Here, microarray gene expression analysis of HepG2 spheroids at different stages of spheroid development demonstrates coordinated regulation of various signaling pathways associated with cellular differentiation, development, and metabolism more similar to that of \textit{in vivo} hepatocytes. This highly differentiated phenotype could be maintained for up to 4 weeks in culture, making chronic exposures feasible. Repeated exposure studies showed an increased sensitivity in identifying several hepatotoxic compounds. Comparative transcriptome analysis in response to exposure with the action of (clofibrate, troglitazone, diethylhexyl phthalate, phenobarbital, tetrachlorodibenzo p-dioxin, imidacloprid, etc.) as well as chemicals not known to be hepatotoxins. Gene expression profiles were determined using a custom approach which measures the expression levels of approximately 1000 landmark genes, and uses a computational model trained to infer the response of all other genes (L1000; Genometry). Each of the 30 chemicals was tested at 3 concentrations, and the transcript profile was determined after 6h of exposure. All three models had significant gene expression changes to most of the chemicals tested. The responses were specific enough to identify matches in the eMAP database (Broad Institute) for the same chemical or comparable agents acting via the same mode of action. For example, the transcriptional signature of ketonoozo (a broad spectrum imidazole antifungal agent), from any of the three hepatocyte models matched the one elicited by other imidazole derivatives in the database, most prominently with miconazole, econazole and sertaconazole. These results indicate that any of the cell types may be useful as an \textit{in vitro} alternative to determine transcript profile data useful to define the chemical mode of action. HepG2 cells appeared to be better at detecting peroxisome proliferators. Disclaimer: "This abstract does not necessarily reflect U.S. EPA policy."
non-steroidal anti-inflammatory drug diclofenac was performed on HepG2 spheroids, human precision cut liver slices, primary human hepatocytes, as well as rat and mouse in vitro and in vivo models. HepG2 spheroids showed activation of stress signaling pathways and genes associated with diclofenac-induced liver diseases with a close similarity to human precision cut liver slices. Activation of genes associated with cholestasis, steatosis, mitochondrial dysfunction, and signaling pathways associated with diclofenac metabolism were identified in the transcriptome analysis. We propose that these phenotypically stable HepG2 spheroid model could serve as an improved alternative in vitro model for studying drug induced liver injury.

Human spheroids are used to a variety of man-made chemicals. Some of it can be bio-activated through biotransformation processes. The need to reduce the number of animals used for toxicity studies implies the development of reliable in vitro tools reflecting as closely as possible the human in vivo situation. In parallel, novel approaches in the field of metabolomics are being developed to seek for metabolic changes in biological systems exposed to chemicals. Ideally, in vitro models should express the largest possible metabolic capabilities, both regarding xenobiotics metabolism (biotransformation) and general metabolism (global approaches, metabolomics). We performed metabolic studies to examine the metabolic pathways and biotransformation rates of a variety of food and environmental contaminants such as bisphenol, phthalate and polycyclic aromatic hydrocarbons (Fluoranthene, Benz[a]pyrene). Concentration ranges were optimized for each contaminant and metabolic profiling was performed on differentiated HepG2 cells using radio-HPLC after a 24-h exposure. The HepG2 cell line expresses high metabolic capabilities, like human primary hepatocytes, with the advantage of having the infinite growth capacity of hepatic cell lines. Metabolomic approaches were investigated based on exposed/control HepG2 cells extracts, in the context of low concentration exposure (as low as 10-12M). Sample extracts were submitted to 1H NMR spectroscopy and NMR data were analyzed by multivariate statistical methods. We were able to discriminate between the control group and all BPA-exposed groups, with the discriminant metabolites suggesting a disruption in energetic and lipid metabolism pathways. Taken together, our data demonstrate that the HepG2 cell line is a valuable model for toxicological studies, taking into account phase I as well as phase II biotransformations, but also highly suitable for the study of subtle metabolic shifts, highlighted by metabolomic approaches.
high-intensity flashes were analyzed by the a-wave fitting model (a-wave analysis). The photopic negative response (PhNR) was also recorded at the same time points. Furthermore, histopathological examination of the retina was conducted at each time point. RESULTS. MNU progressively attenuated all the standard full-field ERGs from 1 day after dosing. This attenuation was relatively more pronounced in the rod response, which originates from the rod pathway, compared with those in the other ERGs. In the a-wave analysis, the sensitivity parameters (S) of the rod and cone a-waves decreased 1 day after dosing and unchanged thereafter. The maximum response parameter (Rmax) in the rod a-wave progressively decreased from 1 day after dosing. In contrast, the Rmax in the cone a-wave transiently increased 1 day after dosing and decreased thereafter. The changes in PhNR amplitude showed a similar time course to that of the cone Rmax. Histopathological examination of the retina revealed degeneration of the photoreceptors. The retinal lesion 1 day after dosing mainly consisted of pyknosis and karyorrhexis in the photoreceptor nucleus; depletion of the photoreceptor nucleus, and shortening and disorientation of the photoreceptor segmental linear a-wave decreased 3 and 7 days after dosing. In terms of retinal eccentricity, the equatorial zone was the most susceptible to MNU; no abnormal photoreceptors were observed in the central fovea, where the cones are tightly packed. CONCLUSION. Our results indicated that MNU affected the photoreceptors especially the rods both functionally and morphologically, leading to the retinal toxicity in monkeys.

453 Investigation of the Effect of UVC Radiation on the Toxicity of Benzalkonium Chloride
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Purpose: To investigate the effect of ultraviolet C (UVC) radiation on the toxicity of benzalkonium chloride (BAK) on human corneal epithelial cells (HCEC). Method: BAK solutions (0.005% and 0.01%) were irradiated with germicidal UVC lamp at 0.1745 J/cm², 0.5233 J/cm², 1.0467 J/cm², 2.0934 J/cm², 4.1868 J/cm², and 8.3736 J/cm². HCEC monolayers were then exposed to the UVC-irradiated BAK solutions for 5 min. After exposure, the cultures were assessed for metabolic activity using PrestoBlue assay; cell viability using confocal microscopy with live/dead/apoptotic dyes; and membrane integrity using immunofluorescent staining for zonula occludens (ZO)-1. Phosphate buffered saline (PBS) was used as a negative control. The original BAK 0.005% and 0.01% were used as positive controls. Results: The BAK toxicity on cell metabolic activity was reduced by UVC radiation in a dose-dependent manner. When the solution depth of BAK was 17 mm, the time needed to completely neutralize the toxicity of BAK 0.005% and 0.01% was 2 hours and 8 hours, respectively, with corresponding dose at 2.0934 J/cm² and 8.3736 J/cm². In the confocal microscopy study, cell viability in the cultures treated with UVC-neutralized BAK was found to be similar to the cultures treated with PBS. After exposure to the PBS and UVC-neutralized BAK, the tight junction proteins ZO-1 were well maintained and demonstrated a normal, continuous linear pattern along the cell-cell junctions. In contrast, the expression of ZO-1 was greatly disturbed by the original BAK. Conclusions: The cell toxicity of BAK can be reduced and even completely neutralized by appropriate dose of UVC radiation. Neutralizing BAK with UVC may be a unique way of detoxifying BAK in ophthalmic solutions; and may be of great value in eliminating the toxicity of BAK residue produced by BAK disinfectants in the food and health care industries.

454 Corneal Wound Healing Is Delayed by Diclofenac in a Human Ex Vivo Front-of-the-Eye Model with a Comparison to Rabbit
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Topical administration of diclofenac (DCF) to relieve pain and inflammation post-surgery delays corneal wound healing. Human and rabbit response to topical DCF (0.1%) was evaluated following an anterior keratectomy (AK) in: 1) a NZW rabbit in vivo study, and 2) ex vivo front of the eye models of rabbit and human, cultured in complete medium on a rotating platform to maintain tissue differentiation for several days. Human eyes were procured with donor consent through Midwest Eye-Banks (Ann Arbor, MI) and The Lions Eye Institute for Transplant and Research (Tampa, FL). The initial wound and progression of wound closure was visualized with fluorescein and the area quantified. The AK rabbit in vivo study revealed that DCF delayed wound closure, with a statistically significant greater wound area than time matched AK controls. In the ex vivo AK eye models, DCF significantly delayed the rate and area of wound closure in rabbit by 80% and human by 50%. To further characterize DCF effects, gene expression changes were evaluated in the ex vivo models. Overall, DCF treatment reduced the number of gene changes in both rabbit (17%) and human (30%) compared to AK only. In rabbit cornea, DCF caused an up-regulation of cytoskeletal remodeling and immune response genes and an absence of stress response genes. In human cornea, DCF suppressed many cytoskeleton and remodeling-related genes from AK. This attenuation reflected growth factors and intracellular signal molecules altered by DCF in both (30%) rabbit and human included cytoskeletal, remodeling, and apoptosis or stress networks. This translational research front of the eye model can predict human response to drugs and wound repair.

455 Müller Glial Cell (MGC) Alterations Induced by the Inhalation of Vanadium Pentoxide
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Vanadium (V) is in the air and the eye is directly exposed to the atmosphere, as well as to the elements that enter into lung and further are distributed through the systemic circulation. Ninety percent of the retina’s glia is Müller glial cell (MGC). Besides other functions this cell provides support and structural repair to the retina. Glial fibrillary acidic protein (GFAP) and Glutamine synthetase (GS) are considered injury biomarkers; therefore, changes in GFAP and GS levels are also considered biomarkers of MGC injury. To study the effect of inhaled V on retina’s MGC, male CD-1 mice inhaled vanadyl (V2O5) [0.02M] 1h twice a week for 4 and 8-week and changes in GFAP and GS expression were measured by densitometry of the retina immunohistochemistry stain for GFAP and GS photomicrographs and statistical differences were evaluated (ANOVA, post hoc Tukey test, p<0.05). GFAP increased at 4-week exposure compared to controls and 8-week group (p<0.05). At 8-week exposure, GFAP decreased to values similar to controls; a reduction in GS expression at 8-week exposure was observed (p<0.05). The 4-week increase in GFAP expression might evidence MGC reactive gliosis; however, its 8-week reduction could suggest a dedifferentiation process as a consequence of the V injury, reason why the GFAP expression was reduced. Given that GS is a specific glial enzyme whose levels are reduced by its substrate, its reduction at 8-week exposure time might suggest that photoreceptors, that produce most of the Glu in the retina, are degenerating in response to the V toxic insult. It is concluded that GFAP and GS can be used as retinal injury markers and that mice that inhaled V have retinal damage where MGC undergoes reactive gliosis and photoreceptor degeneration.

456 Circulating miRNA Biomarker of Retinal Toxicity
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Retinal toxicity is among the leading causes of attrition in drug development and drug-induced ocular toxicity remains a common issue for most therapeutic drugs. Derisking strategy to identify retinal toxicity early on with a specific miRNA biomarker approach will greatly benefit drug discovery programs in decision making, and reduce attrition associated with retinal toxicity. Two retinal injury models: NaNO3 induced retinal injury and laser-induced choroidal neovascularization (CNV) were used to validate circulating miRNAs as potential retinal toxicity biomarkers. NaNO3 was given intravenously once on Day 1 at 0, 10, 30 and 60 mg/kg in rats, and in a negative control group acetaminophen was dosed at 500 mg/kg. Retinal toxicity was assessed by ophthalmic exam and electroretinogram (ERG). We identified 3 plasma specific miRNAs that had dose-related changes upon NaNO3 treatment. At 60 mg/kg, mir-183, mir-182 and mir-96 increased significantly by 15 fold (p<0.001), 3 fold (p<0.01) and 2 fold (p<0.05) respectively, on Day 5 post-dosing. The changes in the 3 miRNAs correlated with the retinal functional alterations, i.e. reduction in b-wave amplitude as measured by ERG, suggesting potential diagnostic value of the identified miRNAs. In addition, there were no EG alteration in the miRNAs levels in acetaminophen treatment group. There were no significant changes in the reference miRNAs: mir-181, mir-92a and mir-192. For rat laser induced CNV model, we delivered different intensities of laser burns or number of laser burns. The choroidal injury was evaluated by isocitric B4 staining of whole mounts at termination on Day 14. mir-183, mir-182 and mir-96 also exhibited laser-dose dependent increase, correlated well to laser-intensity or the numbers of laser-burns in the choroid. In conclusion, this study identified and characterized 3 miRNAs in plasma using two animal retinal injury models. They have the potential to serve as specific and predictable circulating biomarkers for retinal and/or ocular injury, which will be beneficial for ocular de-risking in drug development.
Mutations in the cone-specific cyclic nucleotide-gated channel beta subunit (CNGB3) gene account for about 50% of cases of achromatopsia, an autosomal recessive retinal disease affecting functionality of cone photoreceptors. The phenotype of CNGB3−/− mice is similar to that in humans, specifically loss of cone function and early onset, slowly progressive cone degeneration, making it an appropriate disease model to study both toxicity and efficacy of the treatment. The purpose of this study was to evaluate ocular tolerability and efficacy in rescuing cone phenotype of a recombinant adeno-associated viral (rAAV) vector expressing human CNGB3 (hCNGB3) under control of a PR1.7 cone-specific promoter (rAAV2tYF-PR1.7-hCNGB3). Male CNGB3−/− C57BL/6N/tac mice were administered rAAV2tYF-PR1.7-hCNGB3 via subretinal injection into right eyes at 1 X 10⁹ or 4.7 X 10⁹ vg/eye. One group of mice received subretinal injection of vehicle in the right eyes. Animals were observed for 3 months. Slit lamp biomicroscopy, indirect ophthalmoscopy, scotopic and photopic electroretinography (ERG) luminescence-response series, and light-adapted flicker ERG were performed monthly for assessment of tolerability and cone phenotype rescue. rAAV2tYF-PR1.7-hCNGB3 was well tolerated as assessed by ophthalmic examinations when observed for up to 3 months after subretinal delivery. Starting at 1 month post-dose administration, test article-treated eyes showed an increase in single flash photopic ERG and flicker sensitivity with vehicle-treated eyes, indicating restoration of cone function at 4.7 X 10⁹ vg/eye. No adverse effects were noted on rod function. These results support further development of rAAV2tYF-PR1.7-hCNGB3 as a potential treatment for patients with achromatopsia.
and constituent amounts in products may provide guidance on whether to assess herb-drug interactions (HDIs) experimentally. The literature is replete with reports of various herbal extracts and constituents as potent inhibitors of drug metabolizing enzymes, including the cytochromes P450, as well as transporters. However, without standardized methods for herbal product characterization or in vitro testing, extrapolating these reports to clinically-relevant HDIs is difficult. This lack of a clear definition of risk prevents clinicians and consumers from making informed decisions about the safety of taking herbal products with conventional medications. A logical strategy is to borrow, as applicable, from the testing guidelines for assessing drug interactions established by regulatory agencies (e.g., FDA, EMA). For example, intestinal and hepatic in vitro systems can be used to assess potential HDIs. These data can be incorporated into PBPK models to help forecast clinical relevance. Lastly, a strategy for monitoring post-marketing signals related to potential HDIs is needed. In summary, a framework is needed that describes an integrated and sophisticated approach for assessing HDI potential of dietary supplement ingredients and products.

462 In Vitro Evaluation of Boswellia serrata Extract (BSE) As an Inhibitor of CYP450 Using Cryopreserved and Fresh Human Hepatocytes and Human Liver Microsomes

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As the use of dietary supplements increases, herb-drug interaction (HDI) potential should be evaluated. We have developed an in vitro strategy to assess HDI that uses an integrated liver system yielding physiologic intracellular concentrations of drugs and herbs. B. serrata has been traditionally used for inflammatory diseases but has no reported clinical HDI. Potent in vitro inhibition (IC50>100% across major drug metabolizing enzymes using pooled human liver microsomes (PHLM) have been reported in the literature for levels of BSE as low as 1 μg/mL. This potent level of inhibition by an herbal led us to question the relevance of the results using PHLM. In PHLM, BSE was a direct inhibitor of CYP2C9 and CYP3A4/5 with IC50 = 11 μg/mL and 1.4 μg/mL, respectively, and there was little or no evidence of time-dependent inhibition of either enzyme. We next evaluated the ability of BSE to inhibit CYP2C9 and CYP3A4/5 in pooled suspensions of human hepatocytes, which have robust metabolism and uptake activities but no biliary efflux capabilities. In this hepatocyte suspension system, considerably less direct inhibition of CYP2C9 was observed (IC50 > 50 μg/mL) for BSE. Finally, to represent the in vivo situation with both transporter and metabolic capabilities integrated together, a sandwich-cultured human hepatocyte (SCHH) system was used to assess potential HDI which would yield physiologic intracellular concentrations. In SCHH, direct inhibition of CYP3A4/5 by BSE was observed resulting in an IC50 = 17.2 μg/mL. A maximum 30% decrease in 3-OH-ibuprofen formation, a CYP2C9 specific metabolite, was observed in SCHH treated with increasing concentrations of BSE (IC50 > 75 μg/mL). There are no in vitro reports of drug interactions, despite the PHLM findings. Together, these data suggest that PHLM can provide the potential worst-case scenario highlighting the need to utilize a more physiologically relevant model that integrates transporter and metabolism to assess HDI potential.

463 In Vitro Evaluation of Schisandra Extracts (SE) As Inhibitors of CYP450 and P-glycoprotein (P-gp) Utilizing B-CLEAR® Sandwich-Culture Human Hepatocytes (SCHH)

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Herb-drug interaction (HDI) potential should be evaluated given the dramatic increase in the sale and use of alternative medicines. To assess HDI potential, we utilized a fully integrated hepatic cell system that includes drug metabolism, drug transport, and key regulatory pathways. We investigated SE for hepatocyte cytotoxicity using ATP and LDH assays following 24 or 72 hrs exposures. No marked changes were observed in either assay after exposure with S. chinensis extract (SCE) or S. sphenanthera extract (SSE) at 0.3, 3, and 30 μg/mL. Next, we examined the SE potential to inhibit CYP3A4/5 enzyme activity and P-gp function in our system. Significant inhibition of OH-midazolam formation was observed in SCHH by SCE (IC50=4.9 μg/mL) and SPE (IC50=0.8 μg/mL). Pre-incubation of SCE in SCHH prior to midazolam exposure yielded a 3-fold decrease in the CYP3A4/5 IC50 providing evidence of time-dependent inhibition; an effect not observed with SPE. Significant inhibition of digoxin efflux was observed with increasing concentrations of both SE, reducing the digoxin hepatobiliary clearance 47-72%. Finally, we examined the potential of SE to induce/suppress CYP450 or drug transporter proteins in SCHH following 72 hr of exposure to SCE or SPE. St. John’s Wort, a positive control inducer, induced CYP2B6 and CYP3A4 mRNA and enzyme activity demonstrating that key regulatory pathways in SCHH were functional. Changes were observed in gene expression for both drug metabolizing enzymes and transporter proteins in response to SE. Our results of SPE inhibition of CYP3A4/5 and P-gp are consistent with clinical data that demonstrated significant SPE drug interactions resulting in >60% increase in AUC of tacrolimus. In summary, in vitro results demonstrated that SCE and SPE both had similar inhibition profiles of CYP3A4/5 and P-gp but SCE has a higher potential to cause clinically relevant HDIs as compared to the SPE varietal because of time-dependent inhibition.

464 Safety Evaluation of Fractions Derived from Peltophorum africatum, a Medicinal Plant Used to Treat Inflammatory Pain in Southern Africa

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Based on ethnomedical and long history of use, medicinal plants are often assumed to be safe. However, misadministration of toxic plants is a significant cause of hospital admissions partly because the use of medicinal plants for therapeutic purposes is currently not regulated in most countries. In addition, some plant extracts or biologically active compounds isolated from plants are capable of causing adverse effects when consumed. Crude acetone extracts and fractions resulting from ethyl acetate extracts of dried leaf of Peltophorum africatum were evaluated in vitro cytotoxic effects using Vero (African green Monkey) and liver cell lines (H295R). In 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay methods. Crude acetone extracts of P. africatum had an IC50 value of 103.45±0.41 μg/mL in vero kidney cell lines. The results also indicated that some of the fractions had high toxicity on H295R liver cells (IC50= 7.51-21.34 μg/mL) and vero kidney cell lines (IC50= 5.2-18.96 μg/mL) respectively. The liver is a key metabolic organ in the body while detoxification and metabolism of xenobiotics also occur in the kidney. Decoctions made from leaf extracts of P. africatum is widely consumed in Southern African to treat infections, painful joints and stomach aches among others. The potential risk associated with consumption of herbal products is direct when the plants used in remedies are inherently of a toxic nature or incorrectly prepared in a manner that adversely affects the therapeutic quality of the end product. However, a source of indirect risk is the possible interaction that may occur between herbal products and orthodox medicines during concurrent use. The results indicated that some constituents of extracts of P. africatum are cytotoxic when in isolation. Therefore, it is desirable that more work be done to validate these results and evaluate potential hazards associated with consumption of extracts of P. africatum to justify its therapeutic use.

465 Epigallocatechin-3-Gallate (EGCG) Enhances the Therapeutic Effects of Leptomycin B on Human Lung Cancer Cells

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Chromosomal region maintenance 1 (CRM1) is a nuclear exporter that shuttles cancer specific proteins from the nucleus to the cytoplasm. CRM1 inhibitors, such as Leptomycin B (LMB), have been found to be promising, novel, anti-tumor drugs. The green tea polyphenol, epigallocatechin-3-gallate (EGCG), has been found to have anti-oxidative, anti-mutagenic, anti-carcinogenic, and anti-tumor properties. However, the clinical application of EGCG is still debated. The objective of the present study is to evaluate the combination therapeutic effects of LMB and EGCG, and its molecular mechanisms in human lung cancer A549 cells. Cell viability, gene expression (drug metabolizing enzymes, cell cycle, and apoptosis), and protein expression were assessed using MTT assay, qRT-PCR, and Western blot, respectively. More pronounced cell growth inhibition was observed in cells treated with LMB and EGCG compared to cells treated with LMB alone. In comparison to the vehicle control, p21, CYP3A4, and GPX1 expression levels were increased 13-, 10-, and 5-fold in 5 nM LMB-treated cells, respectively (P<0.05), while survivin was decreased 24-fold (P<0.05). Additionally, p21 was significantly increased (1.6-fold), while CYP3A4 was significantly decreased (1.7-fold) in cells co-treated with LMB (5 nM) and EGCG (20 μM), compared to the respective LMB treatment (P<0.05). The qRT-PCR results for p21 and survivin were further confirmed by Western blot. Finally, no changes in CYP1B1, GSTP1, and p53 at either the mRNA or protein levels were observed in any treatment groups. Our study is the first to show that LMB could possibly be metabolized by CYP3A4 and GPX1. From this study, it is also evident that combination treatment of LMB and EGCG in A549 cells enhances LMB-induced cell growth inhibition through the modulation of drug metabolism and p21/survivin pathways. Future investigations involving cell cycle and reactive oxygen species could further elucidate the role of EGCG and its stimulatory effect in LMB.
Several studies have indicated that kahweol, the coffee-specific diterpene, exhibits anti-oxidative, anti-inflammatory, and anti-carcinogenic activities. Although various bioactivity studies of kahweol have been performed, the molecular mechanisms by which the expression of matrix metalloproteinase-9 (MMP-9) and the invasive-ness of HT-1080 cells are regulated via kahweol remain unclear. This study inves-tigated the inhibitory effects of kahweol on tumor invasion and migration and the possible mechanisms. Kahweol suppresses PMA-enhanced MMP-9 expression at the protein, mRNA, and transcriptional levels through the suppression of NF-κB and AP-1 activation. In addition, kahweol repressed the PMA-induced phosphorylation of Akt, p38 and JNK1/2. Also, kahweol reduced PMA-induced cell inva-sion and migration. These results suggest that kahweol inhibits the PMA-induced invasion and migration of human fibrosarcoma cells via Akt/MAPK/NF-κB and AP-1 signaling.

Inhibitory Effects of Saponins Isolated from the Root of Platycodon grandiflorum on High-Fat Diet-Induced Nonalcoholic Steatohepatitis via Attenuation of Oxidative Stress, Inflammation, and Fibrosis in Rats

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The consequences of precipitously rising obesity rates and lifestyle change worldwide have accelerated the risk of liver injury due to nonalcoholic fatty liver disease (NAFLD), nonalcoholic steatohepatitis (NASH), liver fibrosis, and liver cancer. NASH is hepatic steatosis-induced oxidative stress and metabolic inflammatory disease. This study investigated the inhibitory effects of saponins isolated from the root of Platycodon grandiflorum (Changkil saponins: CKS) on high fat diet (HFD)-induced NASH in rats. CKS has many biological and pharmacological effects, including anti-inflammatory, anti-oxidative, anti-tumor, and hepatoproteective effects. HFD fed with 8 weeks led to mark NASH as assessed by body and liver weight, serum ALT and AST activities, and hepatic lipid peroxidation and collagen content, and histopathological examination. CKS reduced HFD-increased body and liver weights. CKS suppressed HFD-induced serum ALT and AST activities and hepatic malondialdehyde level. CKS attenuated hepatic collagen deposition and collagen content. CKS inhibited HFD-induced mRNA expression of MMP-13, TIMP-1, and TNF-α and protein expression of TGF-β, α-SMA, and collagen type I. Moreover, CKS inhibited HFD-induced COX-2 protein expression via inhibition of NF-κB p65 nuclear translocation and IkBα degradation. Furthermore, CKS restored HFD-reduced Nrf2-mediated HO-1, NQO1, and GST expression. These results indicated that CKS ameliorates HFD-induced hepatic oxidative Stress, inflammation, and fibrosis via induction of Nrf2-mediated antioxidant enzymes.

Inhibitory Effects of Saponins, Especially Platycocin Acid A, from Platycodon grandiflorum on Ovalbumin-Induced Mice and PMA-Exposed A549 Cells

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Allergic airway inflammation is acute and chronic pulmonary disease caused by inappropriate responses to inhaled allergens and is characterized by airway hyper-responsiveness associated with infiltration of inflammatory cells, mucus over-production, and the overexpression of inflammatory cytokines and chemokines. This study investigated the inhibitory effects of saponins isolated from the root of Platycodon grandiflorum (Changkil saponins: CKS) on ovalbumin (OVA)-induced airway inflammation in mice and PMA-induced MUC5AC expression in A549 cells. CKS has many biological and pharmacological effects, including anti-inflamm-atory, anti-oxidative, anti-tumor, and hepatoprotective effects. CKS inhibits the number of inflammatory cells and the levels of IgE, Th1/Th2 cytokines, and MCP-1 chemokine by OVA in bronchoalveolar lavage fluid. Also, CKS suppressed OVA-induced MUC5AC, MMP-2,9, and TIMP-1/2 mRNA expression, NF-κB activation, inflammatory cell infiltration, and mucus production in lung tissue. Moreover, the active component of CKS, platycocin acid A (PA), suppressed PMA-induced MUC5AC mRNA expression by inhibiting NF-κB activation via Akt in A549 cells. These results suggest that CKS or PA inhibited the development of airway inflammation, hyper-responsiveness, and remodeling by reducing allergic responses, and they may be an effective alternative therapy for allergy-induced respiratory disease.

Inhibitory Effects of Saponins Isolated from the Root of Platycodon grandiflorum on Ovariectomy-Induced Bone Loss in Mice via Osteoblast Differentiation Stimulation and Osteoclast Suppression

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Osteoporosis is the result of imbalance both osteoblasts and osteoclasts and is characterized by skeletal degeneration with low bone mass and destruction of the microarchitecture of bone tissue which is attributed to various factors including hormonal imbalance, chronic diseases, and medications. Osteoporosis is a major health problem in postmenopausal women. This study investigated the molecular mechanism of anti-osteoporosis of CKS in vivo and in vitro model. Saponins isolated from the root of Platycodon grandiflorum (Changkil saponins: CKS) has many biological and pharmacological effects, including anti-inflammatory, anti-oxidative, anti-tumor, and osteoblast differentiation effects. After 4 weeks of recovery from surgery, the ovariectomized (OVX) mice were randomly divided into three groups and orally treated with saline or CKS for 3 weeks. CKS suppressed OVX-increased body weight. CKS restored OVX-reduced serum level of alkaline phosphatase, phosphorus, and calcium. CKS improved the bone deterioration of trabecular microarchitecture. Furthermore, platycodin D (PD), active component of CKS, increased osteoblast differentiation via ALP mRNA expression and ALP activity in cells. Also, PD suppressed receptor activator of nuclear factor κB ligand (RANKL)-induced osteoclast differentiation. PD suppressed RANKL-induced TRAP, OSCP, and Cathepsin K mRNA expression via inhibition of c-Fos and NFATc1. PD suppressed RANKL-induced NF-κB activation via ERK1/2, p38, and Akt phosphorylation inhibition. These results suggest that CKS improve estrogen deficiency osteoporosis via osteoblast differentiation stimulation and osteoclast suppression.

Effect of Impressic Acid Isolated from Acanthopanax koreanum on Endothelial Nitric Oxide Synthase Activation in Endothelial Cells


Nitric oxide produced by endothelial nitric oxide synthase (eNOS), key regulator of vascular functions, has anti-thrombotic and anti-atherosclerotic activities in the vasculature. This study investigated the intracellular pathways underlying eNOS activation by impressic acid (IPA). IPA, 3α,11β-dihydroxy-20(29)-en-28-sce acid, is a lupane-type triterpenoid isolated from Acanthopanax koreanum, which has been used as a Korean folk medicine for rheumatism, hepatitis, diabetes, and inflammatory disorders. However, the anti-thrombotic and anti-atherosclerotic activities of IPA and its pathways remain unclear. IPA increased the phosphorylation of eNOS and the production of nitric oxide in a concentration- and time-dependent manner in human endothelial cells. IPA increased the phosphorylation of Akt, ERK1/2, JNK1/2, and p38. Furthermore, IPA increased the phosphorylation of AMP-activated protein kinase (AMPK), and calmodulin-dependent protein kinase II (CaMK II). Also, IPA-induced eNOS phosphorylation was blocked by respective inhibitors of Akt, MAPKs, AMPK, and CaMK II. These results suggest that IPA may have a possible potential of vasoprotective effect, stimulating eNOS phosphorylation and NO production via the activation of Akt, MAPKs, AMPK, and CaMK II.

Protective Effect of Rutacearpine on Tert-Butyl Hydroperoxide-Induced Apoptosis by Upregulation of Nrf2-Mediated HO-1 Expression via Akt Pathway in HepG2 Cells

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Rutacearpine, a quinazolinocarboline alkaloidal compound, is a natural product isolated from Evodia rutacear and has various biological and pharmacological effects, including vasodilation, anti-thrombosis, and anti-inflammatory proper-
ties. However, the anti-oxidative effect of rutacarpine and its intracellular pathway remain unclear. This study investigated the protective effects of rutacarpine on tert-butyl hydroperoxide (t-BHP)-induced oxidative stress in HepG2 cells. Rutacarpine restored t-BHP-reduced cells viability by inhibition of ROS generation. Also, rutacarpine increased HO-1 expression via activation of Nrf2 nuclear translocation. Furthermore, rutacarpine-induced HO-1 expression was reduced by inhibition of Akt phosphorylation. These results suggest that rutacarpine could be beneficial in hepatic disorders by scavenging ROS and to regulate the antioxidant enzyme HO-1 expression via Akt signaling pathways.

Nonalcoholic fatty liver disease (NAFLD) is considered to be the most common hepatic manifestation of metabolic syndromes such as diabetes and obesity. The dysregulation of triacylglycerol metabolism may contribute to the derangement of hepatic lipid homeostasis and chronic liver damage. In this study, we evaluated body weight, liver histology, and hepatic lipid content in high-fat diet (HFD)-fed ICR mice treated with water extract of the stem of Acer tegmentosum ICIR mice treated with water extract of the stem of Acer tegmentosum.

Acer tegmentosum

ICIR mice treated with water extract of the stem of Acer tegmentosum.

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Use of Innocuous Natural Products to Modulate Membrane-Cytoskeletal-Dependent Ras Signaling

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The relationship between plasma membrane order and Ras activation is dictated by interactions involving transmembrane receptor signaling, membrane biophysical properties and cytoskeletal components. Using two distinct intestinal models, we investigated how chemoprotective n-3 polyunsaturated fatty acids (PUFA), e.g., docosahexaenoic acid (DHA, 22:6n-3), and eicosapentaenoic acid (EPA, 20:5n-3) all suppressed the temporal and spatial activation of Ras. For this purpose, giant plasma membrane vesicles (GPMVs) were generated from immortalized mouse colonic epithelial (YAMC) cells in the presence or absence of exogenous PUFA. These vesicles retain the endogenous composition of the plasma membrane without the attachment of the cytoskeleton. Using a membrane order sensitive dye, G-14, we subsequently quantified membrane order in whole cells and GPMVs isolated following treatment with n-3 PUFA vs control. n-3 PUFA increased membrane order in GPMVs, while exhibiting the opposite effect in whole cells, indicating a critical role for the cytoskeleton. In complementary experiments, the functional significance of n-3 PUFA modulation was assessed by monitoring Ras activation using fluorescence resonance energy transfer (FRET) biosensors, targeted to specific membrane ordered (H) or disordered (K) domains. Interestingly, only DHA and not EPA suppressed the temporal activation of membrane ordered and disordered targeted Ras following stimulation with epidermal growth factor. These data demonstrate that chemoprotective n-3 PUFA uniquely modulate cell membrane order and Ras activation in a cytoskeletal dependent manner.

A Comprehensive Toxicological Safety Assessment of an Aqueous Extract of Polypodium leucotomos (Fernblock®)


A commercial product—Fernblock®—prepared by aqueous extraction of the aerial parts of the tropical fern Polypodium leucotomos, has been studied and shows photoprotective properties as an oral and topical agent against the harmful effects of ultraviolet exposure from sunlight. A battery of toxicological studies was conducted in accordance with internationally accepted standards and protocols to investigate the in vitro and in vivo genotoxicity and repeated oral toxicity in rats of Fernblock®, and thereby, extrapolate to predict the safety of chronic oral consumption by humans. No evidence of mutagenicity was observed at concentrations up to 5000 µg/plat and 5000 µg/mL in a bacterial reverse mutation test (AMES) and in vitro mammalian chromosomal aberration test, respectively. Additionally, there was no genotoxic activity observed in an in vivo mouse micronucleus test at concentrations up to the limit dose of 2000 mg/kg bw. Two repeated oral toxicity studies were conducted in male and female Crl(W)BR Wistar rats. In the first study, no mortality or toxic effects were observed and no target organs were identified at doses administered for 14 days by gavage up to the maximum dose of 5000 mg/kg bw/d. Based on these results, a 90-day study was conducted at 0, 300, 600, and 1200 mg/kg bw/d. No mortality or treatment-related adverse effects were observed and no target organs were identified. The NOAEL from the 90-day study was determined to be 1200 mg/kg bw/d, the highest dose tested. Based on these results we extrapolate a level of safe human consumption of 840 mg/day (12 mg/kg bw/d) using a 100-fold safety factor and an average 70 kg person.

Safety Evaluation of the Aqueous Extract of the Leaves of Moringa oleifera in Rats

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Moringa oleifera Lam. is the most widely cultivated species of the monogenic family Moringaceae (order Brassicales), which includes 13 species of trees and shrubs distributed in sub-Himalayan ranges of India, Sri Lanka, North-Eastern and South-Western Africa, Madagascar and Arabia. This plant has been well documented for its medicinal importance for a long time. The stem bark, root bark, fruit, flowers, leaves, seeds, and gum are widely used in Indian folk medicine. The aqueous extract from the leaves of Moringa oleifera was evaluated for its oral toxicity by the oral route, and for the sub-acute toxicity on haematological, biochemical and histological parameters in rats. In the acute toxicity test, M. oleifera extract caused no death in animals even at 2000 mg/kg dose. Oral treatments in rats with this extract at 400, 800, and 1600 mg/kg caused varied significant changes in the total red blood cell (RBC), packed cell volume (PCV), haemoglobin concentration (HB), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), total and differential white blood cell (WBC) count. The extract did not cause any significant change in the level of platelets. In the biochemical parameters, the extract at different doses also caused varied significant changes in the levels of total proteins, liver enzymes, and bilirubin. Clinico-pathologically, changes were also noted in the body weights of the animals. There was slight dullness at the onset of extract administration but no significant changes were noticed in all the organs examined in the course of this study. The study thus showed that the plant is relatively safe both for nutritional and medicinal uses.

Safety Investigation of Herb Extracts: Protective Effects of Phikud Navakot against Oxidative Stress in HepG2 Cells

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Phikud Navakot (PN), which comprises nine species of herbs, has been widely used in Thai traditional medicines for treating symptoms of cardiovascular diseases, hyperlipidemia and cerebrovascular disorder. This study aimed to investigate the effects of hydroethanolic extract from PN to protect against H2O2-induced oxida-
tive stress in human hepatoma cell line, HepG2. The levels of intracellular reactive oxygen species (ROS), glutathione (GSH), and antioxidant enzyme activities were measured as biomarkers of cellular oxidative status. Treatment of HepG2 cells with 500 μM H$_2$O$_2$ for 3 h induced 6-fold ROS formation and caused about 50% decrease in cell viability. However, pretreatment of the cells with PN at 0.01–1 mg/mL effectively reduced intracellular ROS level and attenuated the cell survival in a concentration-dependent manner. In addition, the PN extract also prevented the depletion of total GSH and increased the GSH/GSSG ratio. Pretreatment of PN significantly augmented the activity of antioxidant enzymes such as superoxide dismutase, catalase, and glutathione reductase. These data suggest protective effects of PN against H$_2$O$_2$-induced oxidative stress in HepG2 cells by modulation of GSH level and activation of antioxidant enzymes.

### 477 Mining Maple for In Vitro Anti-Inflammatory and Anti-Hyperglycemic Properties

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Maple syrup is a natural sweetener produced by concentrating the watery sap collected from maple species. Maple sap and syrup extracts have previously reported anti-oxidant, anti-proliferative, anti-radical and anti-mutagenic activities. Recently, our laboratory has been involved in the isolation of bioactive compounds present in maple sap and maple syrup and we have shown that maple syrup extract and their pure constituents inhibit the activities of α-glucosidase and α-amylase enzymes using in vitro models. However, until now, the anti-inflammatory and anti-hyperglycemic activity and the associated molecular mechanisms elicited by maple syrup extract have not been thoroughly studied. In this project, we have focused our attention on investigating the biological activities of novel phenolic-rich extract derived from maple syrup for future nutraceutical application. The in vitro anti-inflammatory effects of a maple syrup ethyl acetate extract (MS-EtOAc) and 15 purified phenolic constituents were evaluated in a lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophage cell model. RAW 264.7 cells were co-treated with 50 ng/mL LDS and MS-EtOAc for 24 hours and then nitric oxide (NO), prostaglandin-E2 (PGE2) levels along with upstream inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (Cox2) gene and protein expressions were quantified. The activation of nuclear factor-kappa B (NF-κB) was measured using luciferase activity. MS-EtOAc downregulated NO, PGE2 and iNOS expression (at 10-100 μg/mL) through suppression of NF-κB transcriptional activation. Among the 15 pure isolates, (E)-3,3′-dimethoxy-4,4′-dihydroxystilbene was most effective in decreasing both NO and PGE2 levels. However, 4-acetylchelate, tyrosol, and protocatechuic acid only reduced PGE2 levels. Our data illustrates that the potential anti-inflammatory activity of MS-EtOAc can be attributed to its unique combination of compounds and not as a result of a single purified constituent alone. Thus, for evaluating anti-hyperglycemic properties, human HepG2 cells were treated with the maple syrup extract for 7 h. Glucose consumption, AMP activated protein kinase (AMPK) activation and its target genes expression were measured. The glucose levels were reduced in the hepatocytes through AMPK activation. Taken together, we demonstrated that a novel maple syrup extract exhibited anti-inflammatory and anti-hyperglycemic activities in vitro.

### 478 Potential Effects of Dietary Novasil Clay on Selected Tissues of Sprague-Dawley Rats

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Aflatoxins (AF) are metabolites of the fungi Aspergillus flavus and Aspergillus parascitus. They occur naturally and are ubiquitous in many staple foods, including maize and peanuts. Among the strains of AF, AFB1 is the most toxic and known human carcinogen. Previous studies have identified that NovaSil clay (NS) has the potential to selectively bind the toxin in the GI tract of animals and reduce AF bioavailability. The objective of this study was to investigate whether the use of NS can prevent aflatoxicoses can adversely affect the nutrient and non-nutrient element levels in selected tissues of Sprague-Dawley (S-D) rats. Twenty male S-D rats, 5-6 weeks old, were divided into two groups: NS-treated and NS-untreated (control) groups. The NS-treated group received 2% of NS in their basal feed whereas the NS-untreated group received the basal feed only. The NS-treated group was euthanized and brain, liver and bone tissues were collected for elemental analysis. Results indicate no significant differences between the NS-treated vs. controls in the levels of 22 of the 23 micro-nutrients and metals analyzed. Strontium (Sr) was the only element with levels significantly different in the liver (NS-treated = 0.015±0.001 ppm vs. control = 0.009±0.000 ppm [p=0.0001]), brain (NS-treated = 0.153±0.058 ppm vs. control = 0.047±0.022 ppm [p=0.0410]), and bone (NS-treated = 78.650±2.357 ppm vs. control = 53.290±1.127 ppm [p=0.0001]) suggesting bioavailability of this mineral. Sr is naturally present in water and foods, such as cereals, grains and sea foods at levels up to 25 mg/kg. Naturally occurring Sr, other than its radioactive isotope, is considered non-toxic. Also, it must be mentioned that Sr is reportedly used in the treatment of osteoporosis. This study suggests that minerals in NS do not affect the liver, bone and brain tissues; indicating that humans ingesting up to 0.25% NS for preventing AF exposure may not experience adverse health effects.

### 479 Studies on the Teratogenicity of Anabasine in a Rat Model


A number of plant toxins have been shown to be teratogenic to livestock. The teratogenic action of some of these alkaloids is mediated by nicotinic acetylcholine receptors (nAChR). However, for many of these alkaloids it is difficult to obtain sufficient quantities of individual alkaloids to perform teratology studies in livestock species. Therefore the objective of this study was to determine if a rat model can be utilized to characterize the teratogenic nature of individual plant toxins that are nAChR agonists. In this study, we evaluated the teratogenicity of anabasine, the toxic compound in tree tobacco, by feeding pregnant rats anabasine-containing rodent chow from gestational day (GD) 6-21. On GD21, the dams were euthanized and the gravid uterus were removed. The gravid uterus and individual pups were weighed. The pups were evaluated for bone malformations including cleft palate and scoliosis. Overall, the results of this study suggest that the rat is not a good model to study the teratogenicity of plant toxins that are nAChR agonists. It is possible that in the rat model, anabasine administered orally via the chow may not result in sufficient reduction in fetal movement to cause the significant malformations observed in livestock species.

### 480 In Vitro Filarial Activity, Cytotoxicity, and Phytochemical Analysis of Crude Extracts of Daniellia oliveri and Psorospermum ferbifugum

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Subcutaneous filariasis, otherwise known as onchocerciasis or river blindness is a debilitating disease of the poor, caused by the filarial nematode, Onchocerca volvulus. It is the world’s second leading infectious cause of blindness. About 37 million people globally suffer from onchocerciasis, with an estimated 120 million at risk. Current control of onchocerciasis relies on mass drug administration of ivermectin, which is only microfilaricidal. Effective control therefore is hindered by the lack of an adulticide, thus emphasizing the need for the search of new products that will target adult worms. The leaves and stem bark of Daniellia oliveri and Psorospermum ferbifugum were collected from Bambui, NW Cameroon based on local ethnopharmacological information. The plant parts were dried at 40°C and sequentially macerated in hexane, methylene chloride and methanol to generate 12 extracts. These extracts were tested for both micro- and macro-filaricidal activities on Onchocerca volvulus based on motility reduction and the MTT/ formazan assay respectively (Comley et al., 1988). Cytotoxicity of all active extracts was assessed on N27 cells using the MTS assay. Of the 12 extracts tested, 8 were active on microfilariae while 2 of 5 extracts tested were active on adult worms. The hexane extract of P. ferbifugum ranked the highest activity on microfilariae (IC50 4.0 μg/mL and IC100 7.8 μg/mL) while the hexane extract of D. oliveri ranked the highest activity on adult worms (IC50 <15.6 μg/mL and IC100 31.3 μg/mL). Importantly, these extracts were more selective for parasites than N27 cells. Phytochemical analysis of these extracts revealed the presence of alkaloids, steroids, flavonoids, saponins and cardiac glycosides. Supported by: - Schlumberger Foundation “Faculty For The Future” Program; - NIH R01AI047194 to RJM and R21AI092185 to APR.
481 Inhibition of Intestinal Cell Proliferation Activity Induced by Jatropha Phorbol Ester May Cause Weight Loss and Death In Vivo

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[Purpose] Jatropha curcas is increasingly noted as a biodiesel feedstock in the world. Jatropha seed contains potential tumor promoter, phorbol esters, for example12-deoxy-16-hydroxyphorbol-4'-(12',14'-butadienyl)-6'-(16',18'-nonanatrienyl) bicyclo [3,1,0] hexane-(15-O)-2'-[carboxylic acid]. [16-O]-3'-[8'-butenioic-10'] acete (DHPB). We previously reported that 2.5 mg per mouse of transdermal DHPB led to weight loss. Dosage above 5 mg per mouse of DHPB caused death in 3 weeks. The mice treated with the same dose of 12-O-Tetradecanoylphorbol 13-acetate (PTA) did not show weight-loss and lethality. Gastrointestinal bleeding and splenic atrophy were observed in the dead mice. Mechanism of DHPB toxicity has not been fully elucidated in this study, assessing DHPB and PMA. [Experimental procedures] DHPB and PMA were tested in the colon cancer and myeloma cell lines. Activity of cell proliferation, cytotoxicity and cell death were analyzed by WST-1 cell proliferation assay, LDL cytotoxicity assay. [Results] High concentrations of DHPB and PMA induced suppression of cell proliferation in Caco-2, HCT-116 and SP2/0-Ag14 dose-dependently. DHPB caused decrease of cell proliferation activity at lower concentration (at pg/mg) as compared with PMA. Both DHPB and PMA did not showed marked LDH leaching cytotoxicity. [Conclusion] These results suggested that high concentration of transdermal DHPB may strongly inhibit replacement of colon cells, leading to cell-death in vivo. This study was supported by JICA/JST, SATREPS (Science and Technology Research Partnership for Sustainable Development), Japan.

482 Chronic Toxicity of Phorbol Ester, Crude Oil, and Biodiesel Fuel from Jatropha curcas


[Purpose] Jatropha curcas attracts rising attention as a biodiesel feedstock in the world. However, Jatropha contains various toxic components, generating concerns about its health effects. One of toxic components is potential tumor promoter, phorbol esters. We previously examined acute toxicity of Jatropha phorbol ester. However, in this study, chronic toxicity of main component of Jatropha phorbol esters, Jatropha crude oil, and biodiesel fuel (BDF) were evaluated in mice. [Experimental procedures] One of the Jatropha phorbol esters (DHPB), Jatropha crude oil, and BDF were applied onto skin of mice twice a week after single application of tumor initiator. As a positive control, 12-O-Tetradecanoylphorbol 13-acetate (PTA); a known tumor promoter, was applied. We set the development of papilloma and death as the end points of experiment. [Results] Latency for papilloma development was 53.0± 0.069 days (DHPB), 50.3± 0.002 mg/day/g body weight (PTA); a known tumor promoter, was applied. We set the development of papilloma and death as the end points of experiment. [Results] Latency for papilloma development was 53.0± 0.069 days (DHPB), 0.014±0.002 mg/day/g body weight (DHPB), 1.29±0.69 mg (0.05±0.002 mg DHPB equivalent)/day/g body weight (crude oil). Jatropha BDF also caused papilloma although it does not contain any phorbol esters. Number of papilloma was 13±2.3/mouse (PTA), 2.3±2.3/mouse (DHPB), and 2.4±1.7/mouse (crude oil). Latency for death was much shorter in PMA, followed by DHPB, crude oil group and control group. LD50 for chronic exposure was 0.012 µg/day/g body weight (PTA), 0.019 µg/day/g body weight (DHPB) and 1.367 mg (0.035 µg DHPB equivalent)/day/g body weight (crude oil). [Conclusions] Chronic exposure to Jatropha phorbol ester and crude oil developed papilloma in mice as well as PMA indicating that Jatropha phorbol ester has tumor promoter activity. Development of papilloma caused marked decrease in survival rate of mice. This study was supported by JICA/JST, SATREPS (Science and Technology Research Partnership for Sustainable Development), JAPAN.

483 Modulation of Multidrug Resistance by Phytochemicals in Breast Cancer

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Cancer accounts for nearly one-quarter of deaths in the United States, exceeded only by heart diseases. Environmental polycyclic aromatic hydrocarbons (PAHs) such as 7, 12- di-methylbenz(a)anthracene (DMBA) induce mammary tumors in rodents. Taxanes (paclitaxel and docetaxel) are the most successful chemotherapeutic agents used for the treatment of breast and ovarian cancer. However, multidrug resistance (MDR) to these anticancer drugs is a major obstacle for the overall response and survival of breast cancer patients. Drug metabolizing enzymes (DMEs) includes phase I metabolizing enzymes, phase II detoxifying enzymes, as well as phase III transporters play central roles in the metabolism, elimination and detoxification of drugs introduced into the body. Many studies have revealed the ability of dietary phytochemicals in vegetables and fruits to reduce the risk of cancer. It is estimated that roughly 50% of cancer patients use some kind of dietary supplements. In the present study, we evaluated the potential of chemopreventive and dietary phytochemicals, curcumin and sulforaphane as broad-spectrum modulators to reverse MDR mechanisms. The wild type (C57BL/6) female mice were exposed to the environmental PAH (DMBA), taxanes and dietary phytochemicals, alone and in different combinations. We assessed the gene expression levels of various drug metabolizing enzymes, drug transporters and receptors. Our studies show that DMBA mediated induction of mammary and hepatic CYP1A1, B1 and AHR gene expressions were suppressed and phase enzyme levels were significantly dropped down by chemopreventive phytochemicals, sulforaphane and curcumin. Our results also show that the phytochemicals inhibited the DMBA and taxane induced hepatic CYP3A11, PXR and mammary P-gp gene expression levels compared to controls, thus increasing the intracellular level of the chemotherapeutic drug. Our findings reveal that administration of the phytochemicals in combination was more efficacious than individual components. Our studies will help us to develop an efficacious and low toxicity dietary and chemopreventive phytochemicals for breast cancer chemotherapy.

484 Exposure Measurement Error and the Impact of Nutrition in Studies of Inorganic Arsenic and Cardiovascular Endpoints


Concern over whether inorganic arsenic (iAs) exposure could increase the risk of cardiovascular disease and other cardiovascular (CV) outcomes has led to increased research over the past decade. Studies of populations outside of the US (primarily Taiwan, China, India, and Bangladesh) suggest that there may be an association between high iAs exposure levels and CV outcomes. These associations are observed consistently only in populations exposed to water with iAs concentrations above 100 µg/L, but not at low-dose iAs exposure (<100 µg/L). We reviewed 49 studies of iAs and CV effects, focusing on the characterization of exposure in each study. We also evaluated how nutritional factors may influence the metabolism of iAs, potentially leading to changes in internal dose of iAs and its metabolites from the estimates reported in the studies. We found that none of the studies that estimated exposure based on the concentration of iAs in water fully considered other sources of exposure, thus leading to an underestimation of iAs exposure. We estimated that exposures could be at least two-fold higher than when estimated based solely on drinking water iAs concentration. Moreover, because nutritional factors affect iAs methylation, the internal dose in studies of unexposed populations is expected to be higher than in well-nourished populations, even at the same level of iAs intake. While our analysis focused on CV effects, we conclude that exposure measurement error is not specific to this endpoint, and the same biases are likely present in studies that investigated other endpoints in the same cohorts.

485 Arsenic Concentration in Nail and Drinking Water Samples from a Brazilian Population Exposed Due to Mining Activity

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The arsenic (As) is the first substance with high priority, and is associated with various types of cancer. One of the most important anthropogenic sources of arsenic in the world is mining, which contributes to contamination of soil, water, air and foods. In Brazil, environmental contamination by arsenic occurs in Paracatu city- MG, due to the operation of a gold mine which have been detected high As concentrations in the environment. However, little is known regarding the risks of exposure of the local population to arsenic . Thus, the aim of this study was to evaluate the concentration of arsenic in nail and drinking water samples from residents of mining region. Were made collection of 600 nail samples and 600 water samples of residents near and distant from mining region (Study approved by the Ethics Committee for Human Research FCFRP-USP-12432813.4.0000.5403). The collections were made on 2013. The concentration of As in nails and drinking water was determined by Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Tetramethylammonium hydroxide was used for nail sample preparation, and water drinking was prepared with dilution in nitric acid for direct analysis. The average concentration of As in nail samples was 221.28 µg L-1, with values found since 1.0 µg L-1 until 31.503.38 µg L-1. The concentration in drinkin water was since 0.1 µg L-1 until 80.0 µg L-1. Some water samples had concentrations above the upper limit recommended by the World Health Organization (WHO). The As concentration in nail samples was upper than values from other populations. These values indicate that the population have high exposure to the element an is subject to its toxic effects. Acknowledgement: Capes, Fapemig, CNPq, UFVJM.
Major human environmental health concern has been associated with inorganic arsenic (As) in drinking water in which dissolved As is highly bioavailable. As arsenic levels in drinking water have been reduced, health concerns have been raised regarding the extent of As exposure via food and other potential sources. The extent of As bioavailability in food is not well characterized while soil relative bioavailability is variable. Dietary and other human exposure media may have lower relative bioavailability (RBA) that present in drinking water. Establishing RBA is important for understanding As exposure potential for various media. RBA for As in soil has been well characterized, but coal fly ash and bottom ash are other potential exposure media that have not been well characterized. A literature search on As bioavailability was conducted to evaluate the contribution of food, soil and coal combustion ash to As exposure. Study inclusion and exclusion criteria were established to evaluate more than 700 identified studies. Approximately 70 provided information useful to address aspects of As RBA. Database strengths include application of in vitro accessibility methods which provide critical information for understanding uptake in terrestrial and aquatic environments; database limitations identified include the lack of reporting of As concentrations in exposure media and quantitative measures of systemic levels in human studies. The main sources of dietary As intake in the U.S. include rice, vegetables, fruits, grains and seafood, with food contributing approximately twice the intake compared to drinking water. Thus when water concentrations are low, RBA of As in various foods determines the relative exposure contribution and helps focus efforts to control exposure from specific foods. In some populations, rice is the greatest source of As. The reported range of As RBA for rice is 40-100%. RBA estimates from the literature for different foods and exposure sources are used to illustrate relative exposures.

**487 Estimating Children’s Soil and Dust Ingestion Rates Using Blood Lead Biomonitoring at the Bunker Hill Superfund Site in the Silver Valley of Idaho**


Soil/dust ingestion rates are among the most sensitive variables affecting children’s health risks at contaminated sites. Estimates of childhood ingestion rates of soil and house dust are largely based on soil tracer methodology, which are limited by analytical uncertainty, small sample size, and short study duration. To address the need for better soil and house dust ingestion rate estimates, we measured the bioavailability and lead concentrations in archived soil and dust samples from the Bunker Hill Superfund Site (BHSS). We adjusted ingestion rates and the soil/dust source partition to achieve predicted geometric mean blood lead levels in children best approximating measured blood lead levels. Bioavailability was measured, using the USEPA in vitro method, in 78 soil and 193 house dust samples. Children’s blood lead surveys were conducted between 1988 and 2002, which captured more than 50% of resident children for 15 consecutive years at the BHSS (n = 5,399). Absolute soil and house dust bioavailability averaged 33% and 28%, respectively. This analysis estimated a combined soil/dust ingestion rates of 64 mg/day for children up to 84 months old. Our results support reducing the default combined soil/dust ingestion rates, currently 109 mg/day in the USEPA – Integrated Exposure Uptake Biokinetic (IEUBK) model by approximately 40% based on concordance between IEUBK model predictions and blood lead observations. Disclaimer: The views expressed are those of the authors and do not necessarily reflect the views or policies of the USEPA.

**488 Lead Exposure in São Paulo, Brazil Blood Levels and Risk Factors Associated**

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INTRODUCTION: In Brazil, there are scarce data on lead (Pb) contamination, especially for the more vulnerable population composed by preschool children. Information on the prevalence of the exposure to lead is essential to formulate public health policies. The aim of this cross-sectional study was to estimate the blood Pb levels (BLL) in children attending Day Care Centers (DCCs) in Sao Paulo, Brazil and evaluating the risk factors associated to this exposure. METHODS: This study included 50 DCCs, totalizing 2,463 children aged 1-4 years. Venous blood samples were analyzed by inductively coupled plasma mass spectrometry with quadrupole (q-ICP-MS) and dynamic reaction cell (DRC-ICP-MS). Prior to analysis by ICP-MS, 200 μL of each blood sample was diluted 1:50 into a 15-mL polypropylene Falcon® tube with a solution containing 0.01% (v/v) Triton® X-100, 0.5% (v/v) nitric acid and 10 μg L-1 of Rh as their internal standard. Questionnaires were applied to the parents and descriptive statistics was used. RESULTS: We present here the preliminary results of 1,973 children attending 47 DCCs. Geometric mean for BLL was 2.2 μg/dL (95% CI: 2.1–2.3 μg/dL) and the 97.5th percentile was 16.2 μg/dL. Furthermore, we found some associations, such as geographic region of BLL, indicating specificity for lead poisoning. Another one was that the higher the mother’s education level, the lower the concentration of blood lead in their children. CONCLUSIONS: The current reference value by CDC is 5 μg/dL, which is based on the 97.5th percentile of the National Health and Nutrition Examination Survey (NHANES)’s BLL distribution in children. Summing up, these preliminary results showed that, considering the Fourth National Report on Human Exposure to Environmental Chemicals by CDC (2013), in Brazilian children, the BLL are much higher than those found in the U.S. children. This study was approved by Ethical Committee (128.943) and funded by FAPESP (Grants 2011/13076-0, 2011/23272-0 and 2012/21840-4).

**489 Evaluation of Hepatic Function Biomarkers and Bone Alkaline Phosphatase in a Population Chronically Exposed to Fluoride through Drinking Water**


Globally, an estimated 200 million people are exposed to high concentrations of naturally occurring fluoride that exceeds the WHO guideline of 1.5 mg/L in drinking water. Chronic fluoride exposure can weaken bone and increase the risk of fractures. Besides, it has been reported the alteration in liver membrane lipids in chronic fluorosis. The aim of the study was to determine the relationship between the fluoride exposure and the bone alkaline phosphatase (b-ALP), which is directly related to bone turnover, dental fluorosis and liver function in an adult population. A cross-sectional study was conducted in 239 participants in the age group between 18 and 77 (mean 46 ± 13.3 years) who living in normal (control) and two endemic fluorosis areas of Chihuahua, Mexico. Dental fluorosis was evaluated according Dean’s index classification, 17% presented moderate to severe dental fluorosis. The concentration of fluoride in drinking water and urine varied from 0.1-5.12 mg/L and 0.34-22.7 mg/g/mL, respectively, almost 46.2% of urine samples contained fluoride levels higher than the Biological Exposure Index of 2 mg/L. Activity of b-ALP in plasma were decreased in endemic fluorosis areas relative to control group (p<0.045). Activities of total alkaline phosphatase, aspartate aminotransaminase, alanine aminotransaminase and gamma-glutamyltransferase in plasma were not related to the concentration of fluoride in urine. In conclusion the chronic exposure to high fluoride exposure had negative effects on b-ALP. Nevertheless had a small but significant impact on bone turnover. Further studies are needed to know the relationship between plasma b-ALP and bone mineralization in the chronic fluoride exposure. Funded by Conacyt (grant 180847).


Risk assessors develop exposure scenarios using facts, data, assumptions, inferences, and professional judgment to develop estimates of exposure, dose, and risk. The U.S. EPA 2004 Example Exposure Scenarios and 2014 Child-Specific Exposure Scenarios Examples present a limited number of exposure scenarios that can be used when conducting risk assessments for EPA and other organizations. To supplement these resources, EPA has developed the Exposure Factors Interactive Resource for Scenarios Tool (ExpoFIRST) to enable assessors to define a broader range of exposure scenarios for various receptor populations and demographic variables based on data from the Exposure Factors Handbook: 2011 Edition (EFH). The tool uses exposure factors from EFH to parameterize user-specified scenarios with respect to route of exposure, medium, receptor(s), timeframe, and dose metric for a contaminant of concern. Assessors can then modify initial parameters as appropriate to account for assessment-specific knowledge and use the tool to calculate deterministic, “screening-level” exposure estimates as point estimates. The tool will be made available to users through the Exposure Factors module of the EPA-Expo-
Formulation Development and Validation of an Analytical Method for a Combination-Dose Formulation of Emtricitabine, Tenofovir Disoproxil Fumarate, and Efavirenz in Support of Rodent Toxicology Studies

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Treatments for acquired immunodeficiency syndrome (AIDS) generally include combination therapies of antiretroviral agents. The regimen of Efavirenz (EFV), Emtricitabine (FTC), and Tenofovir (TDF) is being used for the treatment of HIV-1 infection in adults as well as pediatric patients. However, there is limited nonclinical safety information, specifically in the developing offspring. In this work, a procedure was developed to formulate FTC/TDF/EFV in 1:1:5.3 ratio (consistent with the clinical dose ratio) in 0.5% aqueous methylcellulose for gavage administration in rodents. The analysis method, ultra-performance liquid chromatography coupled with photodiode array detector, was validated over the range 1,000/1,500/3,000 to 24.0/36/57.20 mg/mL of FTC/TDF/EFV in 0.5% methylcellulose for determination of all three compounds in a single chromatographic run. Validation parameters included linearity (r ≥ 0.9997), accuracy (± 6.3%), and precision (± 3.6%). The method was suitable for analysis of formulations with concentrations up to 32/48/96 mg/mL (FTC/TDF/EFV) by diluting with vehicle. Homogeneity was successfully demonstrated on formulations containing FTC/TDF/EFV at 57/5/15 mg/mL (low) and 32/48/96 mg/mL (high) with relative standard deviations ± 4.6%. Stability was evaluated at 57/5/15 mg/mL (low) and 32/48/96 (high) mg/mL for up to 42 days at ambient and refrigerator temperatures. Formulations were stable when stored refrigerated; however, under ambient storage conditions the concentration of the TDF decreased in both lowest and highest dose formulations. Formulations were stable up to 3 hours under simulated dosing conditions (ambient temperature, exposed to air and light).

Comparative Study of Indoor Air Databases and the Veracity of “Background”

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Risk assessors are often challenged to acquire accurate and relevant technical data and references for background levels. To address one aspect of this issue we compared two historical databases used as benchmarks for background indoor air quality in homes and businesses and for the evaluation of soil vapor intrusion, to a more recent dataset collected in homes and businesses adjacent to contaminated sites. The U.S. EPA (EPA) published the results of an indoor air quality study conducted at randomly selected public and commercial office buildings across the U.S. in 2001. EPA’s Building Assessment and Survey Evaluation (BASE) study sampled 100 buildings with a total of 298 sample sets. The New York State Department of Health (NYSDOH) conducted a study of the occurrence of volatile organic chemicals (VOCs) in the indoor air of homes that heat with fuel oil (1997 – 2003). The study included 104 homes, collected more than 600 samples, and analyzed for 69 individual VOCs. Our study collected indoor air samples from 137 sites and compared these data to the “background values” found in the federal and state studies. We collected a total of 794 indoor air sample sets that were analyzed for volatile organic compounds using EPA Method TO-14A and TO-15. Our data set was then compared to the minimum, maximum, means and the 25th, 50th, 75th, 90th, 95th and 99th percentile values in the background data sets. Our results demonstrate that although similar to the background dataset there were significant differences, enough to conclude that current “background” datasets are limited and should be expanded to include additional data. This additional data is readily available through State programs that have been collecting indoor air samples through a variety of programs including soil vapor intrusion assessments. This expanded dataset would be more reflective of actual background, resulting in a more accurate assessment of risk to the human population that it is being applied.

An Analysis of Varskin 5.0 Radiation Dosimetry Software

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The Nuclear Regulatory Commission (NRC) strives to keep radiation exposure as low as reasonably achievable (ALARA) for United States citizens. The NRC has radiation protection software to ensure hospitals are keeping exposure rates low. This research focused on assessing the accuracy of beta dosimetry software distributed by the NRC. The NRC uses Varskin 5.0 (Pacific Northwest National Laboratory), a software package used to estimate ionizing radiation dose to layers of the skin resulting from hot-particle exposure. When comparing the samples from Varskin 5.0, Varskin 4.0, and Duke Universities’ Beta Dosimetry Calculator, the same amount of absorbed doses are expected. Since variation can occur depending on exposure, distance, and shielding, this study was conducted running multiple beta dose calculators, with the radionuclide Fluorine 18 while varying the exposure time and cover density to assess the accuracy of beta dosimetry software. The comparison showed that, while the quantitative uncertainties were large, the tools described here can be useful in narrowing the range of chemicals needing further, more detailed evaluation. This abstract does not necessarily reflect U.S. EPA policy.

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An Upper Bound for Population Exposure Variability

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Tools for the rapid assessment of exposure potential are needed in order to put the results of rapidly-applied tools for assessing biological activity, such as ToxCast® and other high throughput methodologies, into a quantitative exposure context. The ExpoCast models (Wambuagh et al., 2013) comprise such a tool, predicting population median exposures and confidence intervals for the total population and subgroups. However, even if the population median exposure is so low that effects appear unlikely, as predicted by in vitro assays, upper quantiles of the exposure distribution may be large enough to be of concern. It would be extremely helpful to be able to set an upper bound on the likely population variability. We used an analysis of data on urine analytes collected as part of the NHANES (National Health and Nutrition Examination Survey) program to estimate the total variability among individuals of log transformed urinary analyte concentration (creatinine adjusted) for environmental chemicals. This analysis suggested that, for 95% of chemicals like those assayed for in NHANES, the exposure to 95% of the population is less than two-orders of magnitude greater than the median exposure. However, the variance on which this is based is known to overestimate the actual exposure variability (Aylward, et al., 2014), and we showed numerical simulations whose results help to quantify the degree of this overestimate. Finally, we showed the application of this methodology to over 400 chemicals from ToxCast Phase I and II. We compared in vitro concentrations that result in biological activity, converted to equivalent human steady-state exposures (Wetmore et al, 2012), and exposure predictions adjusted for population variability. The comparison showed that, while the quantitative uncertainties were large, the tools described here can be useful in narrowing the range of chemicals needing further, more detailed evaluation. This abstract does not necessarily reflect U.S. EPA policy.

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The Nuclear Regulatory Commission (NRC) strives to keep radiation exposure as low as reasonably achievable (ALARA) for United States citizens. The NRC has radiation protection software to ensure hospitals are keeping exposure rates low. This research focused on assessing the accuracy of beta dosimetry software distributed by the NRC. The NRC uses Varskin 5.0 (Pacific Northwest National Laboratory), a software package used to estimate ionizing radiation dose to layers of the skin resulting from hot-particle exposure. When comparing the samples from Varskin 5.0, Varskin 4.0, and Duke Universities’ Beta Dosimetry Calculator, the same amount of absorbed doses are expected. Since variation can occur depending on exposure, distance, and shielding, this study was conducted running multiple beta dose calculators, with the radionuclide Fluorine 18 while varying the exposure time and cover density to assess the efficiency of Varskin 5.0 versus older dosimetry programs. Our hypothesis was that Varskin 5.0 would account for the real absorbed dose and produce the most precise results. Although all the programs were below regulatory limits (NURG 20.1201), Varskin 5 radiation levels benchmarked between Varskin 4.0 and Duke University’s calculator. We concluded Varskin 5 has a better account for electron energy loss than Varskin 4 due to a more detailed backscatter model. The changes in equations used accounted for the radiation decrease. Also, unknown factors, such as geometry and skin averaging area may have skewed our results. Knowledge from this research will be useful in providing general background data for the use of quantitating radiation exposures.


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This study analyzes temporal trends in the U.S. population for five phthalates (di-buty1 phthalate, DBP; di-isobutyl phthalate, DIBP; butyl-benzyl phthalate, BBP; bis(2-ethylhexyl) phthalate, DEHP; di-isononyl phthalate, DINP) at various percentiles using quantile regression for the National Health and Nutrition Examination Survey (NHANES) 2005-2012. Upper quantiles of these five phthalate exposure levels are critical for the cumulative risk assessment of phthalates from a public health perspective (CHAP on phthalates and phthalate alternatives). The NHANES uses a complex, multistage probability sampling design with over-sampling of ethnic minorities and young children. Statistical analyses took into account unequal probabilities of selection resulting from the complex sample design,
High-Throughput Exposure Modeling of Semi-Volatile Chemicals in Articles of Commerce

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Chemical components of consumer products and articles of commerce such as carpet and clothing are key drivers of exposure in the near-field environment. These chemicals include semi-volatile organic compounds (SVOCs), some of which have been shown to alter endocrine functionality. SVOCs have the potential to accumulate in the indoor environment at high rates, which is correlated with high indoor exposures although it is not well quantified. The ExpoCast project is developing an indoor exposure prediction model (Little et al., 2012) was subsequently utilized in order to evaluate the robustness of the \(y_s\) predictor for chemicals lacking analytical data. For a given SVOC, exposure calculations depend on \(y_s\) and the surface area of its source material. In order to evaluate the indoor exposure model, high-throughput exposure predictions in were then compared with available oral equivalence values calculated from Toxicast high throughput screening assay data, available for 271 chemicals. Results show that both the \(y_s\) and indoor exposure models tend to over-predict their respective values. Future work using Monte Carlo based uncertainty analyses and chemical domain of applicability testing are being pursued to better calibrate the model and reduce uncertainty. This abstract does not necessarily reflect EPA policy.

High-Throughput Exposure Estimation Tool Incorporating ADME Processes

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EPA’s Chemical Safety for Sustainability (CSS) research program has been developing new ways to prioritize chemicals used in consumer products and articles. Prioritization is being addressed from both a toxicity potential (i.e. Toxicast) and exposure potential (i.e. ExpoCast) standpoint. Combining these approaches will form the basis for improved methods for risk-based prioritization of chemicals found in consumer products and articles. The ultimate objective is to identify potentially problematic chemicals before they reach the marketplace or are used as ingredients in products wherein their use would lead to undesirable exposures. Currently, screening-level models, such as the Exposure & Fate Assessment Screening Tool (E-FAST) and the Stochastic Human Exposure and Dose Simulation-High Throughput (SHEDS-HT) model, are used to screen near-field chemical exposures across exposure pathways (inhalation, skin contact, dietary and non-dietary ingestion) for differing exposure scenarios. Model evaluation via comparison with biomonitoring data, however, requires information on absorption and clearance from the body (i.e., metabolic biotransformation). This tool allows for the incorporation of the pharmacokinetics that occur after chemical uptake in the body to estimate internalized dose allowing for better comparison of toxicity and exposure estimates. This is accomplished by incorporating robust methods for route- and chemical-specific dose predictions considering absorption, distribution, metabolism, and excretion (ADME). The tool considers exposures from consumer products and articles accounting for product formulation and use: physical-chemical properties, such as partitioning coefficients; and user demographic information, exposure factors, activity patterns, and use profiles. The tool indicates products likely to be in specific microenvironments, along with the ways people contact chemicals in these products and articles, allowing better screening and ranking of exposure in a high throughput environment.

Development of a Computational Model Describing and Extrapolating Salivary Acinar Cell In Vitro Pesticide Transport

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The use of saliva as a biomonitoring matrix has potential to significantly advance quantitative dosimetry as an integral component of epidemiology. A major limitation has been an inability to predict which chemicals are readily cleared in saliva at levels that can be quantified from occupationally relevant exposure levels. In order to predict clearance, an in vitro mechanistic cellular computational model of salivary pesticide transport has been developed and will enable extrapolation of experimental results obtained from in vitro cell based systems to humans. This mechanistic cellular transport model has been developed describing basolateral, cellular, and apical compartments. Pesticides are distributed among these compartments by diffusion and active uptake and efflux by cells. The model was coded in acsIX using ordinary differential equations. The model was parameterized with a kinetic (0, 1, 2, and 3 hr, 40 \(\mu g/\text{ml}\)) experiment and validated with a dose-dependent (9, 18, and 36 \(\mu g/\text{ml}, 4 \text{ hr}\) experiment using chlorpyrifos as an initial test pesticide in a Transwell in vitro rat salivary acinar cell system. The resulting model simulations fit the data reasonably well, and fit parameters suggest that chlorpyrifos is transported across basolateral and apical cell membranes by passive diffusion. Diffusion coefficients were consistent across chlorpyrifos concentrations tested, suggesting no dose-dependent differences in transport at concentrations tested. Mechanistic parameters from this model will be integrated into pharmacokinetic models to identify ideal chemical candidates for saliva biomonitoring. This experimental and modeling strategy will be further used to evaluate a broader range of pesticides with varying physical and chemical properties. Once established, this approach can be exploited for biomonitoring without the need to conduct more challenging in vitro saliva clearance studies.
Sewage water analysis is a potential method to estimate health status of an entire community. The systemic oxidative stress marker, 8-isoprostane (prostaglandin-like free-radical catalyzed oxidation product) is a biomarker for metabolic disease that is excreted everyday into urban sewer networks. Its measurement in waste water could reflect the vulnerability of a large scale population to develop critical metabolic diseases. The aim of this study is to verify if 8-isoprostane could be assessed in sewage and to compare its level with the synthetic plastic component bisphenol A (BPA). Sewage samples collected from 3 different water plants located in different urban areas in Eastern Europe (IEF, NIE, OAK) and from a water outtake (ZUG) were provided by the Detroit Water and Sewage Department during a three month period. The sewage water was concentrated 25-fold, and 8-isoprostane and BPA were measured using ELISA kit from Detroit R&D, INC. Patterns of 8-isoprostane levels among the three water plants and a water outtake source during the three measured months did not oscillate, in opposite of BPA levels measured over the same time. When comparing communities, levels of 8-isoprostane were -300% higher in NIE and OAK than JEF. These results suggest that the measurement of this human derivate biomarker, 8-isoprostane, may be a tool for metabolic disease risk assessment of a large scale population. Changes of 8-isoprostane levels in sewage could be used as a first warning in a community and could facilitate early intervention to improve the health quality of a whole population and avoid the development diseases.

Monitoring Fusarium Mycotoxins by GC-MS/MS in European Beers

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The presence of mycotoxins in cereal-based products is a significant problem worldwide, and its occurrence is monitored in most countries. Risk arises from the fact that mycotoxins occur naturally in food and are difficult to remove by cooking since they are formed by a normal industrial processing such as brewing. In this line, a multiresidue method has been developed and validated following the SANCO/12495/2011 document for the simultaneous quantification and confirmation of 14 Fusarium toxins in beer by gas chromatography-tandem mass spectrometry (GC-MS/MS) with triple quadrupole analyzer. The method was validated by analysis of beer samples fortified at three different concentration levels (50, 100 and 200 µg/L). Average recoveries (n = 5) ranged from 70 to 110% with relative standard deviation lower than 15%. Spiked blank samples were used as standards to counteract the matrix effect observed in the chromatographic determination. Limits of quantitation (LOQs) ranged from 0.1 to 16 µg/L. The proposed methodology was used for mycotoxin analysis in 114 beer samples produced in several countries. Up to 80% occurrence of mycotoxins was found in samples. Additionally, co-occurrence of mycotoxins was observed in 12% assayed samples. Deoxynivaloenol (DON) was the most detected mycotoxin and it was quantified at mean contents of 31 µg/L. For, mycotoxin risk assessment approach, data showed that probable daily intake was lower than DON provisional maximum tolerable daily intake (PMDTI) (1 µg/kg b.w.) for average consumer. Acknowledgements: This work was supported by the Spanish Ministry of Science and Innovation (AGL2013-43194-P). Y. Rodríguez-Carrasco is grateful for the F.P.U. Grant (AP2010-2940) provided by the Ministry of Education.

Multi-Mycotoxin Dietary Exposure Monitoring in Cameroon Using Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS)


Dietary exposure to multi-mycotoxin and associated effects on the progression of HIV/AIDS in people living with HIV (PLWH) from Cameroon was investigated for the first time. 145 food samples were analysed for 320 toxic and potentially toxic fungal secondary metabolites, as well as 175 urine samples analyzed for 15 mycotoxins and relevant metabolites on the same instrument LC-MS/MS. Sixty-nine metabolites were detected in all studied food commodities including all regulated mycotoxins. Aflatoxin B1 (AFB1) and fumonisin B1 (FB1) were detected in all samples. The most targeted source of AFB1 (overall mean: 47.52; range <LOQ-210µg/kg) is ground-nut products. Total AF levels exceeded FDA, EC, and FAO maximum tolerable limits (MTLs). Maize was major source of FB1 (mean: 508; range 2-2313µg/kg). Also, 11 analytes were recovered individually or in combination in 110/175 (63%) urine samples including all the established urinary biomarkers, aflatoxin M1, FB1, and ochratoxin A. Additionally, viable mycotoxins and metabolites thereof, such as urinary FB2, nivalenol and zearalenone, were determined, some for the first time. Multi-mycotoxin contamination was common with one HIV-positive individual exposed to five mycotoxins, a severe case of co-exposure been reported for the first time. The common nature and high levels of multiple mycotoxins in studied staple foods and urines with mean levels of AFB1 and FB1 exceeding their MTLs and tolerable daily intake, suggesting negative health implications. Keywords: Multi-mycotoxin, Dietary exposure, Biomonitoring, HIV, Biomarkers, LC-MS/MS

Exposure Assessment of Lotion, Stick, and Spray Sunscreen Application


Although sunscreen SPF is calculated assuming a application thickness of 2 mg/cm², studies have shown that users typically apply less than this recommended amount. There is little information regarding the average application thickness of spray and stick sunscreen. We assessed the amount of sunscreen applied across the three sunscreen types (lotion, spray, and stick) by 52 adult volunteers. Volunteers applied lotion and stick sunscreen to both forearms, and the sunscreen tube was weighed before and after application to each forearm. Volunteers applied spray sunscreen to an area with a dry paper towel, twice, and the towels were weighed before and after both applications. In addition, volunteers completed a survey regarding their sun and sun protective behaviors, and provided some demographic information. The geometric means for the application thickness of lotion, spray, and stick sunscreens were 1.1, 1.6, and 0.35 mg/cm², respectively; all application methods were below the recommended value of 2 mg/cm². The amount of lotion sunscreen applied was significantly different between age groups, with older individuals applying more than younger individuals, and the amount of spray sunscreen applied was significantly different between skin reaction groups, with those who sunburn applying more than those who do not sunburn. There was no significant correlation between the amount of sunscreen applied and race, gender, time spent outdoors, number of sunburns in the last year, frequency of sunscreen use, or frequency of reaplication among any application method. Our results suggest that users apply different amounts of sunscreen depending on application type, with the largest application, and therefore greatest protection, afforded by spray sunscreen.

Risk of Dermal Sensitization to Methyliothiazolizinone from Lotion Sunscreen


Sunscreen are an important component of sun protective behaviors and heavy application is necessary to achieve the labeled sun protection factor. However, greater sunscreen application increases dermal exposure to chemicals within the formulation and increases the risk of allergy. The objectives of this study were to evaluate the sensitization risk associated with exposure to the preservative methylisothiazolinone (MI), a known dermal sensitizer. Five commercially available lotion sunscreens by national brands were identified as containing MI by the product label. The MI concentration in the sunscreens ranged from <1 to 5.6 ppm. Based on the recommended sunscreen application amounts and typical sunscreen application the potential exposure to MI was calculated. The acceptable exposure level, i.e., the maximum µg/cm² not expected to induce skin sensitization, was derived from previously reported human repeat insult patch testing results and sensitization assessment factors. A comparison of the acceptable MI concentration to the measured concentrations resulted in margins of safety from 4.5 to 25.4. In conclusion, it is unlikely that the MI concentrations present in sunscreen would induce an allergic response with normal sunscreen application behaviors.
Modern disposable diapers are complex products comprised of multiple layers of materials. Two important parameters considered for Exposure Based Risk Assessment are, i) frequency of diaper use & ii) Ingredient transfer from diaper to skin from a) direct skin contact materials, and b) indirect skin contact materials via migration of components from the diaper to skin via urine rewet. Frequency of use was determined from surveys in 10 countries and diary studies in the US. The mean number of diapers used per day varies by country; the US reported the highest diaper usage. The overall mean usage from the US diary studies was 4.7±1.6 diapers per day with a 95th percentile of 7.25 ± 1.29%; usage was inversely proportional to the body weight. Direct transfer of ingredient from a diaper top-sheet to skin (via Tegaderm tape) was evaluated using a lotioning ingredient (stearyl alcohol) for varying wear times. Mean direct transfer to infant skin ranged from 4 to 6%. Indirect transfer is a measure of skin re-wetting as urine resorbes to the topsheet of the diaper under pressure. Several factors were taken into account to develop a re-wet model to simulate realistic diaper wear conditions for an average 18-24 month old. These include: i) pressure applied by an infant on the diaper under various use scenarios (sitting, lying-back/front, and plopping down), ii) urination patterns (void volume and interval between voids), iii) wear time, and iv) surface area. The mean re-wet (liquid transferred from the diaper to a simulated skin surface - collagen) under diaper wearing conditions ranged from 0.35-0.75%. This re-wet protocol can also be used to determine specific chemical transfers by analysis of the collagen sheet. This poster will provide details of the methods/parameters used to determine exposure factors & a general exposure model to assess exposure to diaper ingredients.

Determination of Polybrominated Diphenyl Ethers and Polychlorinated Biphenyls in Bovine Milk


Polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs) are lipophilic persistent organic pollutants (POP). Their bioaccumulation tendencies and toxicity potentials resulted in discontinuation beginning in 1976 for PCBs, and in 2003 for several PBDE mixtures, respectively. Few studies evaluated the persistence levels of PBDEs and PCBs in dairy products in the US. In this study we compared traditional liquid-liquid extraction (LLE), solid phase extraction (SPE) and QuEChERS methods for extraction, and evaluated gel permeation chromatography (GPC), silica and florisil column adsorption chromatography for sample clean-up. Fourteen PBDE and 19 PCB congeners were measured in eight brands of commercial milk products available in central and northern California by gas chromatography coupled to triple quadruple mass spectrometry (GC-MS/MS). The QuEChERS method followed by GPC clean-up was selected as the sample preparation protocol due to the highest recovery. The average recoveries of targeted PBDE and PCB congeners were 92.2% and 72.6% for skim milk, 89.6% and 64.4% for whole milk respectively. BDE-47 (18.94 ± 10.13 pg/ml), BDE-99 (12.79 ± 12.19 pg/ml), BDE-49 (6.06 ± 3.55 pg/ml) and PCB-101 (41.23 ± 53.80 pg/ml) were the most dominant three PBDE and PCB congeners identified in the milk samples. There were no statistically significant differences (p < 0.05) in concentrations between skim and whole milk. This method is an accurate monitoring method for PBDEs and PCBs in dairy products. Monitoring of trace levels of PBDEs and PCBs is imperative because of their widespread occurrence in the environment and food supply. It is generally accepted that the vast majority of human exposure to POP is through the diet. This method can be used for efficient monitoring to evaluate time trends of POP persistence in dairy products and can be expanded to other food matrices. This study was supported by 1R01ES020202/592 NIH/NEIHS and 2P01ES011260R/68543201 NEIH/NEPA.

Bisphenol A Metabolites Levels in Pooled Urine Specimens from Pregnant Women in Rio de Janeiro, Brazil


The plastic monomer and plasticizer Bisphenol A (BPA) is an endocrine active compound used in the manufacture of polycarbonate plastics and epoxy resin. BPA has applications in everyday consumer products such as baby bottles, toys, dental sealants, eyelash lenses, consumer electronics, digital media, medical equipment, food and beverage can linings and glass jar tops. Approximately 4 million tons of BPA are produced annually. Concerns about reproductive and developmental health risks of exposure to BPA among the general population are increasing. Even if regulatory agencies are concerned with BPA's potential to injury pregnant women, BPA's toxicity in this population is very discussed. BPA metabolites were measured in 276 pooled urine samples collected during the first and second half of pregnancy. Both free (unconjugated) and total (free plus conjugated) BPA concentrations were analysed by online solid-phase extraction coupled to high-performance liquid chromatography–isotope dilution tandem mass spectrometry. Descriptive statistics and non-parametric tests were conducted. Geometric mean (GM) urinary BPA concentration was 4.86 ng/ml [95% confidence interval (CI), 3.33–5.02 ng/ml], and mean excretion was 7.92 µg/day (5th percentile, 4.65 µg/day; 95th percentile, 18.66 µg/day). GM creatinine-standardized concentrations were 2.39 µg/g (16 weeks), 2.87 µg/g (26 weeks), and 3.12 µg/g (birth). Creatinine-standardized BPA concentrations exhibited low reproducibility (ICC = 0.15). Consuming canned vegetables at least once a day was associated with higher BPA concentrations (GM = 2.92 µg/g) compared with those consuming no canned vegetables (GM = 2.95 µg/g). BPA concentrations did not vary by consumption of fresh fruits and vegetables, canned fruit, or store-bought fresh and frozen fish. These results suggest accumulation of BPA in early fetuses and significant exposure during the prenatal period, which must be considered in evaluating the potential for human exposure to endocrine-disrupting chemicals.

Human exposure to bisphenol A (BPA), a weak estrogenic monomer used in the manufacture of some plastics and epoxy can liners, is virtually ubiquitous. Extensive first-pass metabolism of orally ingested BPA by gastro-intestinal tract and hepatic tissues restricts human blood concentrations bioactive BPA to less than ~0.5% of total BPA - picomolar concentrations, even at the upper bounds of human exposure. Absorption of ingested BPA through non-metabolizing tissues of the oral cavity, which would bypass first-pass metabolism, could lead to higher BPA blood concentrations, like those reported some human studies, but are likely due to sample contamination. We hypothesized that if absorption through non-metabolizing oral cavity tissues was significant, higher bioavailability, higher serum BPA concentrations, and faster kinetics would result from exposure in soup, compared to prior studies in solid food or capsules. Serum and urine concentrations of BPA, and metabolites, were measured in 10 adult volunteers following ingestion of 30 µg/kg BW d6-BPA in soup. Absorption of BPA was rapid and complete, with an absorption half-life of 0.45 h, and complete urinary elimination by 24 hours post-ingestion. The maximum BPA concentration in serum, 0.43 nM, was <0.3% of total BPA, and occurred 1.6 h after administration. All pharmacokinetic parameters were consistent with those reported in previous oral pharmacokinetic studies in humans and animals. Pharmacokinetic parameters and pharmacokinetic model simulations of the data were inconsistent with absorption through a non-metabolizing tissue (below 1%). In conclusion, we found clear evidence against, and no evidence for, meaningful absorption through the oral cavity or other non-metabolizing tissues following exposure to BPA in a liquid food.
Previously, a subgroup of the cohort was identified based on self-reported consumption of fish and wildlife. Exposure to PAHs was measured and validated and participants selected based on data from those studies. Participants were divided into two groups; consumers with the highest serum mirex and PCB153 levels that also self-reported consumption of Lake Ontario wildlife (n=27) and non-consumers who were matched by age, sex and geographic area and reporting having never consumed Lake Ontario wildlife (n=16). The objective of the current study was to correlate contaminant levels and leukocyte populations (i.e., B, T and NK cells and macrophages) in these groups. Preliminary results were calculated on data from combining consumer and non-consumer groups. Although the power of this study was limited by the relatively small number of participants, our results show positive correlations between NK cells levels and serum oxychlor (r=0.345, p<0.04) and t-nonachlor (r=0.36, p<0.03) levels. Total serum PCBs were positively correlated with macrophage (r=0.41, p<0.01) and CD8+ (r=0.33, p<0.048) T cell levels while negatively correlated with CD4+ (r<0.359, p<0.03) T cell levels. (Supported in part by ATSDR Grant H75-ATH 298338)

510 PAH Bioavailability from Incidentally Ingested Soil: Influence of Thermodynamics and Metal Coexposure

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Polycyclic aromatic hydrocarbons (PAHs) are a group of hydrophobic organic chemicals which are commonly found in soil and are known to be carcinogenic. Approximately 20 PAH contaminated soils have been collected from around the world and the bioavailability of various PAHs has been determined using the in vivo juvenile swine model. The soils have a wide range of properties, including organic carbon content, metal concentrations, and PAH concentrations. Due to the organic nature of PAHs, thermodynamics is suspected to play a significant role in PAH bioavailability and the organic carbon is predominately responsible for governing the thermodynamic response. Soils are primarily composed of metals, therefore they will contain many different metals at varying concentrations. Both heavy metals and trace metals have the potential to interact with cellular biochemical mechanisms associated with PAHs, including metabolism and transportation. In this study, the role of thermodynamics and metal exposure is examined to further understand the bioavailability of PAHs in soil. The in vivo bioavailability across all soils is relatively low (15 of 20 soils have bioavailability < 10%). The PAH bioavailability from soil does not correlate well with the individual soil properties of soil PAH concentration, organic carbon content, or metal concentration. However, when examining the combined effect in a general linear model, soil PAH concentration is a significant factor (p<0.05 for 3 of 5 PAHs) while soil organic carbon when examining the combined effect in a general linear model, soil PAH concentration, organic carbon content, or metal concentration. However, bioavailability from soil does not correlate well with the individual soil properties of soil PAH concentration, organic carbon content, or metal concentration. Due to this, the bioavailability of PAHs in soil is relatively low (15 of 20 soils have bioavailability < 10%). The PAH concentrations in vivo are significantly higher in Alameda than in Ector. No statistical difference for the concentration of toluene. The mean concentrations of m/p-xylene and o-xylene were significantly higher in Alameda than in Ector. While significant differences are present, well density and activity do not appear to have an effect on all BTEX concentrations.

511 Non-Hodgkin’s Lymphomas in Humans and Dogs in the City of São Paulo, Brazil: Spatial Distribution As a Clue to a Potential Etiologic Role for Environmental Pollution

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Non-Hodgkin’s lymphomas (NHLs) are complex and heterogeneous neoplasms characterized by malignant proliferation of lymphoid cells. Human and canine NHLs share several features. Because dogs share most environmental and living conditions with humans, we reasoned that spatial distributions of NHL cases between the two species would provide some evidence for the role of certain environmental factors, such as pollutants, in the pathogenesis of NHLs. In this study, we retrospectively analysed the spatial distribution of 630 human NHL cases randomly selected from a database of more than 8,000 cases at the Cancer Registry of São Paulo and 579 canine NHL cases diagnosed in five referral veterinary hospitals in the same city. All human and canine incident cases were recorded between 1996 and 2006. We show that human and canine cases of NHL have similar spatial distributions in the city of São Paulo, with a high incidence in the central region, which is the most polluted area of the city, due in particular to the heavy traffic of vehicles. This result suggests that environmental pollutants may play a role in the pathogenesis of human and canine NHLs.

512 A Comparison of Ambient Air BTEX Concentrations between Two Counties with Active Petroleum Wells

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Communities in close proximity to active petroleum wells have concerns over exposures to constituents associated with the exploration process. Volatile organic compounds (VOCs) are associated with such activities, including benzene, toluene, ethylbenzene, and xylene (BTEX). This study evaluates ambient air concentrations of BTEX in an area dense with active oil and gas wells (Ector County, Texas, with 26,559.082 barrels of oil and 4,836.915 MCF of gas produced in 2013) and compares concentrations with a community that has relatively few wells (Alameda County, California, with 12,088 barrels of oil and no gas produced in 2013). Data were obtained from the US EPA Air Quality System web page. Hourly data for both counties were available for the months of April to November, 2013. A student’s-t test was conducted on data from this time period to determine if there were any statistically significant differences in BTEX concentrations between the two areas. The findings are as follows: Ector mean benzene at 0.287 Parts per Billion by volume (PPBv) (95% CI: 0.275-0.299) versus Alameda mean benzene at 0.159 PPBv (95% CI: 0.154-0.164), p<0.0001; Ector mean toluene at 0.452 PPBv (95% CI: 0.431-0.472) versus Alameda mean toluene at 0.463 PPBv (95% CI: 0.446-0.479), p<0.01; Ector mean ethylbenzene at 0.071 PPBv (95% CI: 0.068-0.075) versus Alameda mean ethylbenzene at 0.057 PPBv (95% CI: 0.055-0.059), p<0.0001; Ector mean m/p-xylene at 0.187 PPBv (95% CI: 0.176-0.199) versus Alameda mean m/p-xylene at 0.216 PPBv (95% CI: 0.208-0.224), p=0.0001; and Ector mean o-xylene at 0.062 PPBv (95% CI: 0.058-0.065) versus Alameda mean o-xylene at 0.081 PPBv (95% CI: 0.078-0.084), p<0.0001. Mean benzene and ethylbenzene concentrations are significantly higher in Ector County, although the average concentration is not much higher than that of Alameda County. There is no statistical difference for the concentration of toluene. The mean concentrations of m/p-xylene and o-xylene were significantly higher in Alameda than in Ector. While significant differences are present, well density and activity do not appear to have an effect on all BTEX concentrations.

513 Assessment of Public Health Risks Associated with Petrochemical Emissions Surrounding an Oil Refine

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Refinery operations have been associated with atmospheric emissions consisting of a wide variety of air pollutants, including sulfur and nitrogen oxides, carbon monoxide, volatile organic components, polycyclic aromatic hydrocarbons (PAHs), hydrogen cyanide, greenhouse gases and hydrogen sulfide. The type and quality of the crude oil, refinery process and refined products all influence the variability, composition and amount of emissions from one refinery to another. Subsequently, the potential associated public health risks may also vary and are expected to be dependent on emission constituents and volumes. This study measured exposures associated with petrochemical emissions surrounding Isla Refineria located in Willemstad, Curaçao. Levels of atmospheric PAHs were measured using polyurethane foam (PUF) based passive air samplers deployed in areas surrounding the refinery for 9 weeks during 2011 (n=43) and 2014 (n=30). PUF samples were extracted using methylene chloride by an Accelerated Solvent Extractor (ASE) and cleaned up using silica column chromatography and gel permeation chromatography. Sixty one PAHs, including parent compounds and homologs, were quantified and confirmed on an Agilent 7890A gas chromatograph coupled with a 5975C mass selective detector. In addition, sulfur dioxide and inhalable particulate matter (PM10) data was analyzed from two air monitoring stations in Curaçao from 2010-2014, including daily, hourly, and monthly samples for SO2 and PM10. Mean annual SO2 levels ranged from 47.6 to 155.7 g/m3 in 2011 and 2013, respectively, demonstrating increasing trends. Annual PM10 concentrations ranged from 36.6 to 41.5 between 2011 and 2013, also illustrating a strong increasing trend (R2=0.98). PAH measurements were evaluated on the basis of their species proportion to characterize their emission source and were identified as partially associated with petrochemical emissions in Curaçao.

514 NHL Pathogenesis in Canine and Human Lymphoid Tissues

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Pathogenesis of human and canine NHLs in the same city. All human and canine incident cases were recorded between 1996 and 2006. We show that human and canine cases of NHL have similar spatial distributions in the city of São Paulo, with a high incidence in the central region, which is the most polluted area of the city, due in particular to the heavy traffic of vehicles. This result suggests that environmental pollutants may play a role in the pathogenesis of human and canine NHLs.
The aim of this study was to investigate benzene concentrations in the working environments of workers at gasoline stations. Benzene samples were collected by personal air sampling (n=101) and area sampling (n=20) in the city of Muang, Khon Kaen, Thailand using sorbent tubes connected to a personal pump and analyzed with gas chromatography (GC-FID). Data records were also kept of the amounts of various petroleum products sold. The results of the personal sampling showed that the mean concentration of benzene was 23.85 ppb (ranged 0.03 - 65.71 ppb) and that the highest concentration was found in suburban zone (35.55 ppb), followed by urban zone (18.19 ppb), and rural zone (2.52 ppb). These concentrations were significantly different between each zone (p<0.05). Additionally, results of area sampling showed that the mean concentration of benzene was 42.62 ppb (ranged 7.50 - 50.00 ppb) that the highest concentration was found in urban zone (45.55 ppb) and the lowest was in rural zone (34.24 ppb). Regarding different job functions, the mean benzene concentration in inhaled air of fueling workers (27.29 ppb) was significantly higher than that of cashiers (0.56 ppb). In addition, the positive trend of correlation between benzene concentrations and the amount of petroleum product sold with a type of high benzene content was found and identified by the significant difference between suburban and rural zones of gasohol 91 sold (p<0.05). In conclusion, although the finding of inhaled air concentration of benzene was not higher than the standard, some workers in urban and suburban zone had a potential risk for benzene exposure in long term. Therefore, training on safe working practice to protect gas station employees from benzene exposure should take into account to the different job functions related with the tasks, functions, locations of gasoline stations and the amounts of different petroleum products sold at gasoline stations.

Benzene is a common component of petrochemicals (PTCs), with Phenol, a principal metabolite used as biomarker of exposure. Benzene is implicated in myeloproliferative disorders; with mode of action incompletely elucidated. The role of key micronutrients of the haem pathway (HP); Cu, Fe, and Zn is poorly explored, particularly in regions where these nutrients are limited and exposure to PTCs is rising. One hundred age-matched subjects, comprising 50 gasoline dispensers (GDs) & 50 occupationally unexposed (M CF), were selected from Oyo-Ekiti, Nigeria. Duration of occupational exposure was 2-10 yrs. GDs were classified into 3 groups based on duration of occupational exposure; I, 2-4 yrs., II, 5-7 yrs. & III, > 10 yrs. Phenol was determined in urine by HPLC, haem in whole blood by spectrophotometry. Levels of Cu, Fe, and Zn were assayed in serum using AAS. Phenol levels were very significantly higher in GDs than in control (p<0.001). Phenol also increased with duration of exposure (p<0.05). In contrast haem was significantly lower in the exposed than in control (p<0.05). The micronutrients, Cu, Fe and Zn, key modulators of HP were all very significantly decreased in GDs than in controls (p <0.001 in all cases); Phenol and Fe demonstrated significant negative correlations (r = -0.557, p< 0.001). Haem and Zn also exhibited inverse correlations to phenol (r = -0.38, p = 0.01; r = -0.37, p = 0.01 respectively. Findings suggest intense perturbation of the haem pathway, arising from benzene toxicity. Explanation in part, is drain of Cu, Fe & Zn; vital in the antioxidant system; Cu/ZnSOD; Cytochrome P450 CYP 2E1 and molecular activities. Low Zn level particularly causes cell cycle derangement, impaired p53 function (guardian of the genome), faulty DNA repair, alteration of transcription, (Zn fingers) increased replication errors and genome instability; potentiating myelotoxicity and myeloproliferative disorders.
were received but analysis was hampered by test results not related to pesticide handling, inconsistent formats, poor data quality, and missing information. After extensive cleanup, 25,494 records were identified from 6,990 individuals, of whom 1,637 (23.4%) had at least one occurrence of significant ChE depression (<80% of baseline activity). The data set was consistent with expectations: (a) high ChE levels (baselines) were generally found during non-spray season while ChE depressions occurred mainly in-season, and (b) there was a concordance between geographic density of tests and areas of highest pesticide use. Cases of significant ChE depression were generally followed by a rebound in activity, suggesting that affected workers were no longer engaged in activities leading to exposure. But in some cases ChE activity remained depressed for an extended period of time and in other cases repeated occurrences of significant depression followed by rebound were observed, both indicative of a lack of corrective action. A screening tool is being used to quickly identify these patterns of variation in ChE activity to facilitate our evaluation of the program and provision of toxicological consultation to medical supervisors.

521 Evaluation of ChE Mutations in Lungs of Male Big Blue Mice Exposed to Vanadum Pentoxide by Inhalation for Up to 8 Weeks

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Chronic inhalation of vanadium pentoxide (V₂O₅) increases the incidence of alveolar/bronchiolar tumors in male and female B6C3F1 mice at 1, 2 or 4 mg/m³. The genotoxicity of V₂O₅ has been extensively investigated in the literature with mixed results. In general, tests for gene mutations have been negative. Both positive and negative results were reported for clastogenicity in vitro with some reports suggesting aneugenic potential. In vivo, V₂O₅ was negative in the mouse micronucleus test (erythrocyte) and comet assay (lung). A recent short-term inhalation study in B6C3F1 mice reported an induction of 8-oxodeGuo DNA lesions in lungs. Because 8-oxodeGuo DNA lesions can lead to induction of G → T mutations, we have used groups of transgenic Big Blue (BB) mice (B6C3F1) to test whether V₂O₅ has mutagenic potential in vivo in the tumor target tissue under the conditions of the bioassay. Groups of six male BB mice were exposed to particulate aerosols containing 0, 0.1 or 1 mg/m³ (tumorigenic concentration) V₂O₅ for 4 or 8 weeks (6 hours/day, 5 days/week) and ChE mutant frequencies (MFs) were evaluated in the right lungs. The MFs (× 10⁻⁶) of mice in the 4-week exposure groups were 30 (vehicle control), 39 (0.1 mg/m³), and 24 (1 mg/m³) while the corresponding values in the 8-week exposure groups were 29, 48, and 17, respectively. None of these ChE MFs measured at any time point was significantly higher than the corresponding control MFs (P ≤ 0.1). Additionally, a significant, time-dependent increase in lung weight was noted in mice exposed to 1 mg/m³ V₂O₅ (P ≤ 0.05). Overall, these results suggest that mutagenicity is not likely to be an initial key event in the lung tumorigenicity of V₂O₅.

522 Quantification of Kras Codon 12 Mutations in Lung DNA of B6C3F1 Mice Following Inhalation of Aerosolized Particulate Vanadum Pentoxide

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The role of Kras mutations in the etiology of vanadium pentoxide (VP)-induced B6C3F1 mouse lung tumors is unclear. In a previous study, of the 40 lung tumors induced by chronically-inhaled aerosols of particulate VP and analyzed for Kras, 73% had Kras mutations as compared to 30% of historical control tumors. This study sought to characterize changes in the frequency of Kras mutation following VP exposure and to investigate whether amplification of preexisting Kras mutation is an early event in VP-induced mouse lung tumorigenesis. Male Big Blue B6C3F1 mice (6 mice/group) were exposed to VP by inhalation, 6 hours/day, 5 days/week for 4 or 8 weeks, at targeted concentrations of 0.1, 0.1 mg/m³. Levels of Kras codon 12 GTG to GAT and GTT to GGT mutations were measured in lung DNA by Allele-specific Competitive Blocker PCR. It was hypothesized that a parallel increase in both mutations, relative to their spontaneous levels, would support a mode of action (MOA) involving amplification of preexisting mutation. The median Kras codon 12 GAT mutant fractions (MFs) measured in mice exposed to 0.1, 0.1 mg/m³ VP for 4 weeks were 2.07 × 10⁻⁴, 2.02 × 10⁻⁴ and 1.38 × 10⁻⁴, respectively. The median Kras codon 12 GAT MFs for mice exposed to 0, 0.1, and 1 mg/m³ VP for 8 weeks were 1.06 × 10⁻³, 6.40 × 10⁻⁴ and 1.99 × 10⁻⁴, respectively. No statistically-significant differences were observed among the treatment groups. Median Kras codon 12 GTT MFs were below the limit of accurate quantification (10⁻⁹). Given that 4- and 8-week exposures to a tumorigenic dose (1 mg/m³) of VP aerosols did not produce significant changes in the levels of the two prevalent Kras mutations, these results show that it is unlikely that induction of Kras mutation is an early initiating key event in the MOA for VP-induced mouse lung tumors.
Mice and rats are often used as animal models for testing the in vivo effects, including gene mutations, of chemical exposure. Standard study lengths are 28 days, 90 days, or for cancer bioassays, 2 years in length. Although there is a communal assumption that aging increases mutation in cancer relevant genes, the contribution of aging to oncogenic mutations has not been studied well. In order to study the effects of age on an oncogene, we determined the mutant frequency of Kras codon 12 GGT to GAT and GGT to GTT mutations in B6C3F1 mouse lung tissue at 4, 6, 8, 12, 21, and 85 weeks of age. A previously established allele-specific competitive blocker PCR (ACB-PCR) technique with a mutant fraction (MF) quantification limit of 10^{-5} was used to determine the MF of Kras codon 12. The results show that for the GGT to GTT mutation, the MF was below the quantification limit of 10^{-5} and averaged 1 x 10^{-2} at 21 and 85 weeks of age. The MFs at 21 and 85 weeks were significantly different than the MFs at younger ages of the mice, but not significantly different than each other. In contrast, the GGT to GAT mutation fraction in lung tissue remained below 10^{-5} over the lifespan of the mice and did not significantly increase with age. The data indicate that age itself does not significantly contribute to the accumulation of Kras codon 12 oncogenic mutation and therefore is not a confounding variable in studying the mutational effects of chemical exposure on Kras codon 12 in mouse lung tissue.

### 524 Optimization of Methods and Proof of Principle for Assessing Mutagenicity in the Oral Cavity of Transgenic Big Blue® Rats


Transgenic Big Blue (BB) mouse and rat in vivo mutation assays are available to investigate mutagenic mode of action (MOA) following positive genotoxicity and/or carcinogenicity results. Importantly, these assays permit measurement of mutations in almost any tissue. Novel tissues may require optimization of methods and qualification to new OECD guideline 488 including demonstration of mutation induction with a positive control. For drinking water studies, the oral cavity is often the target tissue of interest, so we developed reproducible methods for tissue isolation, DNA isolation and mutant analysis in various areas of the oral cavity in BB Fisher 344 rats. Due to the small tissue quantity and cartilaginous nature of oral cavity tissues, standard protection and DNA isolation methods of homogenization and dialysis did not consistently yield sufficient DNA of high quality. Modified tissue collection, homogenization and DNA extraction methods were evaluated resulting in a method using liquid nitrogen pulverization, nuclei pelleting, digestion and phenol chloroform extraction. Using these methods, we qualified the oral cavity carcinogen 4-NQO (CAS 56-57-5; 10 ppm in drinking water for 28 days; N=5/group) as a positive control for oral cavity mutagenicity studies. Tissue was collected from inner gingiva with adjacent palate and upper outer gingiva with adjacent buccal tissue. Tissue weights initially varied widely but with refinement, weights of each side were 45.1±10.3 mg for gingiva/buccal and 29.9±9.1 mg for gingiva/palate. Statistically significant increases in MF were observed in gingiva/palate (45.2±8.6 x 10^{-6} vs controls 2277±458 x 10^{-6} in treated) and gingiva/buccal epithelium (51.2±19.6 x 10^{-6} in controls vs 1091±146 x 10^{-6} in treated). These results demonstrate the utility of the transgenic Big Blue rat mutation assay for investigation of oral cavity mutagenicity. Importantly, the methods we developed can be extended to other small and cartilaginous tissues thus expanding the range and utility of the Big Blue Transgenic Rodent Mutation Assay for MOA studies.

### 525 Hexavalent Chromium Does Not Induce Mutations in the Oral Mucosa of Transgenic Big Blue® Rats following Drinking Water Exposures at a Carcinogenic Dose

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Hexavalent chromium (CrVI) has been shown to induce tumors in the rat oral cavity following chronic drinking water exposure at 180 ppm; however the mode of action (MOA) for these tumors is unknown. We conducted a transgenic rodent mutation assay in Big Blue F344 rats, following OECD Technical Guideline 488, to test whether CrVI induces oral tumors by a mutagenic MOA. Tumors in the NTP CrVI cancer bioassay arose from the gingival epithelium surrounding the upper molars. Therefore, we assessed mutation frequency in gingival/palate and gingival/buccal regions of the oral cavity. Because these tissues are not commonly studied in the assay, we optimized collection methods and conducted a positive control study exposing male F344 Big Blue rats to 10 ppm 4-nitroquinoline N-oxide (4NQO), a mutagenic carcinogen in the oral cavity, in drinking water for 28 days. At day 31, 4NQO induced statistically significant increases in mean mutant frequencies in the gingiva/palate (45.2±8.6 x 10^{-6} in control vs 2277±458 x 10^{-6} in treated) and gingiva/buccal epithelium (51.2±19.6 x 10^{-6} in control vs 1091±146 x 10^{-6} in treated). Having established ability to detect mutations in these tissues, we conducted a 28-day dosed water study with 10 ppm 4NQO (positive control) and 180 ppm CrVI. At Day 31, a statistically significant increase in mutant frequency was observed in gingiva/palate region of 4NQO-treated rats (49.8±17.8 x 10^{-6} vs 1818±362 x 10^{-6}, but not CrVI-treated rats (57.8±9.1 x 10^{-6}). Similarly, a statistically significant increase in mutant frequency was observed in gingiva/buccal region of 4NQO-treated rats (39.1±7.5 x 10^{-6} vs 688±250 x 10^{-6}) but not CrVI-treated rats (44.4±25.1 x 10^{-6}). These findings support that CrVI does not act by a mutagenic MOA in the rat oral cavity and demonstrate the utility of this assay for MOA research.

### 526 Detection of ENU-Induced Mutations in the Germ Cells of the Transgenic MutaTM®Mouse


OECD test guideline 488 describes optimum study designs for determination of mutagenicity in a variety of rodent tissues; for germ cells sample time should carefully select the routes of exposure allowing cell type specific mutagenicity assessment. To fully develop the assay, we conducted a positive control study exposing male F344 Big Blue rats to 10 ppm ethylnitrosourea (ENU). Animals were treated orally by gavage for 28 consecutive days at 10 mg/kg/day. On Days 31, 49, 56, 63, 70 and Day 77, animals were necropsied and liver, bone marrow, developing germ cells from the seminiferous tubules and mature sperm from the cauda epididymis/vas deferens or 2) to dose for 28 days and sample mature sperm only a minimum of 7 weeks (mice) or 10 weeks (rat) after last treatment. Option 1 has significant advantages in terms of logistics, duration and cost of the study, but even more importantly, where somatic tissues are investigated alongside germ cells. Option 1 is more ethically justified as it would use up to half the number of animals as Option 2. However, it has been questioned whether all stages of germ cell development are equally covered by the 28 plus 3 day study design to ensure accurate detection of germ cell mutations. We treated male MutaTM®Mice with water or the potent mutagen ethylnitrosourea (ENU). Animals were treated orally by gavage for 28 consecutive days at 10 mg/kg/day. On Days 31, 49, 56, 63, 70 and Day 77, animals were necropsied and liver, bone marrow, developing germ cells from the seminiferous tubules, and mature sperm from the cauda epididymis/vas deferens were isolated. All tissues were examined for mutation in the neutral lacZ transgene using positive selection methods. Clear increases in mutant frequency (MF) were detected in liver, bone marrow and developing germ cells at each sample time. However, an increase in MF was only seen in mature sperm on Day 77, supporting the idea that a 28 plus 3 day study design may not be optimal for robust detection of mutation in sperm cells.

### 527 Impact of Dexrazoxane on Epirubicin-Induced DNA Damage and Apoptosis in Nontumor Cells

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The objective of the present application is to determine whether dextrazoxane in combination with the anthracycline, epirubicin, can modulate epirubicin-induced mutagenicity and apoptosis in non-tumor tissues. Methods: Male mice were divided into six groups consisting of 10 mice each, set up as follows: Group 1: mice served as a control and treated with the vehicle. Groups 2 and 3: mice were treated with 75 or 150 mg/kg dextrazoxane, respectively. Group 4: mice were injected with 10 mg/kg epirubicin alone. Groups 5 and 6: mice were treated with 75 or 150 mg/kg dextrazoxane, respectively, and 10 mg/kg of epirubicin was administered 20 min after dextrazoxane exposure. Animals were sacrificed at 3 or 24 h after the exposure to epirubicin then bone marrow cells were collected. Comet assay and micronucleus test were undertaken to assess DNA damage and repair. Apoptosis was assessed by staining with annexin V and propidium iodide. Oxidative DNA damage was assessed by DCFH-DA probe. Results: Dextrazoxane was neither cytotoxic nor genotoxic in mice at tested doses. Pre-treatment with dextrazoxane reduced epirubicin-induced DNA strand breaks as detected by Comet assay. Pre-treatment of mice with dextrazoxane reduced the frequency of micronuclei formation. Moreover, the mitoderepression induced by epirubicin was also restored in the dextrazoxane

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pre-treatment group. Dexrazoxane also markedly decreased the degree of apoptosis in epirubicin-treated animals. Accumulation of DCF fluorescence was profoundly abrogated by dexrazoxane. Conclusion: Dexrazoxane has a protective role in the abatement of epirubicin-induced DNA damage and apoptosis that reside, at least in part, in its radical scavenger activity. Thus, dexrazoxane can be a promising chemoprotective agent and might be useful to avert secondary tumor in cancer patients and medical personnel exposing to epirubicin.

**528** Dihydroxyacetone Induces Cytotoxicity and DNA Damage in a Dose- and Time-Dependent Manner in an In Vitro Primary Human Skin Cell Culture Model

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Dihydroxyacetone (DHA) is the main ingredient in sunless tanning products that causes the skin browning or tanning effect. In the past, use of products containing DHA was not thought to pose health risks, because DHA interacts with amino groups of proteins of dead skin cells on the outer layer of skin (stratum corneum) to produce the tanning effect. More recent studies suggest that DHA can penetrate beyond the outer skin layer to reach viable keratinocytes, therefore, there is a need to further investigate the potential toxicity of DHA. DHA has been shown to induce DNA damage, block cell cycle progression, and cause apoptosis in an in vitro immortalized human keratinocyte cell line. In this study, we used a primary cell line, normal human epidermal keratinocytes (NHEK), to further assess the cytotoxicity and genotoxicity of DHA. We treated NHEK cells with increasing concentrations of DHA for 1hr, 3hr, and 24hr, and assessed viability by measuring the metabolic capacity of cells using an indicator dye, and performed comet assay to detect genotoxicity. We found that treatment with low DHA doses (≤10mM) reduced cell viability to 60% after 3hr, and by 24hr - 10% of cells remained viable, but cells retained normal morphology and no DNA damage was detected by comet assay. Treatment with higher DHA doses of 25, 50, and 100mM for up to 3hr reduced cell viability to 50%, 40% and 30%, respectively, and caused cells to become spherical. Comet assay revealed that cells had comet tails, indicating DNA damage. After 24 hours, cell viability was reduced to ≤15%, and severe DNA damage was observed by comet assay. We conclude that ≤10mM DHA is cytotoxic, while ≥25mM DHA is both cytotoxic and genotoxic to NHEK cells. Our results are consistent with those reported for immortalized human skin cells. These findings investigating the potential toxicity of DHA will be useful in evaluating the safety of DHA as a commonly used cosmetic ingredient.
Mitochondrial genomes encode 13 proteins that are all essential components of the electron transport chain and are normally present between 1000 and 100,000 copies per cell. However, this number is greatly reduced during specific developmental stages, representing a potential critical window for mitochondrial genotoxic exposure. Mitochondrial DNA (mtDNA) is more susceptible than nuclear DNA (nucDNA) to damage by many environmental pollutants, for reasons including the absence of Nucleotide Excision Repair (NER). NER is a highly functionally conserved DNA repair pathway that removes bulky, helix distorting lesions such as those caused by ultraviolet C (UVC) radiation and many environmental toxins, including benzene. We hypothesized that developmental screening would result in altered mitochondrial function that persists later in life. To test this hypothesis, we exposed 1st larval stage C. elegans to a serial dose of UVC that results in the accumulation of mtDNA damage, while allowing for nucDNA damage to be repaired. We then analyzed mtDNA and nucDNA genome copy numbers, DNA damage levels in both genomes, whole animal steady-state ATP levels, and both whole animal and isolated mitochondrial oxygen consumption rates at multiple time points between 2 and 8 days post exposure. Genome copy numbers were measured with real-time PCR and DNA damage levels by quantitative PCR. ATP levels were measured using a luminescence based ATP determination kit. Oxygen consumption rates were measured using the Seahorse XFe24. mtDNA:nucDNA copy number ratio was lower in UVC treated worms, as were steady state ATP levels. Interestingly, whole animal basal respiratory rates were increased in UVC treated worms, however they lacked spare respiratory capacity. Isolated mitochondrial oxygen consumption rates also indicated a lack of spare capacity. These results indicate that developmental mtDNA damage can alter mitochondrial function later in life, and highlights the potential for a critical window of exposure.

534 Role of Helicases in Removal of Zidovudine (AZT)-Induced Genotoxicity in DNA-Repair Deficient Human Cultured Fibroblasts

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Zidovudine (AZT) is a genotoxic antiretroviral nucleoside reverse transcriptase inhibitor (NRTI) that becomes incorporated into DNA and arrests replication, inducing micronuclei (MN), and centrosomal amplification (CA, >2 centrosomes /cell). In this study we cultured human fibroblasts from normal or DNA repair defective donors having Bloom’s (BLM) or Werner’s syndromes, or xeroderma pigmentosum (XP) with defects in proteins A, B, C, D, E, F and G. To elucidate the role of proteins involved in processing of AZT-induced DNA lesions, each cell type was exposed to AZT for 24 hr, stained with DAPI to reveal micronuclei (MN), and stained with anti-pericentrin to score CA. With the highest dose of AZT (200 μM) none of the cell lines showed cytotoxicity, with the exception of the AZT-treated BLM fibroblasts that had 56% survival after 6 days of exposure. In addition, only the BLM fibroblasts showed AZT-induced MN, with values of 5.97, 6.32, 11.09, and 14.12 % of cells with MN in cells exposed to 0, 10, 100 and 200 μM AZT, respectively (p<0.02). In contrast, normal human fibroblasts, with or without AZT exposure, had ≥1.8 % of cells with MN. The percentage of cells with CA in normal AZT-exposed fibroblasts was 0.73-1.10, and elevated CA values were found in AZT-exposed XBP and XPD cells. CA values of 3.0, 4.5, 5.5 and 4.9 % were found in XBP cells exposed to 0, 10, 100 and 200 μM AZT, respectively (p<0.08). XPD cells had 1.5, 3.4, 2.7, and 1.4 % of cells with CA, upon exposure to 0, 10, 100 and 200 μM AZT, respectively (p<0.03). LXB, XBP and XPD cells are all deficient in helicases required for normal DNA replication, and therefore this study suggests that removal of AZT at the DNA replication fork requires normal helicase activity.

535 Cytokinesis-Block Micronucleus following Telomere Centromere Staining

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Distinguishing between clastogens and aneugens is necessary to evaluate genotoxic risk. CBNM is an international standard for genotoxic evaluation. Micronuclei (MN) can be formed in dividing cells that contain either acentric or whole chromosomes. Introduction of telomere, centromere staining offers the potential to render MN scoring more efficient and sensitive. In this study, we improved the detection of all type of MN leading to a significant reevaluation of clastogenic, aneugenic effect and the sensitivity of CBNM assay following telomere centromere assay. Human cell line (TK6), in mouse cell line (L5178Y) and human lymphocytes (HuLy) were exposed for 3h to various concentrations of aneugen (Vinblomycin) or clastogen (MitomycinC) and a negative control (Pyrene). The highest concentrations of each induced only weak cytotoxicity. Chromosomal aberrations were scored following telomere, centromere staining. Quantification of telomere length was performed using Q-FISH technique. Association of telomere, centromere staining to CBNM made it not only possible to distinguish between clastogens and aneugens but also to increase sensitivity of CBNM assay. In addition higher sensitivity of cell lines to aneugens and clastogens compared to HuLy was demonstrated. Drastic telomere shortening and the loss of telomeric sequence in both cell lines before exposure compared to HuLy was found and was associated with presence of dicentric chromosomes without acentric fragments. These data suggest the important role of telomeres in protecting the ends of chromosomes and preventing chromosome fusion in response to genotoxic agents. In this work, we demonstrate for the first time, application of telomere, centromere staining to assist scoring of induced MN, making a new step in classification of substances and in response to genotoxic risk as it associated the quantification of telomere length with scoring of MN.

536 Telomere Instability in In Vitro Genotoxicity Assessment of Ethyl 4-Hydroxybenzoate

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Telomere length has been proposed as a marker of mitotic cell age and as a general index of human organism aging. Telomeres are essential for chromosomal stability. Critically short telomeres can trigger a persistent DNA damage response that leads to cellular senescence, apoptosis. The impact of telomere status on the response to chemical genotoxic agents has not been studied to any great extent. We investigated the potential correlation of Ethyl 4-hydroxybenzoate exposure in the presence and absence of metabolic activation (S-9), with the mutagenic effect and telomere dysfunction in cell lines with heterogeneity of telomere status. Ethyl 4-hydroxybenzoate was evaluated in screening and regulatory tests. Human cell line (TK6), mouse cell line (L5178Y) and Human lymphocytes were exposed to different doses. Micronucleated cells and induced chromosomal aberrations were scored following telomere, centromere staining. Telomere dysfunction was assessed using
Q-FISH technique. Significant increases were noted in both the Mouse Lymphoma Test (L5178Y) and in micronucleus test (HuLy) in 3-hour treatment with 5-9, and in micronucleus test using L5178Y cells after 3-hour treatment with 5-9 and at two sampling times, short (<5) and long (>5) using TK6 cells. Unstable chromosomal aberrations related to S-phase arrest and genotoxicity of ethyl 4-hydroxybenzoate in the presence of metabolic activation and provides additional evidence that telomere length may be a proxy for underlying inter-individual sensitivity.

PS 537 The Food Processing Contaminant 2, 5-Dimethylfuran Shows Genotoxic and Tumorigenic Potential in V79-Cells and Apc(Min/+ ) Mice

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Sponsor: U. Nygaard.

2,5-dimethylfuran (DMF) is formed during heating of foods, and can be used as a flavoring agent. After metabolic hydroxylation, DMF is a possible substrate for sulfotransferases (SULTs), which could lead to formation of reactive metabolites. Given its molecular structure and chemical similarity to the potential carcinogen furan, there is a need to fill the existing knowledge gap concerning the potential toxicity of DMF. In this study, we aim to evaluate the genotoxic and tumorigenic potential of DMF in models expressing human SULT1. The genotoxicity of DMF was assessed in V79 cells transfected with human SULT1A1 and human cytochrome P450 (CYP) 2E1, using the single cell gel electrophoresis (SCGE) assay that detects DNA strand breaks and alkali labile sites. Cells were exposed to DMF in the range of 0.5 – 200 μM for 30 min. In another experiment, 2 week old C57BL/6j multiple intestinal neoplasia (MIN) mice, with and without human SULT1A1 and SULT1A2, were orally exposed to DMF thrice per week for 8 weeks to assess the tumorigenic potential. The Min mouse model is heterozygous for a mutation in the tumor suppressor gene Apc, leading to spontaneous development of intestinal tumors. Mice were exposed to corn oil, 5, 25 or 50 mg DMF/kg body weight. The SCGE assay is a condition-related assay to detect DNA damage in V79 cells after exposure to DMF. A treatment-related response was also observed in the colon of DMF-exposed Min mice. Exposed male mice showed a significant increase in the number of tumors in the colon compared with unexposed mice, whereas exposed females showed an increased incidence of colonic tumors compared with controls. However, there was no clear effect observed for tumor induction in the small intestine. Interestingly, the effects in cells and mice were largely independent of the expression of human SULTs. The implication of the current findings calls for further toxicological and carcinogenicity studies of DMF.

PS 538 Lack of Genotoxicity/Carcinogenicity of Methylisoeugenol (ME) Compared to Methyleneugenol (MIE)

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ME and MIE are natural constituents of herbal spices and thus part of the human nutrition. ME is hepatocarcinogenic to rodents. Its activation is catalyzed by cytochrome P450s (formation of 1'-OH-ME) and sulfotransferases (SULT, formation of 1'-sulfone-ME). After loss of sulfate, the formed carboxylation can form DNA adducts and is thus responsible for the mutagenicity/carcinogenicity of ME. The hepatic main metabolites of ME are 1'-OH-ME and 3'-OH-ME, whereas MIE did not form 1'-OH-ME, but 3'-OH-ME. Both metabolites were nearly genotoxic in the Ames test performed with SULT-expressing S. typhimurium and formed the same amounts of DNA adducts in this assay. This indicates that both metabolites are substrates for SULTs. However, neither ME nor 3'-OH-ME generated significant amounts of DNA adducts in vivo (mouse) and in vitro (rat hepatocytes). We hypothesize, that this lack of genotoxicity and probably the lack of carcinogenicity of ME is due to the rapid oxidation of 3'-OH-ME to 3'-Oxo-ME and its subsequent detoxification or further reaction to non-genotoxic compounds in contrast to the slow oxidation of 1'-OH-ME. We investigated the oxidation of 3'-OH-ME and 1'-OH-ME in different types of alcohol dehydrogenase (ADH, with NAD+ as cofactor) as well as rat microsomes (NADPH-generating system) and cytosol (NAD+ or NADP) of liver, lung and kidney. In all incubations, the oxidation of 1'-OH-ME was hardly or not detectable. In contrast, the oxidation of 3'-OH-ME was comparatively fast. The catalytic efficiencies [μl min-1 (mg protein)-1] were 3.55 (liver microsomes); 29.7 (liver cytosol) and 777 (ADH) which is 5-97 times the efficiency for the corresponding oxidation or sulfoxidation of 1'-OH-ME. The cytosolic reactions were potentively inhibited by the ADH-inhibitor fomepizole. The results show that the oxidation of 3'-OH-MIE is fast and efficient and may prevent DNA adduct formation. However, subsequent reactions of 3'-Oxo-ME with glutathione, proteins, or via aldehyde dehydrogenase may lead to either detoxification or activation.

PS 539 Dose-Response Relationships for DNA-Adducts Formed by Mono-, Di- and Tri-Chlorobiphenyls: Do Common Indoor and Outdoor PCB Vapor Exposures Pose a Significant Cancer Risk?


Mono-, di- and trichlorobiphenyls are reported to create DNA adducts from active oxygen species generated during liver metabolism, resulting in concerns that exposures to vaporized PCBs from environmental sources may pose an appreciable cancer risk in humans. We analyzed the available published reports on airborne PCB measurements, identified the range of upper bound daily intake of lower-chlorinated species during appropriate human exposure scenarios, and determined associated liver concentrations using a one-compartment pharmacokinetic model. We then compiled dosimetry data from in vitro and in vivo studies that identified significantly increased adduct formation from lower-chlorinated congeners. We assessed the genotoxicity of such adduct formation. We found that DNA adducts were significantly increased from lower-chlorinated PCBs when rats were pretreated with agents that increased oxidative liver metabolism or when administered PCBs at doses orders of magnitude higher than upper-bound doses reported during environmental exposures to humans. We determined that internal doses of lower-chlorinated congeners from estimated upper bound environmental exposures are at least 2-3 orders of magnitude lower than the effective doses necessary for adduct formation. The compiled dosimetry data also indicated that PCB mixtures elicit an attenuated dose-response for enzyme induction and adduct formation when compared to single congeners administered at high tissue doses. Similarly, vaporized PCBs constitute a mixture of congeners that vary in their ease of oxidative metabolism depending on the pattern of chlorination, and competitive inhibition may limit potential for generating reactive oxygen species and significant adducts. Considering these dose-response elements, we conclude that ambient environmental sources of PCBs are not likely to pose a significant risk of liver enzyme induction, DNA adduct formation, or cancer risk in humans.

PS 540 Analysis of Duodenal Crypt Death following Exposure to Cr(VI) in Drinking Water

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Chronic exposure to high concentrations of hexavalent chromium [Cr(VI)] in drinking water induces duodenal tumors in B6C3F1 mice. Available data indicate that these tumors are the result of chronic villus epithelial cell damage followed by crypt proliferation, as opposed to direct effects on stem cells within the crypt compartment. To assess the health of the crypt compartment following Cr(VI) exposure, an OECD and GLP compliant in vivo duodenal micronucleus study was conducted in B6C3F1 mice. Specifically, mice (n=5 per dose group) were exposed to 0, 1.4, 21, or 180 mg/L Cr(VI) in drinking water for 7 days, and their duodena were removed, trimmed using the “Swiss roll” technique, processed routinely for formalin fixation and paraffin embedding (FFPE), stained with Feulgen’s stain, and sectioned to 5-μm. Using image analysis software, micronuclei were counted in 15 or more crypts per animal, resulting in analyses of 3,000 to 5,000 crypt epithelial cells per treatment group. Consistent with increased proliferation, the number of enterocytes per crypt increased 1.7-fold from 40.0 ± 3.5 to 67.1 ± 5.5 in the high dose group; however, micronuclei were not elevated in any of the mice exposed to Cr(VI). Consistent with the lack of micronuclei, X-ray fluorescence microscopy conducted on FFPE 20-μm Swiss roll duodenal sections revealed little to no Cr fluorescence in control mice, whereas Cr fluorescence was localized to the duodenal villi of mice exposed to 180 mg/L Cr(VI). In contrast, Ca fluorescence was detected throughout the duodenal sections. In addition to these data, we also describe results from γ-H2AX immunostaining in the duodenum. Unlike transverse sections that limit analysis to a single point along the duodenum, Swiss roll sections allow for greater longitudinal coverage along the intestine. Together, the data herein support that Cr(VI) does not adversely affect the health of the crypt compartment along the length of the duodenum of B6C3F1 mice.
Models for predicting bacterial mutagenicity are now widely used by pharmacu-
tical sponsors to assess the genotoxic potential of impurities in pharmaceutical products. Models built using machine learning (ML) techniques are commonly trained using balanced datasets where, in this case, equal numbers of compounds are positive and negative for mutagenicity. Building accurate models using ML from biased training data – unequal numbers of positive and negative compounds – can be a challenge. Sarah Nexus is a program for predicting bacterial mutagenicity that uses a self-organising hierarchical network (SOHN). Hitherto SOHN models have been built using data that have little bias; however, if models are built using biased training data, then there is a need to ensure that the model learns sufficiently well about the minor class. If the dataset is biased towards negative compounds, this would result in a model for mutagenicity with depressed sensitivity. We have explored how to use the SOHN engine to improve the sensitivity of models generated from a training dataset biased towards negative compounds, and applied this to models based upon individual strains and combinations of strains of Salmonella typhimurium and Escherichia coli in which the data bias was up to 13:1 in favour of non-mutagens. The best practice approach to model building within the SOHN engine consisted of a panel of models derived from repeated random undersampling of the major class with the number of iterations necessary for the most accurate predictions depending on the bias of the training set. For a compound under test, taking the most confident prediction from the set of models is the procedure rate predictions depending on the bias of the training set. For a compound under test, taking the most confident prediction from the set of models is the procedure.

Cells employ high fidelity DNA repair mechanisms in order to prevent chemical carcinogen induced neoplastic transformation. In this study we investigated the choice of DNA double strand break repair pathways after acute and prolonged exposure to particulate hexavalent chromium (Cr(VI)). The main repair mechanism after acute particulate Cr(VI) exposure is homologous recombination repair (HR). We found prolonged exposure to particulate Cr(VI) causes a dysfunction in the effector step of HR. Therefore, we investigated how particulate Cr(VI) affects the other main pathway of DNA double strand break repair, non-homolo-
gous end-joining (NHEJ). We considered localization and expression of the proteins involved in the sensor, transducer and effector steps in the NHEJ pathway. These proteins included Ku8 as the sensor protein, the DNA PKCs complex as the transducer, and Artemis, XRCC4 and DNA Ligase 4 as the effector proteins. We found that proteins involved in NHEJ increase after 72 or 96 h of exposure to particulate Cr(VI), except XRCC4 and Artemis. XRCC4 and Artemis nuclear expression decreased after 72 h exposure to particulate Cr(VI). Therefore, we conclude from this study that similar to HR, classical NHEJ is dysfunctional after prolonged particulate Cr(VI) exposure. Future work will look into if other repair mechanisms is functional after particulate Cr(VI) exposure and how the cell decides which repair mechanism to use after exposure to Cr(VI). This work was supported by NIEHS grant ES016893 (J.P.W.) and the Maine Center for Toxicology and Environmental Health.
Role of Inflammatory Cytokine IL-6 in Regulating the Genotoxicity of BaP and PhIP in the Breast Cancer Cell Lines


Breast cancer is the most commonly diagnosed malignancy in females. Its aetiology is multifactorial, and the role of environmental exposure to DNA damaging chemicals such as benzo(a)pyrene (BaP) and 2-Amino-1-methyl-6-phenylimidazo[4, 5-b] pyridine (PhIP) is one such factor. Both compounds require cytochrome P450 (CYP) mediated metabolic activation to DNA damaging species, and both induce transcriptional responses through the nuclear receptors Aryl hydrocarbon receptor (AhR) and estrogen receptor α (ERα). BaP and PhIP are mammary carcinogens in rodents. Clinically, IL-6 expression is linked with poor prognosis of cancer and 35% of the deaths in breast cancer are linked with inflammation. The objective of this research was to investigate the molecular toxicology and local activation of BaP and PhIP in the presence of IL-6. DNA damage was measured using the micronucleus assay and CYP expression was assessed by qPCR. Treatment of cells for 24hrs with BaP and PhIP induced micronuclei in both cell lines and the effect was potentiated in the presence of IL-6. On its own, IL-6 treatment failed to induce micronuclei in these cells. Compared to BaP or PhIP treatment alone, the presence of IL-6 treatment induced a slight increase in CYP1A2 expression and significant induction of CYP1B1. These data show that the DNA damaging potential of BaP and PhIP in breast cancer cells is potentiated by the inflammatory cytokine IL-6 and that inflammation-induced CYP expression, specifically CYP1B1, is likely to be responsible for this effect.

Formation of Pyrrolizidine Alkaloid-Derived DNA Adducts from Rat Liver Micosomal Metabolism of Hepatotumorigenic Pyrrolizidine Alkaloids in the Presence of Calf Thymus DNA

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Pyrrolizidine alkaloid (PA)-containing plants are widespread in the world and probably the most common poisonous plants affecting livestock, wildlife, and humans. PAs require metabolism to exert cytotoxicity, genotoxicity, and tumorigenicity. We previously determined that rats administered hepatotumorigenic PAs each produced a set of four DNA adducts, consisting a pair of epimers of 7-hydroxy-9-(deoxyguanosin-N2-yl)dehydrosuccinimidine adducts (termed as DHP-dG-3 and DHP-dG-4) and a pair of epimers of 7-hydroxy-9-(deoxyadenosin-N6-yl)dehydrosuccinimidine adducts (termed as DHP-dA-3 and DHP-dA-4) in the liver. These DNA adducts were not formed in the liver of rats administered the non-tumorigenic PA, platypthylnine, or vehicle control. These results indicate that this set of DNA adducts, DHP-dG-3, DHP-dG-4, DHP-dA-3, and DHP-dA-4, is a common biological biomarker of PA-induced liver tumor formation. In this study, rat liver micosomal metabolism of a series of PAs (riddelline, monocrotaline, lasiocarpine, heliotrine, and senkirkine) in the presence of calf thymus DNA was conducted. By LC/MS/MS analysis, it was found that the same four DHP-dG and DHP-dA adducts were formed, with the exception that DHP-dA-3 and DHP-dA-4 adducts were predominant adducts. In addition, the other DHP-dG and DHP-dA adducts, designated as DHP-dG-1, DHP-dG-2, DHP-dA-1, and DHP-dA-2, were also formed as minor adducts. These results suggest that these DNA adducts formed in vitro can potentially be a common biological biomarker of PA-induced liver tumor formation. (This article is not an official guidance or policy statement of the U.S. FDA. No official support or endorsement by the U.S. FDA is intended or should be inferred).

Endogenous Aldehydes Are Ubiquitous Sources of Widespread DNA Damage in Mice with DNA Repair Deficiencies

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Rationale: Endogenously generated genotoxic aldehydes can chemically damage DNA, corrupting the information encoded in the genome. Despite this threat, the genome is kept largely free of errors due to several mechanisms to detect and repair damaged DNA. An additional crucial layer of protection prevents the exposure of DNA to endogenous reactive aldehydes through enzymatic detoxification. The purpose of this study was to determine if sufficient endogenous aldehydes accumulate to cause DNA damage. Methodology: Mouse tissues were provided by the Patel lab. DNA was isolated, reduced, digested, and injected onto an Agilent HPLC-UV. DNA mono-adducts were quantitated using nanoUPLC-MS-MS (with a limit of detection of 0.5 attomoles). Results: Fanconi anemia (FA) mice that deficient in formaldehyde catabolism (ADH5-/-) and/or acetaldehyde catabolism (ALDH2/-/-), spontaneously develop acute hematopoietic stem cells failure and leukemia. The amounts of endogenous N2-hydroxymethyl-dG and N2-ethylidene-dG in ADH5-/- and ALDH2/-/- mice accumulated remarkably in liver and kidney. Methanol or ethanol intake also resulted in large numbers of DNA mono-adducts. Bone marrow transplantation lowered the DNA mono-adducts back to normal in kidney, but not in liver. FANCd2 repair-deficient mice did not counteract the DNA mono-adducts. Conclusions: Our study showed that reactive aldehydes like endogenously formaldehyde and acetaldehyde are sufficient to cause DNA damage and induce leukemia in mice deficient in both aldehyde detoxification and DNA repair. Exogenous intake of formaldehyde or acetaldehyde can be lethal to FA mice. Although FA DNA repair pathways failed to counteract the mono-adducts, a variety of other more toxic lesions such as DNA-protein crosslinks may be targets. Overall, the data generated in this study provide pivotal information to understand the toxicity and carcinogenicity of endogenous aldehydes.
The Aryl Hydrocarbon Receptor (AhR) is a cytosolic, ligand activated transcription factor, historically studied for its role in xenobiotic metabolism. More recently, AhR activity was shown to play a cytoprotective role against intrinsic apoptotic stimuli. However, the mechanism underlying this cytoprotection remains poorly understood. The studies reported here examined the receptor’s protective role in an alcohol induced liver injury model. Using isolated primary hepatocytes from control (AhR-floxed) and liver-specific AhR conditional knockout (AhR-CKO) mice, we demonstrated that the endogenous agonist, carcinobiotic acid (CA), induces AhR-dependent stanniocalcin-2 (Stc2) expression with concomitant cyto-protection against ER and oxidative stress induced apoptosis. In contrast, the arthropod exogenous AhR agonist 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), failed to induce Stc2 expression nor provide concomitant cytoprotection. Knockdown of Stc2 using siRNA confirmed that the anti-apoptotic properties of CA were absolutely dependent on Stc2 expression. In keeping with the primary hepatocyte findings, CA conferred protection against acute alcohol induced liver injury in mice. Chromatin immunoprecipitation assays revealed that CA treatment, unlike TCDD treatment, promoted recruitment of the AhR to the Stc2 promoter. CA-induced AhR-DNA binding was associated with increased Stc2 expression and markedly reduced liver injury in alcohol treated mice as measured by reduced TUNEL positive liver cells, reduced caspase-3 activity, reduced lipid content in the liver, and lower serum alanine aminotransferase levels. Immunochemical results in TUNEL positive liver cells, reduced caspase-3 activity, reduced lipid content in the liver and lower serum alanine aminotransferase levels. Immunohistochemical results in whole liver and with primary hepatocytes are consistent with Stc2 being secreted and functioning as an autocrine and/or paracrine signaling protein at the plasma membrane. Collectively, our data reveal a novel mechanism involving Stc2 that is dependent on AhR activation by CA capable of providing cytoprotection against hepatic injury and subsequent cell death.

**Inhibition of Serum-Deprived Autophagy by Chloroquine Increases Nitric Oxide Production and Promotes Endothelium-Dependent Vasorelaxation**

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Chloroquine (CLQ) is an antimalarial drug recently proposed as a adjuvant chemotherapy strategy for cancer and vascular diseases. CLQ inhibits autophagy by blocking autophagosomal formation and cell recycling. In some conditions, CLQ stimulates nitric oxide (NO) production. Here we show the CLQ-sensitive inhibition of autophagy by serum deprivation in Human Umbilical Vascular Endothelial Cells (HUVEC) as demonstrated by the accumulation of Acidic Vacuoles Organelles (AVOs), conversion of microtubule-associated protein 1 light chain (LC3)-I to LC3-II and SQSTM1/p62 degradation. The inhibition of autophagy by CLQ also increased NO production, protected HUVEC from the increase in superoxide generation by vascular Angiotensin II (ANG II) and sustained cell proliferation under serum-deprivation. The increase in NO bioavailability promoted endothelium-dependent vasorelaxation in vitro experiments employing rat aorta rings. The use of NO synthase inhibitor L-NAME abolished the effects of CLQ and confirmed its role on these processes. These findings support the modulation of NO levels by autophagy and its impact on endothelial function.

**Impact of Dosing Volume and Seeding Density on the Cell Death Triggered by Lysosomotropism**

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*In vitro* cytotoxicity assays are among the most commonly used methods to determine compound activity. Recently lysosomes have been shown to play a role in cytotoxicity and lysosomal membrane permeability has become an emerging mechanism for cell death. Compounds that are basic and lipophilic can accumulate in lysosomes (lysosomotropism) via pH partitioning, potentially compromising the lysosomal membrane integrity and releasing cathepsins that can trigger cell death directly or indirectly via mitochondria. Since the degree of toxicity is presumably correlated to the amount of compounds accumulated in each individual cell, we hypothesize the cytotoxicity is related to the total amount of compound/cell rather than the *in vitro* exposure concentration. ARPE19 (human retinal epithelial cells) was employed and the total amount of compound/cell varied by either altering dosing volume or seeding density. Impedance technology was utilized to monitor the cell viability kinetically. All eight lysosomotrophic compounds induced dose dependent toxicity. An increase in the dosing volume from 50μl to 200μl increased toxicity at constant concentrations. Similarly, decreasing the cell seeding density from 20000 cells/well to 5000 cells/well also increased toxicity. To confirm the cell death is indeed prompted by the lysosomal accumulation we pretreated the cells with bafilomycin A, which prevents compound sequestration in lysosomes. Bafilomycin A demonstrated a delay of the toxicity for certain compounds supporting the role of lysosomal accumulation in the toxicity observed. Our preliminary data also revealed different cell lines responded to lysosomal compounds disparately, presumably due to differences in lysosomal volumes between cell lines. This data highlights the importance of dosing volume and seeding density when comparing toxicity across platforms. Further work is focused on understanding how cellular lysosomal content contributes to the response to lysosomotropic compounds.
The biological consequences of exposure to pipérine nitroxides is a concern, given their widespread use in manufacturing processes and their potential use in clinical applications. Our previous study reported that TEMPO ([2,2,6,6-tetramethylpiperidine-1-oxyl, a low molecular weight free radical, possesses pro-oxidative activity in L5178Y cells. In this study, we investigated and characterized the role of reactive oxygen species (ROS) in TEMPO-induced toxicity. L5178Y cells were treated with TEMPO at concentrations of 1-5 mM for 4 hours. The pro-oxidative property of TEMPO was evaluated by the determination of intracellular ROS and reduced glutathione (GSH) levels. TEMPO-induced apoptosis was assessed by caspase 3/7 activities and FITC Annexin V apoptosis assay. The protein expression of B-cell lymphoma-2 (Bcl-2) protein family members and mitogen-activated protein kinase (MAPK) signaling pathways were determined by Western blot assay. As results, time- and condition-dependent intracellular ROS production and GSH depletion were observed in treated cells. TEMPO also induced apoptosis as demonstrated by increased caspase-3/7 activity, increased proportion of annexin V stained cells, and decreased expression of anti-apoptotic proteins including Bcl-2, Bcl-xL and Mcl-1. N-acetylcysteine, a ROS scavenger, attenuated the ROS production and apoptosis induced by TEMPO. Moreover, Western blot analysis revealed that TEMPO activated c-Jun N-terminal kinases (JNK), the key member in MAPK signaling pathway. Addition of S6P00125, a JNK-specific inhibitor, blocked TEMPO-mediated JNK phosphorylation and also attenuated TEMPO-elicited apoptosis. These findings indicate that both ROS production and apoptosis induced by TEMPO. [Conclusion] Based on the results, we confirmed the concept that a genetically modified model based on FRET system can be used for detecting apoptosis.

Alcohol consumption can cause alcoholic liver disease (ALD), which is a major cause of morbidity and mortality in the United States. Chronic alcohol consumption causes a pro-oxidant environment in the liver and increases hepatic lipid peroxidation. Acrolein (ACR) is the most reactive and toxic aldehyde generated through lipid peroxidation. ACR forms protein adducts and triggers endoplasmic reticulum (ER) stress and hepatocyte apoptosis, which are recognized etiologic factors in ALD. Moreover, recent evidence has established the critical role of the gut-liver axis in ALD pathogenesis, wherein alcohol-induced gut barrier dysfunction contributes to liver injury. This study investigates the pathogenic role of acrolein as a major mediator of intestinal barrier dysfunction and hepatic ER stress and injury in ALD. Accumulation of ACR adducts was seen in response to alcohol consumption in mouse livers and intestines. Acrolein adduct accumulation correlated with hepatic steatosis, JNK activation, ER stress, apoptosis and liver injury. We used cultured hepatic and intestinal cells to examine the direct in vitro effects of acrolein exposure in comparison to alcohol exposure. Alcohol-induced in vitro intestinal effects were mimicked by ACR in vitro in intestinal Caco2 cells: specifically, ACR down-regulated tight junction proteins, resulting in disruption of TEER (transepithelial electrical resistance), and increased FD-4 permeability. Similar to alcohol, in vitro acrolein exposure in hepatic cells triggered ER stress and induced apoptotic cell death, indicating that acrolein may mediate the effects of alcohol. Notably, these effects were attenuated by acrolein scavengers suggesting their therapeutic potential in ALD.
conclude that FB1 modulates apoptosis in a complex dose-dependent regulation of pro- and anti-apoptotic molecules, which may predispose cells to neoplastic transformation.

**560 Expression and Methylation Analysis of Apoptosis-Related Genes Survivin and Bcl2L13 in Mice Subchronic Exposure to Inhaled Benzene**

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Objective: Benzene is a ubiquitous pollutant both in the workplace and in the general environment. The hematotoxicity effects induced by benzene exposure are associated with abnormal bone marrow cell apoptosis. The objective of this study is to investigate the expression level and the CpG islands methylation status of apoptosis-related genes Survivin and Bcl2L13 in mice exposed to benzene. Method: C57BL/6j male mice were randomly divided into 3 groups: high concentration (100 ppm), low concentration (1 ppm) and control group. A subchronic benzene exposure test, which was 8 hours per day, 5 days per week, for a total of 13 weeks, was carried out using the dynamic inhibition control equipment. At the end of exposure tests, bone marrow cells were collected. Cell cycle and apoptosis were detected by flow cytometry. DNA methylation status of promoter of Survivin and Bcl2L13 were determined using the Matrix-assisted Laser Desorption/Ionization-Time of Flight (MALDI-TOF), and mRNA expressions were quantitatively analyzed using real-time PCR. Results: Compared with the control group, the percentages of apoptosis cells in both exposure groups were obviously higher (P<0.01). Cell cycle arrest at S phase was observed in low concentration group. The cell percentages at S phase and G2/M phase were significantly lower in high concentration group. Survivin mRNA expression was significantly reduced in low concentration group. Both Survivin and Bcl2L13 mRNA expressions were significantly suppressed in high concentration group. The low methylation levels of CpG islands of Survivin and Bcl2L13 promoters were observed in all groups. Moreover, the methylation levels were not impacted by benzene exposure. Conclusion: Subchronic exposure to benzene induced apoptosis, changed the cell cycle, and decreased the mRNA expression of apoptosis-related genes Survivin and Bcl2L13 in mice bone marrow cell. But Subchronic exposure to benzene didn’t impact the methylation status of promoter regions of Survivin or Bcl2L13 genes in mice.

**561 New Therapeutic and Research Tool: Cytoprotective Inhibitors for Apoptotic Endonucleases**

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Endogenous cellular DNases/endonucleases responsible for fragmentation of DNA before, and mostly, after cell death are traditionally called “apoptotic.” However, their role is shown well beyond apoptosis, in all kinds of cell death. Two endonucleases, deoxyribonuclease I (DNase I) and endonuclease G (EndoG), are the most active and abundant apoptotic endonucleases in the body. They are known to mediate irreversible cellular death induced by toxic, hypoxic and radiation injuries to the cells and tissues. Genetic inactivation of these endonucleases is almost universally bioprotective against cell injuries. However, neither inhibitors nor high-throughput methods for screening of high-volume chemical libraries in search of DNase inhibitors are available. We developed the first of a kind high-throughput DNase assay based on a near-infrared fluorescence (NIRF) oligonucleotide probe. The assay was shown to be sensitive to DNase I and EndoG, very reliable (Zc=0.5), operationally simple, and has low operator, intra- and inter-assay variabilities. The assay was used to screen a chemical library, and several potential DNase I and EndoG inhibitors were identified. After comparison, two hit DNase I inhibitors and two EndoG inhibitors were selected, confirmed by a secondary (plasmid incision) assay, and characterized by the mechanisms as competitive or uncompetitive inhibitors. The new DNase inhibitors protected against cisplatin-induced kidney cell death and docetaxel-induced prostate cell death in vitro. The inhibitors suppressed DNase activity in live mice at 5 mg/kg as determined by an intratral use of the same NIRF probe. They were not toxic at up to 25 mg/kg concentration in vivo as measured by fourteen blood markers of organ function. These new compounds are the first clinically-relevant candidates for universal amelioration of tissue injury induced by drug intoxications, traumas or diseases. They also can be used as a research tool to study mechanisms of cell death.

**562 In Vitro Analysis of the Role of CFTR in the Induction/Response to Oxidative Stress in NRK-52E Cells Exposed to NaF**


Fluoride (F) is abundant in the environment and is the most electronegative element. F is able to generate free radicals which interact with a large number of biomolecules inducing cellular damage; so it is of interest to know the molecular mechanisms of action of Fluoride in several cellular processes and their potential toxic effects over different kind of cells. These effects may involve CFTR, a membrane protein that can transport glutathione out of the cell since F has been shown to keep this channel open. NRK-52E cells were exposed to 0, 0.5, 1, 1.5, 2, 4, 6 and 8 mM Sodium fluoride (NaF)/-CFTR Inhibitor (5mM, CFTR-(inh)-172). After 24h, Reactive Oxygen Species (2’7’ dichlorofluorescein diacetate), Lipoperoxidación (BODIPY 581/591 C11) and Apoptosis Necrosis (Annexin V-IP, caspase-3) levels were determined using flow cytometry and kits. The induction of expression of related genes to antioxidant response (HO-1, SOD-2, GST-pi and PPAR-alpha) and NF2 transcription factor was determined by RT-PCR. Our data show that exposure to fluoride /-CFTR inhibitor induced ROS (32/24% at 1.5 mM F), LPO (20/10% at 1.5 mM F) and GST-pi (8 fold at 1 mM F) were observed. These results confirm that exposure to F causes cell damage by ROS/LPO production and leads to apoptosis. Interestingly, CFTR appears to be involved in cytoprotection from oxidative stress even if this protein seems not protecting from apoptosis. We also observed that Fluoride induced expression of antioxidant response genes in a concentration dependent manner. Funded by CONACyT (grant 152416).

**563 Antiproliferative Activity Of Leaf Extract of Spondias mombin L.(Anarcardiaceae) on Human Colon Cancer Cells Lines (CaCo2)**

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Ethanol leaf extract of Spondias mombin L (Anarcardiaceae) used traditionally as anti tumor agent was evaluated. Studies on the Phytochemical screening and in vitro antiproliferative activity against human colon cancer cell lines (CaCo2) were carried out. The extract was fractionated by chromatographic techniques using n-hexane, methanol, ethyl acetate, acetone and distilled water. Thin Layer Chromatography (TLC) was used to identify active fractions. The antiproliferative activity of active fractions was determined using Cell Death Detection Enzyme Linked Immunosorbent assay with cisplatin, a known anti cancer chemotherapeutic agent as positive control. Phychochemical screening revealed the presence of flavonoids, saponin, tannin, phenols, anthraquinone, alkaloids, phlobatannin and glycosides. All the fractions demonstrated significant antiproliferative activity against CaCo2 cell lines. Each of the chromatographic fractions (1-5) from S. mombin showed significant cytotoxic effect on CaCo2 cells with fraction 2 having the minimum 50% growth inhibition concentrations (IC50). Further purification and separation of this fraction produced six fractions tagged S1 to S6. All these fractions also showed cytotoxicity in CaCo2 cells with least IC50 of 0.051 µg/ml produced by fraction S4. This finding suggests that the induction of apoptosis may be one of the mechanisms through which the fractions are exerting their antiproliferative activity in the cancer cell lines. Further studies are on going on detailed characterization and extensive biological evaluation of the most active component of this extract.

**564 Evaluation of the Cytotoxicity of Electronic Cigarette Refill Fluids by Four In Vitro Assays and Three Cell Lines**

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The electronic cigarettes are a new type of product designed to deliver nicotine to the respiratory system. The commercial market for electronic cigarettes has increased significantly. However, the evaluation of the cytotoxicity of electronic cigarette refill fluids and its main chemical components are still limited. Our study
aimed to choose optimal in vitro methods for assessing the cytotoxicity of refill solutions for commercial electronic cigarettes. This study compared lactic dehydrogenase (LDH) activity, 5-bromo-2-deoxyuridine (BrdU) assay, WST-1 assays and CCK-8 assay for assessing the cytotoxicity of electronic cigarette refill fluids, and evaluated the sensitivity of Chinese hamster ovary (CHO) cells, human lung adenocarcinoma epithelial cell line (A549 cells) and Immortalized human bronchial epithelial (BEAS-2B) cells to electronic cigarette refill fluids-induced cytotoxic effects. The results indicate that WST-1 and CCK-8 assays are preferable to LDH activity assay and BrdU assay for assessing the electronic cigarette refill fluids -induced cytotoxicity, and CCK-8 assay might be more sensitive than WST-1 assay. The IC50 values in BEAS-2B cells treated with electronic cigarette refill fluids were lower than IC50 values in A549 cells and CHO cells, indicating BEAS-2B cells are more sensitive to electronic cigarette refill fluids -induced cytotoxic effects. refill solutions of 17 commercial brands of cigarettes were tested on BEAS-2B cells using the CCK-8 assay as the chosen method. The results show that different electronic cigarette refill fluids have varying cytotoxicity outcomes. According to the correlative relationship between IC50 values and the amounts of nicotine, 1,2-propanediol and glycerol, respectively, cytotoxicity induced by electronic cigarette refill fluids was not due to these main chemical components of electronic cigarette refill fluids.

565 Selective Toxicity of Plant-Based Analogues against Resistant Colorectal Cancer
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Plant based molecules have shown beneficial toxicity against colon cancer. Harnessing these beneficial effects, we designed and synthesized eight novel poly cyclic heterocycles, with pyrimido[1,2:1,5]-pyrazolo[3,4-b]quinoline framework and evaluated them for anticancer activity against colorectal cancer (HCT-116 and S1), breast (MCF-7 and MDAMB-231), ovarian (ov2008, A2780) and hepatocellular carcinoma (HepG2) cancer cell lines. In addition, we also evaluated cytotoxic effects on non-cancer cells including Madin Darby canine kidney (MDCK), primary mouse embryonic fibroblast cells (NIH/3T3) and human embryonic kidney cells (HEK293). The results of the screening studies revealed compound 5, a 4-chloro-2methyl pyrimido[1′,2′:2,1]pyrazolo[3′,4′:3,4]-quinoline framework that exhibits ten to fifteen times more selectivity and potent cytotoxic activity against colon cell lines at sub-nanomolar concentrations compared to other cell lines. This was also confirmed by changes in morphological status of the cells at sub-nanomolar concentrations of compound 5. Moreover, we found concentration dependent changes in mitochondrial membrane potential of HCT-116 with compound 5 leading to apoptosis and chromosomal DNA damage. Additionally, all the pyrimido[1′,2′:2,1]pyrazolo[3′,4′:3,4]-quinoline derivatives were found to not be a substrate of ABCB1 and ABCG2 transporters. Gene-chip analysis revealed that Compound 5 produces significant change in signaling pathways leading to cell survival and progression. In sum, this work has led to the discovery of a novel 4-chloro-2-methyl pyrimido[1′,2′:2,1]pyrazolo[3′,4′:3,4]-quinoline with potent cytotoxicity and apoptosis-inducing properties on colon cancer cells, without being a substrate of ABCB1 or ABCG2 drug resistance factors. More mechanistic and pharmacokinetic-pharmacodynamic [PK-PD] directed studies integrated with rigorous toxicity studies would be conducted to bring Compound 5 or similar molecules in the clinics.

566 Mass Spectroscopic In Vitro Assay for Screening Different Types of Cytotoxicity
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Changes in protein expression as a cellular response to chemical exposure have been well established. Current methods for monitoring cellular responses usually require the use of specific reagents and/or labor-intensive experimental procedure. In this study, a new concept on using mass spectral pattern to distinguish different cellular responses resulting from chemical exposure is demonstrated. The concept is based on the ability to acquire a unique mass spectral pattern directly from a specific cell culture by using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). For the proof of concept, specific cell lines were exposed to chemicals that were known to cause different types of cytotoxicity. After the incubation with selected chemicals, the cells were collected and washed. The whole cells were then ready to be measured directly by using MALDI-TOF MS. In comparison to many conventional in vitro assays, less number of cells was needed, plus the same procedure could be applied to monitor different types of cytotoxicity. The results demonstrate distinguishable and reproducible spectral patterns can be obtained from different chemical treatments. The developed method is fully compatible with the current approach for high throughput screening of cytotoxicity.

567 Galactosylated Poly(ethylene glycol)-Lithocholic Acid Selectively Kills Hepatoma Cells, While Sparing Normal Liver Cells
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Delivering drugs selectively to cancer cells but not to nearby normal cells is a major obstacle in drug therapy. Recent reports suggest that lithocholic acid (LCA) is a potent anti-cancer drug that selectively kills cancer cells, sparing normal cells. Although LCA holds great promise for killing cancer cells, it is usually inappropriate for in vivo use because of its low solubility, short shelf life, slow circulating half-life, and rapid renal clearance from the body. Conversely, the afore-mentioned defects can be overcome through a process called PEGylation. In this study, LCA was converted to two forms of poly(ethylene glycol) (PEG) conjugates, viz., PEG-LCA (PL) and lactobionic acid (LBA)-PEG-LCA (LPL). The latter form contains a galactose ligand in LBA to target the hepatocytes. Both forms are self-assembled to form nanoparticle formulation, and they have high potency to kill HepG2 cancer cells, sparing normal LO2 cells, similar to the function of LCA as reported before. Besides, LPL had high specificity to mouse liver cells in vivo. Western blot results confirmed that the cell death occurred through apoptosis induced by LPL nanoparticles. Further loading of anti-cancer drugs inside the core of LPL nanoparticles as a drug delivery vehicle will be of great importance to evaluate the potential of this system. This work was supported by National Research Foundation Grant (2012M3A9B6055304) funded by the Ministry of Science, ICT & Future Planning.

568 The Role of Autophagy in Usnic Acid-Induced Toxicity in Hepatic Cells

The use of usnic acid and usnic acid containing products is associated with acute liver failure; however, mechanistic studies of hepatotoxicity caused by usnic acid are limited. In this study, we investigated and characterized the possible mechanisms, especially the role of autophagy in usnic acid’s toxicity in human HepG2 cells. Usnic acid caused apoptosis as demonstrated by an increased caspase-3/7 activity and an increased sub-diploid nucleus formation. Usnic acid induced autophagy as demonstrated by the conversion of LC3B-I to LC3B-II, degradation of P62, and an increased number of puncta. Inhibition of autophagy by treating cells with autophagy inhibitors (3-methyladenine or chloroquine) or by small interfering RNA against Atg 7 aggravated usnic acid-induced apoptosis and decreased cell viability, indicating that autophagy plays a protective role against usnic acid-induced toxicity. Moreover, usnic acid activated the MAPK signaling pathway. Usnic acid-elicited apoptosis was enhanced and autophagy was decreased when JNK was suppressed by a specific inhibitor. Additionally, inhibition of autophagy decreased the activity of JNK. Taken together, our results suggest that usnic acid perturbs various interconnected signaling pathways and that autophagy induction is a defensive mechanism against usnic acid-induced cytotoxicity.

569 The Role of ER Stress and Store-Operated Calcium Entry in Usnic Acid-Induced Toxicity in Hepatic Cells
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Usnic acid and usnic acid containing products have been used for antimicrobial, antiviral, antiparasitic, antimycotic, and antiproflliferative purposes and marketed in the United States as dietary supplements aiding in weight loss. In this study, we investigated and characterized molecular mechanisms of usnic acid induced toxicity in human hepatoma HepG2 cells. Usnic acid caused ER stress as demonstrated by an increased expression of ER stress markers by real-time PCR and Western blots. The expression level of typical ER stress markers such as CHOP, ATF-4, p-eIF2α, and spliced XBP1 was significantly increased. Usnic acid inhibited the secretion of Gluc, an established ER stress reporter. Moreover, an ER stress inhibitor 4-phenylbutyrate attenuated usnic acid-induced apoptosis. In addition, usnic acid significantly increased the cytosolic free Ca2+ concentration. Exposure to usnic acid markedly upregulated the protein expression of calcium release-activated calcium channel protein 1 (ORAI1) and stromal interaction molecule 1 (STIM1), the two major molecular components of store operated calcium entry (SOCE), with ORAI1 contributing to the process of usnic acid-induced ER stress and hepatoxocity. Taken together, our results suggest that usnic acid induced ER stress in HepG2 cells at least partially via activation of the Ca2+ channel of SOCE.
Involvement of Regulated Necrosis in the Pathogenesis of Drug-Induced Acute Liver Failure


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Acetaminophen (APAP) is one of the most commonly used medications as analgesic and antipyretic, though APAP overdose can lead to fulminant liver injury. Various types of cell death in the damaged liver are linked to APAP-induced hepatotoxicity, and of these, hepatocyte necrosis has been shown to be involved in disease pathogenesis. Recent studies have shed light on a novel concept of cell death, called receptor-interacting protein kinase (RIPK)-dependent necrosis, which is regulated by RIPK1 and/or RIPK3. RIPK-dependent necrosis has been implicated in a variety of disease conditions. However, its involvement in APAP-induced hepatocyte death remains elusive. Here, we showed that RIPK1 phosphorylation, which is a distinctive feature of RIPK-dependent necrosis, was induced by APAP, and the expression pattern of RIPK1 and RIPK3 in the liver overlapped with that of CYP2E1, whose activity around the central vein area has been demonstrated to be critical for the development of APAP-induced hepatic injury. Moreover, necrostatin-1, a RIPK1 inhibitor, successfully protected mice against APAP-induced hepatotoxicity, which was corroborated by reduction in necrotic area and suppression of the level of liver enzymes and cytokine expression. It is noteworthy that RIPK1 inhibition decreased the APAP-induced production of reactive oxygen species in hepatocyte cytosol and mitochondria under the condition of APAP exposure. These data demonstrated that a RIPK-dependent necrotic mechanism operates in hepatocyte death in APAP-injured liver, and the therapeutic intervention of this key pathway may have promise for the treatment or prevention of drug-induced acute liver failure.

The Cytochrome P450 Inhibitor Proadifen (SKF-525A) Disrupts Autophagy in Primary Rat Hepatocytes


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The cytochrome P450 inhibitor proadifen (SKF-525A) is commonly used to study drug metabolism and toxicity. By using Western blot and immunofluorescence staining, we unexpectedly found that proadifen caused remarkable accumulation of microtubule-associated protein light chain 3 II (LC3-II) in primary rat hepatocytes, indicating that the turnover of autophagosomes was disrupted. Chloroquine showed no effects on proadifen induced LC3-II accumulation, suggesting that proadifen blocked autophagic flux. Flow cytometry analysis of Lysotracker® Red DND-99 showed that proadifen caused a sharp increase of acidic organelles, which are predominantly lysosomes/autolysosomes in hepatocytes. Immunofluorescence staining of LC3-II (GFP fused Lamp1) (a specific protein marker for lysosomes) (a specific protein marker for lysosomes) and LC3-II showed that co-localization between these two proteins was abolished by proadifen, indicating that autophagosome-lysosome fusion was blocked. The proadifen concentrations (2, 5, 10, 15 and 20 µM) and treatment durations (1, 4 and 24 h) tested here were well within the range of those used for drug metabolism and toxicity studies. The ethoxyresorufin O-deethylase (EROD) activity, a specific indicator of CYP1A1 activity, was significantly decreased in hepatocytes treated by proadifen. As autophagy disruption can profoundly affect drug toxicity, particularly hepatotoxicty, our findings may provide an alternative explanation for the differential effects of various CYP inhibitors in modulating a same drug’s toxicity. Our data also indicate that proadifen may not be an ideal inhibitor for probing the relation between CYP mediated metabolism and toxicity in primary hepatocytes.

Population Modeling of Modified Risk Tobacco Products

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Simulation models have long been used to predict public health impacts of cigarette use and of tobacco control policies. With the US Tobacco Control Act and the FDA’s Draft Guidance for Modified Risk Tobacco Product (MRTP) applications, the use of these models has recently expanded to potential MRTPs such as electronic cigarettes (e-cigs). MRTPs could reduce mortality among smokers who switch to them, but risks being debated include dual use without significant reduction in cigarette use, increased total initiation to tobacco products, relapse of former smokers, and role as a gateway to smoking. In order to explore the magnitudes of possible population impacts, we expand a cigarette simulation model to include a potential MRTP (e-cig) and associated inputs: effects on use transition rates, cigarette use levels, and relative health risks of the MRTP. The cigarette model incorporates effects of age, gender, use levels, and time since quitting, and it was calibrated with historical US tobacco surveys and demographic data before extension to include e-cigs. A product use history generator is used to simulate individuals, who are then aggregated over a large random sample. This approach provides increased flexibility than Markov automata models, for example, modified rates can reflect changing cigarette use levels over time. There are only limited literature data, let alone product-specific data, on which to base e-cig model inputs, while the technology and public perception of e-cigs are evolving rapidly. In order to reflect the high uncertainty in long-term prediction and to weigh benefits vs. risks, we model a broad range of probability-weighted scenarios, with justifiable but hypothetical input ranges. Outcomes from example scenarios show a broad range of possible long-term net population mortality effects of e-cigs, relative to a no-e-cig scenario.

Extension of the Margin of Exposure (MOE) Approach for the Prioritization of Tobacco Smoke Toxicants


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We previously presented margin of exposure (MOE) as a tool for prioritisation of individual tobacco smoke toxicants, a useful approach for toxicants with data sets which meet our criteria for MOE calculation. For toxicants without such data sets, it was not possible to calculate an MOE, preventing prioritisation. Here we describe expansion of our approach to widen the data sets used, based on mode of action (MOA) reviews (where possible) to generate a point of departure (POD) allowing calculation of an MOE. It was imperative that we established a structured approach to determine the most appropriate studies, based on the following key criteria: 1) Route of Exposure, 2) Lesion Type, 3) Study Length and 4) Data Type. Inhalation is the gold standard route of exposure to generate MOEs for tobacco smoke toxicants, although in the absence of published studies, MOEs could be derived from alternative routes of exposure. We assume that lung lesions following
inhalation exposure are most applicable, followed by additional lesions relevant to the MOA. Ideally, the study length should be chronic (104 weeks plus), followed by sub chronic (13 weeks plus) and then acute (4 weeks). Where data sets do not meet BMDS criteria, alternative PODs should be explored on a case-by-case basis, e.g. NOAEL or LOAEL. MOEs generated from data sets not meeting our original criteria should be coupled with clear narratives highlighting limitations. Previously we were unable to assess hydroquinone (recommended by ToBReg for mandatory monitoring) due to the absence of inhalation data but using data from chronic gavage studies in rats and mice that led to lesions in kidney, liver and thyroid, we have been able to calculate MOEs in the range of 338-2976 suggesting that hydroquinone is a high priority for exposure reduction research. Our expansion of criteria for MOE calculations now enables evaluation and prioritisation of tobacco smoke toxicants for which the original data sets precluded assessment by BMDS.

### 575 Consumer Exposure and Risk Assessment of E-Liquid Ingredients in E-Cigarettes: A Practical Framework for Industry and Regulators

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A harmonized consumer exposure and risk assessment approach is needed when evaluating e-cigarettes to promote effective communication and support scientifically-based decision-making across industry and regulatory bodies. A general consumer exposure and risk assessment framework was established to toxico logically evaluate ingredients directly added to e-liquids for use in e-cigarettes. The framework describes a novel approach to estimate exposure from inhalation, oral, and dermal routes. Approaches are dependent on available information and include methods for use with e-liquid formulation or aerosol data. E-liquid engineering design and human use parameters were included in exposure models. Various exposure equations are considered and evaluated in this general framework. Three scenarios (inhalation, oral, and dermal) focus on situations when only e-liquid data would be available for risk assessment. The remaining six scenarios (four inhalation, one oral, and one dermal) focus on situations when machine-generated aerosol data would be available for risk assessment. Once an estimated exposure level is determined, the potential risk from exposure to an e-liquid ingredient is evaluated using margin of exposure and/or hazard quotient approaches. However, when data are limited or unavailable, alternative risk assessment approaches are recommended, including use of the threshold of toxicological concern, quantitative structure-activity relationships, and surrogate compounds. The e-liquid and aerosol-based deterministic evaluation approaches presented in this framework are useful for industry and regulators when assessing the potential exposure and risk associated with individual ingredients added to liquid mixtures.

### 576 Outdoor Air Pollution and Health Impact Assessment, a Local Approach

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Quantifying the impact of air pollution on the public’s health has become an increasingly critical component in policy discussion. Several studies found that current levels of air pollutants in urban areas are associated with health risks, namely of cardiovascular diseases and lung cancer. Recently outdoor air pollution was classified as carcinogenic to humans. The impact of the effects at the individual level may appear low compared to other risk factors. However, since the whole population is exposed, this impact results in a non-negligible public-health burden. The European Directive 96/62/EC requires Member States (MS) to design appropriate air quality plans for zones where the air quality does not comply with the established limit-values and to assess possible emission reduction measures to improve concentration levels. The new Directive encourages the use of numerical models and the incorporation of more ‘integrated’ approaches, that bring together air quality and health aspects in the current assessment methodologies of air quality plans. Despite the air quality improvements observed over the last years there is still a continued wide-spread of exceedances within MS, particularly regarding particulate matter (PM), nitrogen oxides (NOx) and ozone (O3). Portugal is not an exception. Recently, significant associations were found between daily mortality and morbidity endpoints and short-term exposures to PM and NOx in Oporto district, stressing the need of a close research. The aim of the project MAPLIA is to analyse the feasibility of an integrated assessment bottom-up approach for air quality planning, tailored to the local features of Oporto city. This work is supported by Fundação para a Ciência e Tecnologia (FCT) under the grant PTDC/ AAG-MAA/4077/2012.

### 577 Risk from Traffic-Related Air Pollution in Schools: Beyond Distance to Roadway

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California, a state with historically poor air quality, amended its State Education Law in 2003 to bar school construction within <500 ft of heavily trafficked roadways. In 2013, after the Seattle School District announced plans to renovate and reopen a school close to the interstate, local media implied Washington children were not adequately protected because Washington had no such regulations. To address this, the school was evaluated for pollution concentrations and subsequent risk of health effects using site-specific data. Seattle has historically acceptable air quality as it lacks meteorological facilitators of poor air quality prominent in California (air stagnation/inversion, increased temperature/sunlight, and lack of precipitation). In addition, topography near the school was not conducive for air stagnation and hourly PM2.5 air samples collected continuously over 4 days outside school windows (5.7±3.5 µg/m3) were similar to those at a nearby monitoring station (5.9±2.1 µg/m3) and well below regulatory limits. Interstate traffic data from January 2013 indicated daily PM2.5 concentration peaks did not correlate with the number of vehicles on the roadway. While the number of trucks peaked at 520/hr between 10 and 11 am and the total number of vehicles peaked at 9040/hr between 4 and 5 pm, neither peak correlated with the maximum PM2.5 concentration/ hr (8.1 µg/m3) observed between 9 and 10 pm, which was likely due to burning wood for heat. The PM2.5 concentration, on the other hand, indicated air quality at the school should be similar to downtown Seattle levels, which are below criteria pollutant regulatory limits. In addition, school activity patterns indicated the majority of time at school is indoors, where the outdoor pollutant levels are reduced through filtration of incoming air. In summary, risk from traffic-related pollution was proportional to exposure levels, much less in Seattle than California, and not observed above regulatory thresholds at the site of the Seattle school. Funded by Seattle Public Schools

### 578 Petroleum Coke Calcining Facility Emissions and Human Health Risk Characterization


Petroleum Coke Calcining processes including calcining, handling and storage of raw petroleum coke may result in Particulate Matter (PM) and gaseous emissions. Concerns have been raised over the potential association between particulate and aerosol pollution and adverse respiratory health effects including decrements in lung functions in both the susceptible and general population. This risk characterization evaluated the exposure concentrations of ambient air pollutants namely PM10, suspended dusts and gaseous pollutants from a petroleum coke calcining facility. The ambient air pollutant levels were collected from the sites through monitors installed at multiple locations in the vicinity of the facility. Specifically, the exposure levels of PM10, Carbon black in PM10, suspended dust and SO2, CO, NOx were examined. The measured and modeled particulate levels in ambient air along with gaseous pollutant levels from the calcining facility were compared to standards protective of public health. The results indicated that no exposure levels at collected locations were higher than the public health limit of 150 µg/m3 24-hour or annual average for PM10. Exposure levels of the modelled SO2, CO, NOx concentrations were also below public health air quality standards. These results demonstrate that emissions from calcining processes involving petroleum coke are well controlled, are below regulatory standards, and are protective of public health.

### 579 Refined Assessment of PAH Exposure and Potential Cancer Risk from Biomass Burning through Internal Dosimetry

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The current study deals with the assessment of the cancer risk attributable to exposure to PAHs, under an increased use of biomass for space heating in Greece in the winter of 2012-2013. The study incorporated ambient air PM sampling in several sites, as well as chemical analysis of PAHs and levoglucosan, used here as the most specific tracer of biomass combustion. Internal exposure to PAHs was...
estimated taking into account the deposition of the respective PM fractions across the human respiratory tract (HRT) and the respective PAHs concentration of the respective PM fractions. Deposition at different regions of the HRT was estimated using the Multiple-Path Particle Dosimetry (MPPD) model. Potential cancer risk due to exposure to the mixture of urban ambient air PAHs was calculated using the toxicity equivalence factor (TEF) approach using as basis the benzo(a)pyrene (B[a]P) cancer potency. Cancer risk was estimated from the integral of the TEQ of the different size of PM daily deposited across different HRT regions, by a slope-factor equal to 0.25x10^(-6) ng/kg bw/day, and a factor, initially derived by the B[a]P Inhalation Unit Risk (equal to 0.88x10^(-6) ng/m³). This refined exposure and risk characterization methodology allowed us to identify the significant differences experiencing the several age groups, as well as people living in different areas within an urban agglomeration. Estimate lung cancer risk was above 10^(-4) for the areas affected by biomass combustion. Age dependent differences in the estimated risk were mainly attributed to the respiratory physiology differences, favoring the deposition of smaller (and more toxic) particles in children. PM emitted particles, were found to be more toxic (in term of PAHs content) than the ones emitted from traffic sources.

580 Risk-Based Groundwater and Surface Water Investigation to Evaluate Potential Environmental Impact of Coal Ash Management Practices at Coal-Fired Power Plants

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Citizen and environmental groups have often publicly raised concerns about the potential impact that coal ash management practices at coal-fired power plants may have on the environment. Here we were able to collect and analyze surface water and groundwater samples from two power plants where coal ash is stored and potentially impacting the environment. The data collected was compared to risk-based screening levels for both groundwater and surface water. Drinking water wells searches were conducted, and monitoring wells were installed to evaluate the potential for impact to groundwater in areas the proximity to the plants where groundwater is used as a source of drinking water. Potential impacts on surface water were evaluated by identifying the closest surface water bodies, in both cases a small stream and a large river adjacent to the power plant, and collecting surface water samples both upstream and downstream of the facility. Groundwater and surface water samples were analyzed for a comprehensive list of inorganic constituents. Data were evaluated by comparing to risk-based screening levels for the protection of human health and the environment. Data were also evaluated for the presence/absence of coal ash release indicators, boron and sulfate. For both facilities the results indicated no adverse impacts on human health and the environment from either surface water or groundwater uses resulting from the coal ash management practices at the power plants.

581 A Health Impact Assessment of Petroleum Extraction in Ghana

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In Nigeria, data are scarce for the toxicity potentials as well as human health risk potentials of heavy metals in aquatic species. This study investigated the acute (96h) toxicity, bioconcentration and 28-day bioaccumulation potentials of lead (Pb(NO3)2) and cadmium (CdCl2) in a shrimp—Palaemonetes africanaus of a Nigerian habitat in a laboratory model. A human health risk assessment was also conducted based on the levels of Pb and Cd in the organism and habitat waters. Acute exposures of Pb and Cd produced mortalities with LC50 of 0.5 mg/L and 0.7 mg/L, respectively. In dissected P. africanaus, the fleshy muscle significantly (P<0.05) bioconcentrated Pb from day 0 to 28. The Bioconcentration Factors for Pb and Cd were 369 L/kg and 330 L/kg of whole shrimp tissue, respectively. Significant (P<0.05) increases in concentrations of Pb and Cd were recorded for the exoskeleton, fleshy muscle and whole shrimp tissues from day 0-28. In the human health risk assessment, the hazard quotient for Pb and Cd were less than 1, but Hazard Index for combined Pb and Cd exposure for a riverine child was (1.06) while for combined Pb and Cd exposure for a city inhabitant (9.2 x10^-6). In conclusion, Pb and Cd are toxic to, and bioconcentrated in various tissues of P. africanaus to considerable levels, with the potential for non-carcinogenic health effects mainly due to cadmium in riverine children; and that less of these heavy metals will accompany consumption of seafood by humans if the flesh is separated from other the scales before shrimp consumption.

582 Toxicity, Bioaccumulation, and Human Health Risk Assessment of Lead and Cadmium in a Shrimp—Palaemonetes africanaus

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Anthropogenic activities are known to generate products that are of public health importance. Natural spices especially of plant origin are known for their medicinal and nutritional values. Since they are used for the preparation of traditional cuisines. Information is sparse on the risk associated with the consumption of these spices. The present study has determined the levels of heavy metals (cadmium, lead and mercury,) and fifteen polycyclic aromatic hydrocarbons(PAHs) in six commonly used spices in Nigeria namely Negro pepper (Xylopia aethiopica), Ashanti pepper (Piper guineense), African nutmeg (Monodora myristica), Calabash nutmeg (Monodora tenusfolia), and Traditional glutamate (skype) which is a fermented product of mesquito plant (Poropisiss africana). The heavy metals were analysed with Atomic Absorption Spectrophotometer while Gas chromatography coupled with flame ionization detector (GC-FID) was used for the analysis of PAHs. Maximum concentration level 0.04 and 8.97mg/kg were observed for cadmium and lead respectively while mercury was not detected in any of the samples. The target hazard quotient (THQ) for Pb and Cd in the samples analyzed ranged from 0.001 to 0.013 which is well within the safe level limit of THQ which is less than 1. Pyrene was the most abundant of the PAHs in the spices with the highest concentration of 562mg/kg. Benzo(a)pyrene a human carcinogen ranged from 0.68 to 4.07mg/kg and exceeded the (EU) acceptable range (1-10µg/kg). The lead level though higher than the international standards range (0.05-1.5) mg/kg in food and food products, however has THQ within acceptable limits. Indiscriminate consumption of these spices could serve as a source of exposure to potential risks due to the high level of Benzo(a)pyrene.
In its review of the U.S. EPA toxicological review of inorganic arsenic (iAs), the National Academy of Sciences identified cancer endpoints among the highest priority health effects of concern and stated the need to consider evidence that early life exposure to arsenic may increase the risk of adverse health effects. To more fully evaluate the transplacental carcinogenic potential of iAs, a detailed assessment of the role of in utero iAs exposure in carcinogenesis was conducted. Eleven papers describing 9 separate studies with mice were identified; all but 2 were conducted by a single laboratory. Detailed analyses of methods and neoplastic results were conducted across studies, as well as with spontaneous tumor rates and findings in humans exposed to iAs in drinking water. Methodological limitations included lack of control for potential litter effects; possible confounding due to reduced water consumption with induction of a stress response; and no consideration of spontaneous tumor rates. In particular, at 85 ppm iAs, reported rates of liver adenomas (11-23%) and lung adenomas (13-18%) in male mice were within reported background incidences of ≤28% and ≤42%, respectively. Across studies, our analysis found 1) dose- and duration-dependent findings were absent; and 2) both intra- and inter-laboratory concordance of findings were lacking. Further, the incidences of bladder, lung, and skin tumors (the tumor types most consistently reported in humans exposed to high iAs levels), were not consistently increased in the animal studies. Finally, analysis of blood concentrations showed that they are not relevant to human health risk assessment. Specifically, in pregnant mice exposed to 85 ppm iAs, total speciated blood arsenic levels were ≤17x higher than those reported in highly exposed humans. Thus, the available studies do not support the conclusion that in utero-only iAs exposure causes cancer in later life, particularly at environmentally relevant doses; nor do the data indicate early life-stage susceptibility.

**586 Novel Analytical Method to Measure Formaldehyde Release from Heated Hair Straightening Cosmetic Products: Impact on Risk Assessment**

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In Europe, Formaldehyde (FA) is permitted for use in personal care products at concentrations ≤0.2 g/100 g. According to the Cosmetic Ingredient Review (CIR) Expert Panel products are safe when formulates (≥37% saturated solution of FA in water) concentration does not exceed 0.2 g/100 g (0.074 g/100 g calculated as FA). Our method captures and collects the FA released into the air from heated cosmetic products. On a total of 72 market samples analyzed, 42% showed FA concentrations very close to or above the threshold value of 0.074%, whereas 11 samples, negative using the official method of reference, were close to or above the threshold value. This may pose a health problem for occasional users and professional hair stylists. The values of FA equivalents released from the selected hair straightening products transformed into ppm (FA mg/1.23) extrapolated to the hypothesized volume of 1 m3 (FA mg/1.23) 1 m3, for the exposure of the consumer and the operator, are mostly far above the limits established by DFG (0.3 ppm) and OSHA STEL (2 ppm, 15 min) for both stylists and customers receiving a 100 g (median 67.48 ppm) or a 35 g (median 23.62 ppm) treatment, respectively. Even when the FA concentration in the product is below the 0.074% limit, the concentration reached in the air was about 75 ppm, much higher than the OSHA limit (PEL, 0.75 ppm).

**587 Screening-Level Risk Assessment for Biocides in Household Products Using Domestic Exposure Factors**


Household product is the main exposure media of human exposure to biocide substances. To manage biocide active ingredients contained in household products according to risk level, screening risk assessment was conducted for biocides in domestic household products corresponding to disinfectants and preservatives (product types 1, 2 and 6 of the EU Biocidal Product Directive). Total 144 active ingredients, that could be found toxicity values from ECHA or US EPA database, were selected from among the EU BPD active substances list. To estimate potential exposure from domestic household products, exposure factors were gathered from web-based consumer usage pattern survey and by measuring usage amount of the household products. Predicting exposures for individual consumers in each household product were calculated using inhalation and dermal contact exposure algorithms assuming a worst case scenario. To screen the risk for individual pair of substance and product, the margins of exposure (MoE) were determined. For screening risk assessment, it was assumed that target-use in household could be contained at 1% in all household products, which was found to be the maximum level for disinfectants and preservers. As results, numerous active substances and household products were derived below MoE 1000. The lowest MoE for dermal or inhalation exposure obtained for the following products and biocides: indoor black mould remover (chlorophacinone, formaldehyde) and indoor air freshener using spray (Cylbuthrin, 4,5-Dichloro-2-ocetyl-siohazolone). Especially, MoE of spray type was estimated relatively lower risk level than others. Each ingredient was categorized in prohibition or restriction on use to household products according to its risk level. Through the evaluation of risk for each active ingredients usable for household products, we could pre-screen the biocide active ingredients in household product before launching into the market and derive safe concentration in each product type and formulation type of household products. It is expected to be very useful in pre-market safety assessment and management for household products.

**588 Hypothesis-Based Weight-of-Evidence Evaluation and Risk Assessment for Naphthalene Carcinogenesis**


Inhalation of naphthalene causes olfactory epithelial nasal tumors in rats (but not in mice) and benign lung adenomas in mice (but not in rats). The available human studies have not identified an association between naphthalene exposure and increased respiratory cancer risk. The question of naphthalene’s carcinogenicity in humans, therefore, depends entirely on experimental evidence from rats and mice. We evaluated the respiratory carcinogenicity of naphthalene in rodents and its potential relevance to humans using our Hypothesis-Based Weight of Evidence (HBWoE) approach. We weighted all of the data from each realm of investigation (i.e., epidemiology, animal, toxicokinetics, genotoxicity, and other mechanistic data), allowing the datasets to inform interpretation of one another. We systematically compared and reviewed data relevant to key elements in the hypothesized modes of action (MoAs) for naphthalene carcinogenesis to determine which is best supported, and conducted a dose-response analysis based on the most likely MoA. Although the specific mechanism of action for naphthalene carcinogenesis in rodents is not entirely clear, our HBWoE analysis supports a mechanism that involves initial metabolism of naphthalene to the epoxide, followed by GSH depletion, cytotoxicity, chronic inflammation, regenerative hyperplasia, and tumor formation, with possible weak genotoxicity from down-stream metabolites occurring only at high cytotoxic doses. Our results strongly support a cytotoxic threshold MoA in the rat nose, and our dose-response analysis (which incorporated recent physiological-based pharmacokinetic [PBPK] model results) suggests this MoA is not relevant in human respiratory tissues at typical environmental exposures. Our analysis il-
lustrates how a thorough WoE analysis can be used to support an MoA even when a mechanism of action cannot be fully elucidated. A cytotoxic threshold MoA for naphthalene-induced rat nasal tumors should be used to determine human relevance and guide regulatory and risk management decisions.

589 EFSA’s Risk Assessments of Bisphenol A Using the Weight-of-Evidence Approach and an Improved Methodology on Uncertainty

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The EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) has assessed the risks for public health related to the exposure to BPA from foodstuffs and other sources. Exposure to BPA was estimated by exposure modelling taking into account different sources (food and non-food) excluding medical devices and routes of exposure (oral, inhalation and dermal) in the EU population. Additionally, urinary biomonitoring data were evaluated and compared to the exposure estimates. The uncertainty in exposure estimates was assessed both for the modelling and the biomonitoring approaches. Evidence of potential health effects from animal and human studies published between 2010 and 2012 was evaluated using a structured weight-of-evidence (WOE) approach, taking into account their strengths, weaknesses and relevance. Based on this approach the CEF Panel concluded on the relative likelihood of different BPA effects. For the likely effects i.e. changes in the liver, kidney and mammary gland, benchmark dose-response modelling was used to identify points-of-departure (PODs). Using data on interspecies differences in toxicokinetics and PBPK modelling, the PODs were converted into an oral Human Equivalent Dose (HED) to derive a temporary Tolerable Daily Intake (t-TDI). In addition, structured expert elicitation was used to assess (i) the likelihood of other possible health effects occurring at lower doses and to assess (ii) the need for an extra uncertainty factor to cover these endpoints when setting a t-TDI. Finally, the exposure estimates were compared to the proposed t-TDI taking into account the assessment of their respective uncertainties and to provide a risk characterisation for consideration by the European Commission. This is one of several initiatives by EFSA to improve methodology in the treatment of uncertainty and to increase transparency in risk assessment.

590 Probabilistic Modeling of Phthalate Risk

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The cumulative risk of phthalates was modeled recently as three case studies by the CPSC Chronic Hazard Advisory Panel. This analysis reports specific toxicity and exposure calculations that significantly improve the reliability of such cumulative assessments. We identified points of departure (PODs) for several common toxicity endpoints and performed probabilistic exposure modeling of the phthalates from several years of National Health and Nutrition Examination Survey (NHANES) data. Two key issues need to be taken into account when predicting potential cumulative risks from phthalate exposures. First, a common mechanism of toxicity should be demonstrated. The effects themselves should be consistent and adverse, not biomarkers of exposure or hypothetically related to a broadly-defined syndrome. Second, the exposure data and model should reflect current exposures. The pattern of phthalate use has changed over the last ten years. Further, an upper bound (UB) estimate of cumulative exposure cannot be predicted by summing 95th percentile exposure estimates for individual phthalates – this overestimates the true UB of cumulative exposure. A more realistic estimate of cumulative exposure can be achieved using a probabilistic approach. A Monte Carlo assessment enables combining exposures to multiple phthalates based on the distribution of individual metabolites in the population to calculate an overall distribution for phthalates based on the most recent NHANES biomonitoring data. For this exercise we used PODs for nipple retention, anogenital distance, and hypospadias as possible common toxicity endpoints. Five phthalates were evaluated: dibutyl phthalate, diisobutyl phthalate, butylbenzyl phthalate, di-2-ethylhexyl phthalate, and diisononyl phthalate. The total UB exposure to the five phthalates is less than half of that predicted from summing the 95th percentile exposures of the individual phthalates. UB hazard indices for all modeled common male reproductive toxicity endpoints are less than one.

591 Noncancer Toxicity of Carcinogenic Chemicals As a Risk Driver for Toxic Waste Site Cleanup Decisions

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Ordinarily, the non-cancer toxicity of carcinogenic chemicals receives comparatively less consideration when conducting a human health risk assessment at hazardous waste sites. Carcinogenicity is assumed to be the “risk driver,” and cleanup decisions are based on cancer risk alone. Recently new toxicity evaluations of several carcinogenic chemicals have caused this assumption to be reconsidered. We identified five volatile organic compounds (VOCs) frequently found at sites where non-cancer toxicity exposures [hazard quotient (HQ)] are significant (HQ greater than 1), but result in cancer risks within the 1E-6 to 1E-4 cancer risk management (RM) range. These include the 2011 USEPA and provisional peer-reviewed toxicity values (PRTVs) non-cancer assessments for trichloroethene (TCE) and 1,1,2-trichloroethane (TCA), respectively, and the 2014 OECD non-cancer assessment for benzene. In addition the USEPA toxicity assessments for methylene chloride and tetrachloroethene also exhibit HQ’s greater than 1 at exposures in the RM range for cancer. We present several case studies to demonstrate examples where hazard rather than cancer risk was the risk driver for cleanup decisions. At Sites A and B, potential vapor intrusion issues from modeled benzene and 1,1,2-TCA soil vapor data resulted in a HQ of 1.6 and 12, respectively, while cancer risks were within the RM range. At Site C, detected indoor air TCE concentrations resulted in a HQ of 2, while the cancer risk was at the low end of the RM range. Additionally, the potential for adverse health effects from exposure to these chemicals is heightened due to the exposure period of non-cancer, of particular concern is the potential developmental effect from short-term (weeks) TCE exposure and shorter time period in which DTSC and USEPA consider when evaluating non-cancer hazard compared to lifetime for cancer. These case studies illustrate that non-cancer threshold may now play more of a role in RM decisions and have important implications on site risks and cleanup.

592 Launch of Forward Risk Assessment Calculator for Chemical Contaminants following Superfund Guidance

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The U.S. Environmental Protection Agency Office of Remediation and Technology Innovation (OSRTI), through an inter-agency agreement with Oak Ridge National Laboratory (ORNL), has developed an online risk calculator for assessment of environmental media. This tool allows the user to enter concentrations in environmental media (soil, sediment, groundwater, surface water, fish, produce, beef, and milk) for the calculation of risk (cancer and hazard index) utilizing the best available toxicological information for an array of chemicals. This tool is analogous, yet more comprehensive, than the existing Regional Screening Level (RSL) calculator. Daily intakes (chronic or subchronic) are calculated and combined with toxicity data to produce risk (cancer and hazard index) results. Users have the ability to not only select chemicals from a pick list and enter concentrations, but to also upload a simple data file containing media designation, exposure point concentrations, chemical name, Chemical Abstracts Service (CAS) number and detection status. Baseline exposure assumptions are provided as defaults, however, the user may alter and save site-specific exposure parameters, chemical parameters, and/or toxicity values in a file for future use. Once risk results are obtained, the output may be saved with site-specific data for later modification. Output will be formatted into a RAGS part D template, including toxicity metadata. This new tool will be useful for concerned citizens, risk assessors and risk managers.

593 SADA: A Free Geospatial Human Health Risk Tool

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The Spatial Analysis and Decision Assistance (SADA) freeware program is a joint research and development effort between the Oak Ridge National Laboratory and the University of Tennessee. For nearly two decades, SADA has enabled environmental risk assessors (over 18,000 registered) to situate risk and decision analytics entirely within a spatial context. SADA represents a substantial integration of toxicological data, risk models, and advanced geospatial methods, resulting in new approaches for directly developing risk informed sample designs, remedial designs,
cost analysis, and uncertainty analysis within an open modeling environment. In the upcoming Version 6, supported by the U.S. EPA, modernization to the chemical risk models includes new analytics like mutagen cancer risk equations, TCE and Vinyl Chloride specific equations, updated forward risk calculators, expanded landuse, ProUCL guidance, and new Regional Screening Level tools. Radionuclide risk upgrades include exposures and risk from inside rooms and outside buildings. Dose assessment modules informed by the EPA Dose Compliance Concentrations for Radionuclides site and forward dose calculator will be available for the first time. Risk Upgrades will be compliant with RAGS Part E and Part F. Integration of the LandScan USA and Global high resolution population distribution models are also underway. Introduction of population data will enable risk assessors to consider receptor proximity and exposure to contamination at very fine spatial scales. In addition to risk, substantial expansions in SADA’s graphics capabilities will allow users to visualize contamination and risk with respect to elevation, infrastructure, and the built environment within realistic 3D scenes. This level of spatial awareness may allow clearer picture of public exposure and help inform response decisions. Additional plans are to deepen the demographic detail, ostensibly leading to identification of sub-populations particularly vulnerable to site specific exposure and disease.

595 Assuring Safety without Animal Testing Concept (ASAT): Systems Toxicology Supported Data Infrastructure for Human Risk Assessment


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595 Assuring Safety without Animal Testing Concept (ASAT): Systems Toxicology Supported Data Infrastructure for Human Risk Assessment

According to the Assuring Safety Without Animal Testing (ASAT) principle, risk assessment may become possible without the need for animals (Fentem et al., 2004). The ASAT concept takes human disease mechanisms as starting point and investigates if activation of these in vitro models, upon chemical exposure, can be used to identify toxicological hazard and set reference doses for human risk assessments. Very high dose levels are employed that may inflict toxicity to these animals, yet having no relevance to human exposure scenarios. A concept that could redefine traditional toxicity testing is human exposure-based dose level selection. Currently, in vitro toxicity tests comprise control, low-, mid- and high-dose groups, based on test guideline criteria that have changed little in the past 40 years. A more relevant, exposure-based and 3Rs-focused approach would be to limit the high dose level to a fixed multiple of human exposure; this assessment assumed a limit of 1000-times the predicted maximum Tier 1 (worst-case) human exposure level. Utilising the wealth of toxicity data generated for agrochemicals, a dataset was established to evaluate how this approach would have impacted the toxicity testing programmes of existing substances. Chronic dietary exposure scenarios were considered for the 100 most recent agrochemical reviews published by EFSA (http://www.efsa.europa.eu/en/publications.htm) to obtain ADI, critical NOAEL, and predicted chronic dietary exposure. The results showed that for the chemicals with established ADIs, 70% produced no toxicity at 1000-times the predicted maximum human exposure level. In the remaining cases, 1000X human exposure exceeded the critical NOAEL but 70% were lower than the critical LOAEL. In these cases, further analysis was completed to understand the effects that occurred with respect to the level of concern they might represent in relation to predicted human exposure. This research investigates potential refinements that human exposure-based testing could provide to the safety assessment of agrochemicals from a 3Rs and scientific perspective. In the future, this database will be increased in terms of the number of agrochemicals evaluated and also the types of human exposure scenarios (acute dietary, non-dietary) assessed.

596 Nonclinical Safety Assessment of a Monoclonal Antibody against CD47

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CD47 is a cell surface protein that functions as a regulator of phagocytosis mediated by cells of the innate immune system. CD47 binds to SIRP-alpha, a receptor on innate immune cells that delivers an inhibitory signal for phagocytosis. Effective phagocytosis requires silencing the CD47/SIRP-alpha inhibition pathway, and cancer cells exhibit an increased expression of CD47, presumably to prevent phagocytic elimination by innate immune cells. Thus, when CD47 is blocked from interacting with SIRP-alpha, pro-phagocytic signals dominate, which results in phagocytosis of the cancer cells. Hu5F9-G4 is an IgG4 monoclonal antibody that targets CD47 and has been shown to accelerate phagocytosis of Hu5F9-G4 containing cancer cells. Hu5F9-G4 is being developed as a novel therapeutic for cancer. In support of administration of Hu5F9-G4 in clinical trials, a comprehensive toxicology program was conducted in cynomolgus monkeys. The primary treatment-related finding observed in monkeys was anemia, as reflected in decreased red blood cell (RBC) counts and hemoglobin levels. The anemia, however, was considered related to the pharmacological activity of Hu5F9-G4 since CD47 plays major role in the normal clearance of aging RBCs. Therefore, administration of Hu5F9-G4 likely accelerates the process of elimination of aging RBCs by blocking CD47 on aging RBCs. Pilot toxicology studies revealed that the anemia associated with Hu5F9-G4 could be managed using a priming/maintenance dose schedule, where a low dose of Hu5F9-G4 is administered in week 1 (priming dose) followed by twice-weekly dosing at higher doses (maintenance doses). This presentation will describe the strategy in establishing the priming/maintenance dose schedule in the toxicology program to support the clinical administration of Hu5F9-G4 for the treatment of cancer.

597 Safety and Biodistribution Assessment of sc-tAAV2.5IL-1Ra Administered via Intra-Articular Injection in a Mono-Iodoacetate-Induced Osteoarthritis Model in Wistar Rats


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Interleukin-1(II-1) is an important inflammatory and catabolic cytokine in Osteoarthritis (OA) pathophysiology and represents a potential treatment target. The IL-1 receptor antagonist(IL-1Ra) gene therapy using a self-complementary adeno-associated viral vector, sc-tAAV2.5IL-1Ra, has been shown to express therapeutic levels of IL-1Ra in knee joints of animals. This study was conducted to assess the safety and biodistribution of sc-tAAV2.5IL-1Ra administered intra-articularly. Rats were dosed with mono-iodoacetate(MIA) to induce OA and three days later were administered vehicle, rat IL-1Ra vector (5x105, 5x104, or 5x103vg/kg) or human IL-1Ra vector (5x104vg/kg). Rats(8M/8F) were euthanized at days 7, 26, 91, 180 and 364. The OA model was established in the MIA-dosed knees as evidenced by chondrocyte necrosis and arthritis lesions. Vector genomes persisted in the injected knee for up to a year with only limited vector leakage to systemic circulation and tissues outside the injected knees. Transgene IL-1Ra expression was observed in the knee, albeit inconsistently. No local or systemic toxicity was observed in rats attributed to vector administration as evaluated by clinical sign, body weight, feed consumption, serum cytokines, and clinical and microscopic pathology through day 364. Antibodies against the vector developed in a dose-dependent manner, peaked at days 26 and 91, and then declined. Only vector expressing human IL-1Ra, but not the rat vector, induced a small splenic T cell immune response to vector capsid in male rats. Taken together, the gene therapy vector (ACD). All data was stored in a knowledge base. By exploiting this information for ACD, it was possible to discern sensitizing (e.g. eugenol, Bandroswick’s base) from non-sensitizing compounds (e.g. benzoic acid, hexane), as defined by enrichment of clinically defined disease gene sets in in vitro genomics datasets. Moreover, the strongest sensitizers most profoundly activated these gene sets. Currently, the knowledge base is expanded by incorporation of physiology-based pharmacokinetic modeling to judge the relevance of in vitro concentrations in relation to in vivo exposure scenarios. Finally, we are expanding the approach towards the development of biokinetic data models for other disease areas (cholesterol, hyperuribinemia), factoring in cellular pathway signaling.
has a favorable safety profile and warrants clinical development for OA treatment.
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598 Toxicity and Biodistribution of a Conditionally Replicative Adenovirus Vector, CRAd-S-pk7, Administered Intracerebrally to Hamsters
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A promising strategy for treatment of tumors is the use of conditionally replicative oncolytic virus vectors that have been engineered to specifically target and kill tumor cells. Once such vector currently under development for treatment of malignant glioma is the adenovirus vector CRAd-S-pk7. A study was performed to evaluate the toxicity of CRAd-S-pk7 administered as a single intracerebral dose to hamsters. Animals were assigned to 4 dose groups and were administered an injection of vehicle or of CRAd-S-pk7 at 2.5 x 107, 2.5 x 108, or 2.5 x 109 viral particles (vp)/animal. Animals were used for toxicology evaluations and to assess the biodistribution and immunogenicity of CRAd-S-pk7. CRAd-S-pk7 had no effect on clinical signs of toxicity, mortality, body weight, food consumption, or organ weights. Transient small increases in leukocyte, neutrophil, and/or monocyte counts and fibrinogen levels, and a decrease in albumin level were observed on Day 6 for male hamsters in the high dose group. An antibody response against the AdE1a portion of the vector was observed in all dose groups of animals treated with CRAd-S-pk7. Vector DNA was detected in the brains and at the incision sites of treated hamsters, as well as at very low levels in blood and tissues of a few animals, indicating that CRAd-S-pk7 was able to enter the systemic circulation. The presence of vector in those tissues was not correlated with microscopic changes that would suggest toxicity of the vector. Test article-related microscopic changes (particularly inflammation) were observed that were consistent with viral disease affecting the central nervous system; these changes decreased in incidence and severity over time, indicating that recovery was in progress. Because of the minor microscopic changes seen in the low dose group, which were not associated with any observed neurological dysfunction, a No Observed Adverse Effect Level was not identified for the CRAD-S-pk7 vector under the conditions of this study.

599 Toxicity and Toxicokinetics of PEGPH20 in the Monkey and Rat

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PEGylated recombinant human PH20 hyaluronidase, PEGPH20, depolymerizes hyaluronan (HA) which accumulates in the extracellular matrix of many tumor types. Four week toxicity and toxicokinetic studies were conducted with PEGPH20 dosed IV twice weekly at 0.2, 2 and 10.5 mg/kg in monkeys and 0.5, 5 and 25 mg/kg in rats. The recovery phase was 7 and 4 weeks, respectively. PEGPH20 was well tolerated in both species. There were transient decreases in body weight, food/water consumption and slight changes in clinical pathology. The plasma half-life of PEGPH20 in Day 25 was 16.7 to 78.1 hrs in monkeys and 3.28 to 24 hrs in rats. Consistent with the pharmacology of PEGPH20, immunohistochemistry demonstrated a reversible decrease in tissue HA levels. In monkeys, there was a slight to moderate increase in heart rate but values remained within normal range and there were no ECG findings. At the end of dosing, there was a reversible decrease in range of motion of the joints in sedated animals as assessed by limp angle measurements. However, animals exhibited normal cage activity and there were no related histology findings. Radiography showed reduced muscle mass of the thigh in one high-dose monkey that was partially reversible and had no histological correlate. Histological changes were limited to PEG-related vacuolation in the liver. In rats, increases in APTT were shown to be due to assay interference by the PEG-moiety and not PH20 hyaluronidase-related. There was an increased incidence of a background finding of minimal cardiomyopathy in male rats at ≥5 mg/kg. Other histology changes were limited to PEG-related vacuolation in the spleen at 25 mg/kg. In summary, all findings were not considered adverse, and the no observed adverse effect level of PEGPH20 was 10.5 mg/kg in monkey and 25 mg/kg in rat which are ≥3,500-fold the current clinical dose of 3 μg/kg.

600 Human-Recipient Bone Morphogenic Protein 4 (hrBMP4): Safety Profile after 7-Day Continuous Intracerebral (IC) and IV Administration in the Mouse
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BMP4 is a protein member of the Bone Morphogenic Protein family, part of the transforming growth factor-β (TGF-β) superfamily that acts by binding to specific receptors (BMPR1a, 1b, 2) also present in Glioblastomas cancer stem cells. BMP4 causes an overhauling of the cells’ carcinogenic program, activating normal pro-differentiation mechanisms that leads to increased cell maturation and, in turn, blocks the reproduction of tumorigenic cells. To investigate any potential adverse effect, hrBMP4 was administered, 20 to 1000μg/kg, by continuous infusion over a 7 days period by intracerebral (IC) or intravenous (IV) administration to CD-1 mice using Alzet micro-osmotic pumps and the animals were carefully observed for a period of 15 days thereafter. No mortality related to the biological effect of hrBMP4 occurred, and no significant signs of toxicity were recorded, either as clinical signs, body weights, food intake, clinical pathology, or at gross examinations. By histological examination, slight ventricular dilation was observed in most animals at the highest IC dose, generally associated with mild periventricular edema. The systemic exposure was limited (in the picogram range) after IC and IV administration. From the results obtained it was concluded that hrBMP4 given as continuous IC administration for 7 days to CD-1 mice do not exert local or systemic toxicity even following a relatively extended period of observation. 1S. G. Piccinillo, B. A. Reynolds, N. Zanetti, G. Lamorte, E. Binda, G. Broghi, H. Brem, A. Olivi, F. Dimeco and A. L. Vescovi. Bone morphogenetic proteins inhibit the tumorigenic potential of human brain tumor-initiating cells. Nature 444, 761-765 (7 December 2006)

601 Pharmacokinetic Profile of RPH-001, a Recombinant Humanized Monoclonal Antibody against VEGF following Administration by Intravenous Infusion in the Cynomolgus Monkey
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RPH-001 is a recombinant humanized monoclonal antibody to human vascular endothelial growth factor (VEGF). It is being developed as a biosimilar to Bevacizumab (Avastin®). As an angiogenesis inhibitor, its mode of action will be to block VEGF from binding to its receptors, on the surface of endothelial cells leading to a reduction in vascularization, growth and the formation of metastasis. This comparative investigation looked at the pharmacokinetic profiles of both RPH-001 (the investigational drug) and Bevacizumab (the comparator) following a single 90 minute intravenous infusion administration in the male Cynomolgus monkey; at dose levels of 2, 10 and 50 mg/kg. Blood samples for pharmacokinetics were collected: pre-dose, at the end of the 90 min infusion and at 3, 6, 12, 24, 48 and 96 h after the start of infusion, and on Study Days 7, 10, 14 and 21 for analysis by ELISA. All 3 dose levels tested for both the investigational drug and the comparator were not associated with any toxicity. Systemic exposure (AUClast and Cmax) increased linearly with dose for both RPH-001 and the comparator with a similar linearity. Cmax was achieved 3h after the start of infusion for both drugs. Clearance, distribution volume and elimination rates were similar for RPH001 and the comparator, at all 3 dose levels. The results from this study showed good pharmacokinetic profile comparability between RPH-001 and the comparator, Bevacizumab which will support the dosing regimen for the first in man clinical trial of RPH-001.

602 Unexpected Platelet Decreases in Cynomolgus Monkeys Induced by a Therapeutic Monoclonal Antibody
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Effects of monoclonal antibodies (mAbs) on platelets have been infrequently reported in the literature. CH12, a mAb directed against epidermal growth factor receptor variant III (EGFRVIII), is a promising therapeutic agent for human hepatocellular carcinoma and gliomas xenografts with EGFRVIII expression. In a nonclinical toxicity study, CH12 caused dose-related reversible decreases in platelet
counts and thrombocytopenia in cynomolgus monkeys after single intravenous (IV) infusion at dose levels of 25 or 200 mg/kg compared to the concurrent vehicle control. Therefore, additional in vitro studies were conducted to understand this unexpected effect induced by CH12 in cynomolgus monkeys. In vitro, after incubation CH12 with washed platelets (WPET) did not induce dose-related binding and activation (CD62P expression) of platelets were found in cynomolgus platelets. Moreover, CH12 induced activation of cynomolgus platelets was correlated with its binding to platelets. In contrast to the effects in cynomolgus platelets, CH12 neither bound to nor activated human platelets at concentrations up to 10 mg/mL. Further investigations indicated that unlike intact CH12, the Fc or Fab fragment of CH12 did not bind to cynomolgus platelets, while the F(ab'2) fragment of CH12 was found to be essential to the binding and activation of cynomolgus platelets. These data suggest that the effect of CH12 on platelet decrease occurred in cynomolgus monkeys may be species-specific and irrelevant for humans. Further studies are needed to identify the mechanism underlying this unexpected effect.

**603 Comparative Nonclinical Assessment of the Potential Biosimilar PF-06439535 and Bevacizumab**

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Bevacizumab is a recombinant humanized monoclonal antibody targeting vascular endothelial growth factor (VEGF). Bevacizumab is approved worldwide for treatment of metastatic colorectal cancer, non-squamous non–small cell lung cancer, metastatic kidney cancer, glioblastoma, and in some regions cervical, metastatic breast, and head and neck cancers. It is also approved for treatment of all stages of ovarian cancer in combination with chemotherapy, and for treatment of non–small cell lung cancer in combination with chemotherapy and radiation. Three bevacizumab molecules, Union-sourced bevacizumab (bevacizumab-US and bevacizumab-EU, respectively) and test article-related effects were limited to physeal dysplasia of the growth plate (exon 45), and SRP-4053 (exon 53) had no effect on behavior or on cardiovascular, skeletal, smooth, and cardiac muscle myofibers. Exon skipping by PMOs is a promising, disease-modifying approach to DMD. PMOs are members of a unique class of exon skipping compounds based on modification of the natural subunits of nucleic acids. PMO subunits contain phosphorodiamidate-linked, 6-membered morpholino rings forming the uncharged backbone, each of which carries a nucleobase (adenosine, cytosine, guanine, or thymine) to provide the sequence-specificity for exon skipping activity. Eteplirsen, SRP-4045, and SRP-4053 are designed to restore the open reading frame of dystrophin mRNA by skipping exons 51, 45, and 53, respectively. The PMO backbone confers improved PK properties over other classes of RNA therapeutics and allows common synthetic, purification, formulation, and analytical methods to be used for all PMO drug candidates. These 3 PMOs were all metabolically stable in hepatic microsomes of mice, rats, monkeys, and humans. In vitro protein binding was low (≤40%) in all species and significant inhibition of the major human CYP isozymes (CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP3A4, and CYP3A4/S) was not observed. Probe substrate activity and mRNA data from human hepatocyte incubations showed no induction of CYP2B6 or CYP3A4 by any PMO tested, and only weak induction (far less than omeprazole) of CYP1A2 by eteplirsen. Eteplirsen was not a substrate or inhibitor of key human drug transporters (OAT1, OAT3, OCT1, OCT2, OATP1B1, OATP1B3, P-gp, BCRP, MRP2, and BSEP) at physiologically relevant concentrations. Plasma t1/2 of radiolabeled eteplirsen was 6 hours, Vd was 175 mL/kg, and CL was 348 mL/hr/kg in male mice after a single IV injection of radiolabeled eteplirsen (120 mg/kg). Dose-normalized exposure parameters in toxicity studies were comparable within species for all PMOs, but were quantitatively higher in monkeys vs. mice, and no plasma accumulation was observed after repeated IV dosing. A 39-week primate study of eteplirsen at doses up to the MFD showed that renal findings for PMOs do not progress with longer dosing durations and identified no new target organs. These results suggest that, as a class, PMOs are not expected to exhibit sequence-specific toxicities and should be well tolerated in boys with DMD at therapeutic doses. In ongoing clinical trials of eteplirsen there have been no drug-associated serious adverse events or evidence of renal toxicity after once-weekly IV infusions of 30 mg/kg or 50 mg/kg doses through 144 weeks, consistent with its safety profile in NHPs.

**604 Safety of Phosphorodiamidate Morpholino Oligomers (PMOs) for Treatment of Duchenne Muscular Dystrophy (DMD)**


DMD is a rare and fatal, X-linked neuromuscular disorder affecting approximately 15,000 boys in the U.S., caused by mutations in the DMD gene that disrupt the open reading frame of the encoded dystrophin mRNA. This prevents production of dystrophin, a critical protein in maintaining stability of skeletal, smooth, and cardiac muscle myofibers. Exon skipping by PMOs is a promising, disease-modifying approach to DMD, which might benefit up to 80% of the patient population. In safety pharmacology studies, the PMOs eteplirsen (exon 51 skipping), SRP-4045 (exon 45), and SRP-4053 (exon 53) had no effect on behavior or on cardiovascular, respiratory, renal, or hepatic systems after single IV injections up to the maximum feasible dose (MFD, 320 mg/kg) in nonhuman primates (NHPs). Kidney was the primary target organ in repeat-dose studies (once-weekly IV injections for 12 weeks) in NHPs, consistent with renal excretion as the major elimination pathway for PMOs. Microscopic findings of renal tubular basophilia in the cytoplasm, with or without vacuolation or degeneration were typically noted, which were considered non-adverse and showed evidence of reversibility during recovery phases. The NOAEL was the MFD for all three PMOs (320 mg/kg). No adverse effects on the male reproductive system or on complement activation were observed. Plasma exposures (AUC) increased with dose and no accumulation occurred after repeated dosing. A 39-week primate study of eteplirsen at doses up to the MFD showed that renal findings for PMOs do not progress with longer dosing durations and identified no new target organs. These results suggest that, as a class, PMOs are not expected to exhibit sequence-specific toxicities and should be well tolerated in boys with DMD at therapeutic doses. In ongoing clinical trials of eteplirsen there have been no drug-associated serious adverse events or evidence of renal toxicity after once-weekly IV infusions of 30 mg/kg or 50 mg/kg doses through 144 weeks, consistent with its safety profile in NHPs.

**605 Hepatotoxicity of LNA Gaper Antisense Oligonucleotides Is Mediated by RNase H1 Dependent but Nonspecific Preferential Downregulation of Very Long Pre-mRNA Transcripts**


Along with improved target affinity, antisense oligonucleotides (ASOs) with locked nucleic acids (LNAs) seem to exhibit an increased propensity to cause severe hepatotoxicity compared to MOE based ASO. In order to better understand the sequence specific mechanism, a single bolus dose of well tolerated as well severely toxic LNA ASOs were administered to mice. Poorly tolerated LNA ASO are profoundly hepatotoxic as evidenced by severe serum transaminitis or death within 48 to 72 hours. In addition to reducing their intended mRNA target, hepatic transcriptome profiling revealed that hepatotoxic ASOs also robustly decreased many unintended transcripts prior the onset of transaminase increase. While the overall nature of off-target transcripts affected varied across toxic ASO, a subset of mRNAs was found to be commonly downregulated by toxic ASOs. Unexpectedly, commonly downregulated mRNAs were much longer at the pre-mRNA as well as more profoundly downregulated than shorter off-target pre-mRNA. When hepatoxic ASOs were administered to mice specifically lacking functional RNase H1 in hepatocytes, we observed a profound amelioration of the hepatotoxicity. This amelioration was also associated with robust blunting of on-target antisense effect as well as a normalization of long pre-mRNA transcripts level in the liver.

**606 Pharmacokinetic (PK) Properties of Phosphorodiamidate Morpholino Oligomers (PMOs) for Treatment of Duchenne Muscular Dystrophy (DMD)**


Exon skipping by PMOs is a promising, disease-modifying approach to DMD. PMOs are members of a unique class of exon skipping compounds based on modification of the natural subunits of nucleic acids. PMO subunits contain phosphorodiamidate-linked morpholino rings forming the uncharged backbone, each of which carries a nucleobase (adenosine, cytosine, guanine, or thymine) to provide the sequence-specificity for exon skipping activity. Eteplirsen, SRP-4045, and SRP-4053 are designed to restore the open reading frame of dystrophin mRNA by skipping exons 51, 45, and 53, respectively. The PMO backbone confers improved PK properties over other classes of RNA therapeutics and allows common synthetic, purification, formulation, and analytical methods to be used for all PMO drug candidates. These 3 PMOs were all metabolically stable in hepatic microsomes of mice, rats, monkeys, and humans. In vitro protein binding was low (<40%) in all species and significant inhibition of the major human CYP isozymes (CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP3A4, and CYP3A4/S) was not observed. Probe substrate activity and mRNA data from human hepatocyte incubations showed no induction of CYP2B6 or CYP3A4 by any PMO tested, and only weak induction (far less than omeprazole) of CYP1A2 by eteplirsen. Eteplirsen was not a substrate or inhibitor of key human drug transporters (OAT1, OAT3, OCT1, OCT2, OATP1B1, OATP1B3, P-gp, BCRP, MRP2, and BSEP) at physiologically relevant concentrations. Plasma t1/2 of radiolabeled eteplirsen was 6 hours, Vd was 175 mL/kg, and CL was 348 mL/hr/kg in male mice after a single IV injection of radiolabeled eteplirsen (120 mg/kg). Dose-normalized exposure parameters in toxicity studies were comparable within species for all PMOs, but were quantitatively higher in monkeys vs. mice, and no plasma accumulation was observed after repeated IV dosing. PMOs, as a class, are therefore expected to have good exposures and low drug-drug interaction potentials at therapeutic doses in patients with DMD.
Triennary N-acetyl galactosamine (GalNAC, GN3), is a high-affinity ligand for the hepatocyte-specific asialoglycoprotein receptor (ASGPR). When conjugated to 2’-MOE antisense oligonucleotides (ASOs) with either phosphorothioate (PS) or a combination of PS and phosphodiester (PO) linkages in the backbone (PS/PO), the GN3 ligand has been previously shown to enhance the potency to multiple targets by 6- to 10-fold in mice. GN3-conjugated MOE ASOs have high affinity for mouse ASGPR, which results in enhanced ASO delivery to hepatocytes. Until recently, this increased potency was only demonstrated in mice, but here we demonstrate increased potency of a GN3-conjugated ASO in non-human primates (NHP). ISIS 681257 is a GN3-conjugated MOE ASO inhibitor with a PS/PO backbone that targets human apolipoprotein(a) [apo(a)]. Apo(a) is a distinct protein component of Lipoprotein(a) [Lp(a)], a genetic variant of low-density lipoprotein (LDL), and has proatherogenic properties. Elevated Lp(a) levels in humans are associated with increased risk of cardiac death, myocardial infarction, stroke, protein (LDL), and has proatherogenic properties. Elevated Lp(a) levels in humans are associated with increased risk of cardiac death, myocardial infarction, stroke, protein (LDL), and has proatherogenic properties. Elevated Lp(a) levels in humans are associated with increased risk of cardiac death, myocardial infarction, stroke, protein (LDL), and has proatherogenic properties. Elevated Lp(a) levels in humans are associated with increased risk of cardiac death, myocardial infarction, stroke, protein (LDL), and has proatherogenic properties. Elevated Lp(a) levels in humans are associated with increased risk of cardiac death, myocardial infarction, stroke, protein (LDL), and has proatherogenic properties. Elevated Lp(a) levels in humans are associated with increased risk of cardiac death, myocardial infarction, stroke, protein (LDL), and has proatherogenic properties.
function through uncoupling of the electron transfer chain and sustained energy demonstrated that propolis exerted a dose-dependent inhibition of mitochondrial toxic effect as an adjunct to cancer drugs such as doxorubicin. Using the BBS, we moderate toxicity potential. The predicted serious ADRs frequency was 10.5%. Its of the crude extract) propolis ranked in toxicity index group 4 which displayed a geted mechanism of action in pancreatic cancer cells using the Bioenergetic Balance induced cardio- and liver-toxicity, but the exact mechanisms of action involved in antibacterial and anti-inflammatory properties, little is know about its toxicity potential studies. nicotine is recognized to improve cognitive function by stimulating brain nicotinic acetylcholine receptors (nAChRs), which can modulate the release of neurotransmitters. Because of its complex and variable chemical composi- nicotine and are generally regarded as having a low potential for toxicities of severe polycythemia for at least 65 days prior to scheduled euthanasia. Mortality was not associated with frank organ toxicity. A significant levels at ≥50 mg/kg were attributed to ex-vascular congestion and/or increased cellularity in large, highly vascular organs at necropsy; increased cellularity in bone marrow; and findings associated with mortality and surviving animals at these doses had dark, whole body discoloration in vivo. For tumour induction, Balb/c female mice were in an immune-competent model. For tumour induction, Balb/c female mice were in tumor size observed after 21 days of treatment. Hence, our results provide an evidence that 6SA might exert anti-tumoural effect by inducing activation of the immune system in an immune-competent in vivo model.

**612 Encenicline (EVP-6124), a Selective Nicotinic Acetylcholine Receptor Partial Agonist, Does Not Demonstrate Nonclinical Abuse Potential**

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Nicotine is recognized to improve cognitive function by stimulating brain nicotinic acetylcholine receptors (nAChRs), which can modulate the release of neurotransmitters. Various compounds, such as nicotine, can elicit different properties leading to abuse potential. Of the nAChRs, the α7 receptors have a low affinity for nicotine and are generally regarded as having a low potential for abuse. Encenicline (EVP-6124) is a novel and selective α7 nicotinic acetylcholine receptor partial agonist that has improved cognition in nonclinical pharmacology models and clinical studies for the treatment of schizophrenia, Alzheimer’s disease, and other cognitive disorders. Novartis of encenicline’s mechanism of action and the abuse potential associated with some nAChRs, additional evaluation of encenicline was warranted. A battery of nonclinical studies to assess drug abuse and dependence were conducted in Sprague Dawley rats including cross generalization study, rats were trained to differentiate between nicotine (up to 0.56 mg/kg, s.c.) and saline with subsequent testing sessions evaluating lever responses between nicotine, vehicle, and encenicline (up to 3.0 mg/kg, s.c.). The relative reinforcing study evaluated the effect of encenicline (up to 3.0 mg/kg, i.v.) in cannulated rats conditioned to self-administer cocaine. Physical dependency was assessed in rats dosed with encenicline (up to 120 mg/kg/d, p.o.) for 30 days, followed by a 7 day non-dosing cessation phase. Encenicline did not demonstrate any signs of abuse potential or dependency in rats based upon a standard battery of nonclinical abuse potential studies.

**613 Propolis Activity and Toxicity Profile Using Mitochondria Bioenergetics and Behavior**


Propolis is a natural resinous hive product manufactured by honeybees. Despite the fact that propolis has been used in human health since antiquity for its antifungal, antibacterial and anti-inflammatory properties, little is known about its toxicity profile. Its anti-oxidant properties have been demonstrated to reduce doxorubicin induced cardio- and liver-toxicity, but the exact mechanisms of action involved in these effects remains elusive. Because of its complex and variable chemical composition it is important to better understand the synergistic pharmacological and phar- maco-toxicological activities it may have. In the present study we used a crude sol- vant-extract of propolis to test its activity/toxicity profile with the Cellessis profiler using the Mitostream® technology and to further elucidate its mitochondria-tar- geted mechanism of action in pancreatic cancer cells using the Bioenergetic Balance Screen (BBS). We demonstrated that the toxicity profile of Propolis was highly dependent on the dose range used. At the initial range used (0.001% to 0.1% of the crude extract) propolis ranked in toxicity index group 4 which displayed a moderate toxicity potential. The predicted serious ADRs frequency was 10.5%. Its activity profile suggested both anti-viral properties and a potential cardio-protective effect as an adjunct to cancer drugs such as doxorubicin. Using the BBS, we demonstrated that propolis exerted a dose-dependent inhibition of mitochondrial function through uncoupling of the electron transfer chain and sustained energy production through the glycolytic pathway in CFPAC-1 pancreatic cancer cell line. This effect was not associated with loss of cell viability but synergized doxorubicin anti-proliferative activity. This is the first demonstration of a direct activity of prop-olis extracts at the mitochondrial electron transfer chain, which combined with its anti-oxidant properties may provide a mechanism for hepato- and/or cardio-pro- tective effects of anti-cancer drugs.

**614 Hematocrit (HCT) and Hemoglobin (HGB) Responses to 3-Month Oral Dosing of AKB-6548, an Inhibitor of Hypoxia-Inducible Factor Prolyl-Hydroxylase (HIFPH), in CD-1 Mice**

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AKB-6548 is a once-daily, oral, small molecule HIFPH inhibitor in development for treatment of anemia associated with chronic kidney disease. Inhibition of HIFPH coordinates iron mobilization and erythropoietin production to increase erythrocyte production. In clinical trials through 42-day Phase 2a, AKB-6548 was well tolerated and gradually elevated HGB. In prior non-clinical studies, AKB-6548 was generally well tolerated in rat and dog during 6-9/months of dosing, with modest increases in HGB at doses chosen to minimize severe polycythemia and mortality associated with large organ vascular thrombosis. It remained un- known whether animals could adapt to prolonged high doses of AKB-6548 pro- ducing very high HCT values. In a 3-month study in CD-1 mice, AKB-6548 was dosed up to 200 mg/kg/day. There were no AKB-6548-related changes in body weight or food consumption and no clinical signs in surviving mice. Statistically significant rises in HCT and HGB were observed at ≥50 mg/kg by Day 28, with severe polycythemic levels of HCT at ≥100 mg/kg which continued to rise through necropsy (Day 93) in all mice (range: 71% to 100% HCT). Elevations in HGB levels at ≥150 mg/kg ranged from 15.4 to 30.2 g/dL. Drug-related mortality oc- curred between Days 37 and 91 in fewer than 7% of severely polycythemic animals. Mortality and surviving animals at these doses had dark, whole body discoloration at necropsy; increased cellularity in bone marrow; and findings associated with vascular congestion and/or increased cellularity in large, highly vascular organs including liver, lung, and spleen. All findings at ≥100 mg/kg were attributed to ex- ceptionally high erythrocyte mass associated with the pharmacology of AKB-6548 at high doses. Mortality was not associated with frank organ toxicity. A significant number of mice were able to adapt to large increases in HGB and extended inter- vals of severe polycythemia for at least 65 days prior to scheduled euthanasia.

**615 Carcinogenicity Assessment of the Pan-Caspase Inhibitor, Emriscan, in Tg.rasH2 Mice**

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Emriscan, formerly IDN-6556, is a small molecule that is currently being evalu- ated in clinical trials to reduce hepatic injury and liver fibrosis. Since emriscan is an irreversible pan caspase inhibitor, it is a potent inhibitor of apoptosis and caspase-mediated inflammation. Thus, it was important to determine whether em- riscan promotes tumorigenesis in a humanized mouse model. Tg.rasH2 mice received Lab Diet formulated with 10, 20, and 75 mg/kg/day of emriscan, for 26 weeks. At terminal sacrifice, blood was collected for clinical pathology analysis and tissues were collected, fixed in formalin, processed to slide, and evaluated micro- scopically. There were no treatment related deaths or overt signs of toxicity for the duration of the study. There was no evidence of a carcinogenic effect in the peripheral blood leukocyte counts. Liver microgranulomas, which are background lesions, were slightly increased, especially in males. Increases in the incidence of the activated germinal centers were seen in the spleens and mesenteric lymph nodes of male and female mice, and in the mandibular lymph nodes of male mice. Atrophy of ovaries and testicular degeneration were also seen in emriscan treated animals. Although several non-neoplastic lesions were observed, there was no evidence of emriscan-related tumor formation in any tissue. In addition, none of the non-neo- plastic lesions were considered pre-neoplastic. Thus, the potent pan-caspase inhibi- tor, emriscan, is not considered carcinogenic.
616 Chronic Toxicity Assessment of the RANKL Blocker, RPH-203, following Subcutaneous Administration in the Cynomolgus Monkey

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Interactions through the pathway of the receptor activator of nuclear factor k (RANK), its ligand RANKL and Osteoprotegerin significantly contribute to the mechanism of bone metastasis development. The RANKL blocker, RPH-203 is under development for the treatment of bone metastasis, which is a common complication of many solid tumors. The chronic toxicity of RPH-203 was investigated following weekly dosing by subcutaneous administration in the Cynomolgus monkey for up to 32 weeks, followed by a 6 week recovery period. 72 Cynomolgus monkeys were randomly distributed into 4 treatment groups and received RPH-203 at doses of 0, 3, 10 and 30 mg/kg. An interim group of animals was sacrificed after a 13 week treatment period and subsequent 4 week recovery period. All the animals were subjected to standard procedures for chronic toxicity studies. CNS observations, blood pressure, ECG, respiratory rate measurements and ophthalmoscopy investigations were performed at regular intervals. Blood samples for clinical pathology, toxicokinetics, determination of Tand B cells, immunoglobulin levels and bone tissue turnover biomarkers levels were collected at regular intervals. DEXA scans to assess bone mineral density were undertaken on all animals. Results from the study showed that there were no clinical signs, skin reactions and body weight and food consumption changes. Physiological and clinical pathology investigation parameters and Immunoglobulin levels and T and B cell populations were unaffected by treatment with RPH-203. Toxicokinetic data showed systemic exposure to RPH-203 increased with dose, with no accumulation of RPH-203 with time. Bone formation and resorption biomarkers showed a trend towards a reduction, over the course of the treatment period. There were no histological changes noted at the end of the 32 week study conclusion. The weekly subcutaneous administration of RPH-203 for up to 32 weeks was well tolerated in the Cynomolgus and not associated with any toxicity.

617 Bone Marrow and Lymphoid Toxicity of a Chemokine Receptor-1 (CCR-1) Antagonist Is Associated with Centromeric Disruption during Chromosomal Segregation

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CCR-1, a target for immune-mediated diseases, mediates inflammatory leukocyte trafficking. In 3-day studies, BI 639064 was well tolerated in rats up to 1000 mg/kg/day and in dogs up to 30 mg/kg/day. At ≥30 mg/kg/day in dogs, decreases in reticulocytes and leukocytes and clinical signs of decreased motor activity continued post-dosing leading to euthanasia. In a 4-week rat study, decreases in leukocytes increased in severity with dose and time resulting in mortality by day 7 in the 1000 mg/kg/day group. Degeneration/regeneration of intestinal epithelium was evident at ≥100 mg/kg/day. In the 4-week dog study, ex vivo inhibition of neutrophil CCR-1 receptor internalization was maximal 1 mg/kg/day. Sudden and rapid decreases in leukocytes, with no changes to T-cells, were observed by day 8 at doses of 5 but not 1 mg/kg/day resulting in early euthanasia of most dogs. These effects reversed in a 5-day survivor. Histopathology in both species included bone marrow hematopoietic cell depletion and lymphoid depletion most severely in the thymus. BI 639064 was negative in the Ames test and the in vito human lymphocyte chromosome aberration (HIA) test, despite a dose dependent increase in centromeric disruption and low incidence of endoreduplication. BI 639064 was positive in an in vito micronucleus assay and an in vito micronucleus test in CHO cells where anti-kinetochore staining confirmed aneugenicity. Kinase profiling showed hits to MAP4K and STK17a suggesting a possible role for kinase inhibition in the mechanism of aneugenicity. These data demonstrate primary toxicity of BI 639064 to hematopoietic and lymphocytic systems and intestinal epithelium characterized by a sudden onset with a steep dose response. Based on these data and low anticipated therapeutic exposure multiples, BI 639064 was not progressed to clinical Phase 1. Additional research is necessary to confirm the mechanism of hematotoxicity.

618 Characterization of the Renal Toxicity Induced by a Novel Polyoxin Analog

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AZ TC, a novel polyoxin analog, was evaluated in rat toxicity studies to determine its renal toxicity profile versus the standard comparator compounds, colistin and polyoxin B (PMB). AZ TC was administered to male Wistar rats for 2 or 7 days of repeat dosing, via subcutaneous injection, qid. Recovery was assessed at 14 and 28 days following cessation of treatment. Clinical signs, clinical pathology, acute kidney injury biomarker, macroscopic and microscopic observations were recorded. Toxicokinetic analysis of plasma and terminal kidney (whole organ homogenate) samples was conducted, with additional MSI-MALDI evaluation of selected kidney samples. Vasculature of proximal tubular epithelial cells was the primary microscopic finding after 2 and 7 days of dosing, with additional degenerative changes after 7 days. Tubular basophilia accompanied by mononuclear cell infiltrates were the predominant findings after 14 and 28 days of recovery. Microscopic findings were associated with significant changes in kidney injury biomarkers, with peak increases after 7 days of dosing. TK data demonstrated preferential and continued accumulation of AZ TC in kidney over the duration of dosing. We further observed that high levels of AZ TC were detected even during the recovery phase at both 14 and 28 days post-dosing. MALDI imaging indicated that AZ TC preferentially accumulated in the cortical portion of the kidney. Improvement of the severity of degenerative tubular changes and normalization of urinary biomarkers indicates reversibility of kidney injury following cessation of treatment. The persistence of tubular basophilia after 1 month of recovery may indicate persistence of low-grade toxic injury or represent a reparative response.

619 Nonclinical Safety Assessment of the Gamma Secretase Inhibitor Avagacestat

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Avagacestat (AVA, BMS-708163) is a γ-secretase inhibitor with selectivity toward proteolysis of amyloid precursor protein (amyloid-β peptide formation) over Notch substrates, a family of transmembrane signaling proteins that regulate cell differentiation in multiple tissues. To support clinical evaluation as a potential therapy for Alzheimer’s disease, the nonclinical safety of AVA was assessed in a series of studies with daily oral dosing in rats (≥6 months) and dogs (≥1 year). The primary AVA-related toxicity findings in rats and dogs were attributed to mechanism-based inhibition of Notch signaling and included decreases in peripheral lymphocytes (B-cells in dogs and T-cells in rats) and morphologic changes in lymphoid tissues, ovaries, small intestine, and bone (rats only). These Notch related effects demonstrated clear exposure-response relationships in each species, with the dog being the most sensitive. In dogs, gastrointestinal toxicity was dose-limiting and was accompanied by decreases (> 50%) in blood levels of hairy enhancer of split gene-1 (HES 1) mRNA, a downstream marker of Notch inhibition. Other target organs included kidneys, and adrenal and pituitary glands in female rats. Microscopic changes in the adrenal and pituitary glands of rats were associated with alterations in circulating reproductive hormones including decreased estradiol and progesterone, and increased gonadotropins (luteinizing and follicle-stimulating hormone) that were considered secondary to a direct AVA-related disruption of ovarian follicular development. After 1 year in AVA-treated dogs, brain levels of amyloid-β peptides were reduced at all doses providing evidence of sustained pharmacodynamic activity. All AVA-related target organ effects in rats and dogs were partially or fully reversible after a ≥3-month recovery period. In summary, the nonclinical safety profile of AVA supported Phase 2 clinical studies in elderly Alzheimer’s patients.
620 Experimental Study on Toxicity and Toxicokinetics of dl-PHPB
H. L. Tan, L. Jiang, W. Xiaoliang, and W. Anping.

Objective: To observe the toxicity and toxicokinetics of new candidate drug Potassium 2-(alpha Hydroxypentyl) Benzoate (dl-PHPB) in Beagle dogs. Vascular and muscular stimulation, hemolysis and sensitization were tested. SD rats were divided into the doses of 10,30,90 mg/kg groups and beagle dogs at 6, 18, 54mg/kg for 30 days by intravenous injection. On 1st day and 30th day, blood samples were collected at 3min, 0.25, 0.5, 1, 2, 4, 6,24h after administration and analyzed with validated HPLC-UV method, using DAS software to analyze the data. Results: dl-PHPB had no obvious vascular or muscular irritation and injury effects, no evidences of hemolysis and aggregation on rabbit erythrocyte and no allergic reactions on guinea pigs. Following intravenous injection of dl-PHPB at the dose of 41 mg/kg to 210 mg/kg, slight to severe toxicity was showed in beagle dogs. Repeatedly intravenous injection of dl-PHPB for 30d in SD rats could dose-dependently cause slight increment of the liver weight, elevation of serum globulins. At the dose of 54mg/kg showed slight toxicity in beagle dogs: trembling, head tremors, increased BUN and Cr. There was no toxicologically relevant finding at the end of recovery period. More than 90% of dl-PHPB was converted dl-NBP within 1h. On 1st day, its active metabolite (dl-NBP) AUC0-∞ was 2.9, 7.2, 32.5g/h/ml, respectively. On 30th day, dl-NBP AUC0-∞ was 2.9, 8.1,38.7g/h/ml, respectively. There was a slight difference of dl-NBP concentration at the same time point on day 1 and day 30. Conclusion: The non-toxic reaction dose in rats is 30 mg/kg, in Beagle dogs is 18mg/kg.

621 Differential Effects of FIAU, FIRU, and DDC on Functional and DNA Content Endpoints in HepatoPac™ and Huh7 Cells

Fialuridine (FIAU) was evaluated in the 1990s for the treatment of HBV, and 5/15 patients died of hepatic failure. While humans are sensitive to FIAU toxicities, the usual preclinical test species (rat, mouse, dog and monkey) are resistant. This human-specific sensitivity is consistent with reports that humanized-liver TK-NOG mice, but not wild-type mice, are sensitive to FIAU, and that FIAU affects functional endoints (FE) in human, but not rat, HepatoPac in vitro systems. We have further investigated in vitro FIAU species sensitivity differences and compared results to FIRU (FIAU diastereoisomer) and zalcitabine (DDC). We measured nuclear (nuDNA) and mitochondrial (mtDNA) content in the promising non-multiplicating and relatively stable primary hepatocyte HepatoPac system and in the Huh7 human hepatoma cell line. FE results for FIAU in HepatoPac (e.g., IC50 ≤ 10 μM for human vs >100 μM for rat cell urea synthesis) generally recapitulated the previously published greater sensitivity in human vs rat; while FIRU and, surprisingly, DDC had no effects on FE in either species. FIAU decreased mtDNA, nuDNA, and mtDNA/nuDNA similarly in both rat (IC50: 17, 135 & 30 μM, respectively) and human (IC50: of 8, 68 & 9 μM, respectively). FIRU had minimal effects on mtDNA and nuDNA. In Huh7 cells, FIAU and FIRU had little effect on mtDNA or mtDNA/nuDNA. In contrast, DDC reduced both mtDNA and mtDNA/nuDNA by >90% (IC50: ~3 μM each). These results suggest that stable cultured hepatocytes of both species appear to be sensitive to mtDNA synthesis inhibition by FIAU but that a greater excess of mitochondrial functional capacity may contribute to the greater apparent resistance to FIAU observed in rat hepatocytes. Understanding such apparently greater resistance of human HepatoPac to DDC vs FIAU, and of Huh7 cells to FIAU vs DDC together with data in humanized mouse models with such drugs, is expected to improve understanding of these promising models and improve integrated safety screening of novel antiviral nucleosides.

622 In Vitro Toxicity Assessment of the Nucleoside Analog Fialuridine Using Micropatterned Primary Hepatocyte Cocultures and Discovery of a Nontoxic Isomer

Fialuridine, a nucleoside analog designed for the treatment of hepatitis B, failed in clinical trials due to fatal hepatotoxicity. Both in vitro assays as well as preclinical in vivo tests in mice, rats, dogs, and primates could not predict its hepatotoxicity potential. Its toxicity is thought to be mediated by human-specific mitochondrial disruption after long-term exposure. We used the micropatterned primary hepatocyte coculture model, HepatoPac™, to assess fialuridine-mediated toxicity in vitro. Five human hepatocyte donors were exposed to 4 doses of increasing concentrations of fialuridine over 9 days in serum-free medium. Toxicity was evaluated by ATP-content, mitochondrial activity and urea production. To all human hepatocyte donors evaluated, fialuridine was toxic at clinically relevant concentrations but non-toxic to rat HepatoPac™ cultures. The most sensitive donor showed IC50 values that were below the human plasma Cmax of fialuridine (0.5 μM). Interestingly, several commercially available batches of ‘fialuridine’ affected hepatocyte viability differently. Further analysis by 1H-NMR spectroscopy revealed that some of these batches did not contain fialuridine but its 2’ epimer, i.e. respect to the base, the fluorine atom is oriented at the opposite face of the sugar ring. Remarkably, no toxicity was found mediated by this fialuridine-isomer (tested up to 100 μM). Further studies include determination of triphosphate formation and unraveling the mechanism of toxicity by focusing on early signs of mitochondrial dysfunction. Moreover, human-specific toxicity will be proven by examining the toxicity profiles of fialuridine in dog and cynomolgus monkey HepatoPac™. Our results indicate that HepatoPac™ is a useful model to identify non-acute hepatotoxicants, i.e. nucleoside analogs, and study their mechanism of toxicity.

623 Nonclinical Safety Assessment of BMS-902483, a Novel Alpha-7 Nicotinic Acetylcholine Receptor Partial Agonist

BMS-902483 is a novel alpha-7 nicotinic acetylcholine receptor partial agonist that was under development for the treatment of cognitive deficits in schizophrenia and Alzheimer’s disease. The safety of BMS-902483 was evaluated in a comprehensive nonclinical toxicity program that included genetic and phototoxicity studies, a single-dose cardiovascular study in dogs, and 1-month repeat-dose oral toxicity studies in mice and dogs. BMS-902483 was not genotoxic or phototoxic. BMS-902483 was clinically well tolerated in mice (~200 mg/kg/day) with findings limited to decreases in minute ventilation and body weights. In dogs, pronounced clinical toxicity was observed at ~30 mg/kg/day and the kidney, liver, and heart were identified as the primary target organs. Key BMS-902483-related findings were partially or fully reversible and included: 1) tremor, salivaion, and vomiting; 2) QT prolongation and increased systemic and left ventricular systolic blood pressures; 3) increased inflammatory cell (slight to mild) and macrophage infiltrates (minimal to moderate) in the liver with increases in serum ALT, AST, alkaline phosphatase, and total bilirubin; and 4) moderate acute renal tubular necrosis and mild regeneration with increases in serum BUN, creatinine, and phosphorus and decreases in urine volume and specific gravity. Clinical observations and cardiovascular findings were consistent with an adverse effect on renal and liver toxicities or the sensitivity of dogs to these findings are currently unknown. Although these data suggested that BMS-902483 could be safely administered as part of a Phase I clinical study, the nonclinical safety profile was considered insufficient to support further development for chronic use in humans due to concerns that these liver and renal toxicities would be dose- and time-dependent.

624 Cardiac and Neurological Toxicity of G Protein-Coupled Receptor 119 (GPR119) Agonists in Monkeys

Dual acting GPR119 agonists promote insulin secretion, reduce food consumption and present a therapeutic strategy for the treatment of type II diabetes. However, toxicological challenges have impeded preclinical development. We describe here...
the toxicity of two structurally related GPR119 agonists (A and B) in sub-chronic and chronic toxicity studies in monkeys. Gastrointestinal (GI) tract and heart were key target organs of toxicity for both compounds. Clinical signs of toxicity manifested as dose-dependent increase in GI distress (inappetence, ileus, dehydration), decreased activity, hunched posture and decreased body temperature, which led to moribund euthanasia of several monkeys at high doses. For both compounds prominent clinical pathology changes included decreases in red cell parameters (RBC count, hemoglobin and hematocrit, with a regenerative increase in red cell dispersion width). The effects on heart observed at necropsy included dose-dependent increase in absolute and relative heart weight with or without accompanying cardiac hypertrophy. Additionally, for compound A only, multifocal mid brain lesions, characteristic of Wernicke's encephalopathy, were observed at the high dose in the 3-month studies and progressed to lower doses in the 9-month study. Investigative studies of glycogen biosynthetic and degradation pathways with compound A did not explain the mechanism of cardiac hypertrophy, nor was there any benefit of intramuscular thiamine supplementation on the mid brain lesions, which were hypothesized to be secondary to inappetence and Vit B deficiency. However, metabolic perturbations evidenced by increased plasma and cerebrospinal fluid lactate, and decreased serum folate and cobalamin were observed. Thus, metabolic perturbations secondary to GI distress may have contributed to cardiac and CNS toxicity of these GPR119 agonists.

625 Acute Toxicity Assessment of PAC-1, a Novel Anticancer Agent, in Beagle Dogs and in Ames Test
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PAC-1 is a novel proap cese 3 activator and effective anticancer agent. It crosses the blood-brain barrier and hence has a unique property to treat gliomas after oral administration. The toxicity of PAC-1 was studied in Beagle dogs after single oral (Phase 1; free base form of PAC-1 at 50, 100, 200 and 1000 mg/kg) and 7 days oral (Phase 2; free base form of PAC-1 at 50 and 100 mg/kg/day and salt form of PAC-1 at 100 mg/kg/day) administration. There was no mortality. A single and 7-days administration of PAC-1 produced different signs of toxicity – decreased activity, vomiting, soft stool, diarrhea, severe body weight losses, severe decrease in food consumption and changes in hematology parameters more pronounced at 100 mg/kg/day for 7 days (both free base and salt forms) and neurological symptoms at 100 mg/kg/day for 7 days (salt form). After 7 consecutive daily doses of PAC-1 (free base) at 100 mg/kg/day the systemic exposure (AUC) and Cmax values were substantially lower compared to these levels after a single dose of PAC-1 (free base). Administration of the salt form of PAC-1 did not result in greater absorption. There were no test article-related pathology findings. The Highest Non-Severely Toxic Dose of PAC-1 was 50 mg/kg/day after 7-day administration which provided a good safety profile for potential first in human efficacious dose. The mutagenic potential of PAC-1 was evaluated by Ames test. Several strains of Salmonella typhimurium (TA98, TA100, TA1535, TA1537) and Escherichia coli in the presence and absence of S9 activation were used. In the confirmatory assay, the dose levels were 5, 15, 50, 150 and 500 µg/plate in all tester strains (with and without S9 activation) except the TA1537 (with S9 activation). The dose levels tested in TA1537 strain with S9 activation were 15, 50, 150, 500 and 1500 µg/plate. Positive mutagenic responses were not observed in any tester strains. PAC-1 was concluded to be negative in the bacterial reverse mutation assay. This study was sponsored by Vanquish Oncology, Inc.
Biochemical and cellular assays led to the identification of a cohort of compounds that these compounds inhibit ROCK/MRCK in an ATP competitive manner.

Inositol requiring enzyme-1α (IRE-1α) is a trans membrane stress-sensing and signaling molecule that controls the UPR. Numerous perturbations of protein folding contribute to ER stress. Downstream enzymatic activity is selectively activated during times of cellular stress, primarily during disease states and thus, inhibition of this pathway may impact tumor growth. A novel, first-in-class IRE-1α inhibitor, MKC4500, was evaluated in a preliminary 5-day oral toxicology study in Sprague Dawley rats administered 25, 75 or 225 mg/kg/day (n=4/sex/group). Toxicokinetic evaluations were performed on Day 1 and 5 of the study. There were no test article related deaths or clinical observations. At 25 mg/kg/day in females only, there was a decrease in body weight gain and a slight elevation in liver enzymes (1.36x). At 75 mg/kg/day, there was a decrease in body weight gain, slight changes in hematologic parameters and ALT levels (-1.6x), minimal sinusoidal hypercellularity in the liver (females only) and minimal findings in the lymphatic lymph node. Test article-related findings observed only in the high dose group included decreased food consumption and body weight loss, increased leukocytes, hemolytic anemia, increased bilirubin and TG levels, decreased serum protein and cholesterol levels, increased creatinine and BUN and electrolyte alterations. Histopathology changes were noted in the stomach, intestines, kidney, liver, spleen, lymph node, thymus and adrenal glands. Dose dependent increases in systemic exposure was achieved with Cmax at approx. 2.8 hours post dose. Females generally showed greater exposure than males with accumulation at the highest dose in both sexes. Based on a NOAEL of 75 mg/kg/day in this study and exposure levels in early xenograft studies, preliminary safety data indicate there may be sufficient coverage for further evaluation on oncology therapeutic indications.

630 Investigating the Mechanism of Action for a Novel Therapeutic to Mitigate Traumatic Brain Injury (TBI) Using Rat Brain Cells

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There are nearly 10 million new traumatic brain injuries (TBI) every year on a global scale, of which 1.7 million occur in the U.S. Our military personnel face TBI while serving in the call of duty for our country. TBI compromises not only the health of the warfighter but the subsequent quality of life. In the injury treatment process, oxygen is considered a critical component in pain management, mitigating inflammation and the subsequent healing. The main objective of this work is to investigate the beneficial use of a novel material the proprietary polyoxymonoglycerol material termed OX-66. This work determines the cytotoxicity, the potential mechanisms of cellular repair of DI-TNC1 rat astrocytes, and H19-7 rat hippocampal neuronal cells exposed to OX-66 following an in vitro "TBI" (stretch/strain) model. Initially, our results indicate that 1) the viability of healthy H19-7 and DI-TNC cells did not change at 48 hours after applying OX-66. In addition, at certain concentrations, results showed improved improvement on cell viability; 2) At 48 hours after being injured mildly, moderately and severely, both H19-7 and DI-TNC showed improved viability. This work is the foundation of work to follow evaluating potential formulations for use in Phase I clinical trials.

631 Preclinical Development of Novel Dual Inhibitors of Rho Kinase and MRCK As Anticancer Agents

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Cancer cells that become more motile and gain the ability to invade other tissues form metastases that are typically much more difficult to treat than the primary tumor from which they originated. Given the clinical failure of anti-invasive strategies, we hypothesized that targeting cancer cell motility might be a more effective approach to block the development of distant metastases. The Rho-associated kinases (ROCK) and myotonic dystrophy-related Cdc42-binding kinases (MRCK) are two related protein kinase families that regulate stress fiber formation and play critical roles in cancer cell migration. Hence, we screened in-house library of small molecules to identify dual inhibitors of the ROCK and MRCK kinases. In vitro biochemical and cellular assays led to the identification of a cohort of compounds that effectively target both kinase families with different potencies. Biochemical studies using recombinant proteins and computational docking studies confirmed that these compounds inhibit ROCK/MRCK in an ATP competitive manner. Fluorescent microscopy revealed loss of stress fibers in the treated cancer cells and normal fibroblasts. These compounds effectively inhibited migration of various cancer cell lines. NCI-60 cell line screening and in-house studies revealed that inhibition of ROCK/MRCK by these compounds also induced cell cycle arrest or apoptotic cell death in a cell line specific manner. In vitro metabolism studies with mouse, human and rat liver microsomes revealed the major metabolites. Based upon the above studies, we identified one compound (DF4) for further development. Preliminary MTD studies of DF4 in mice revealed that it is well tolerated up to 20mg/kg when administered every other day for 2 weeks. Taken together, these studies indicate that DF4 is a lead compound that can be further developed as a potent, effective dual inhibitor of the ROCK and MRCK kinase families for adjuvant use as strategy to block the development of metastases in newly diagnosed cancer patients.

632 Bromodomains and Extraterminal Domain Inhibitors Induce a Loss of Intestinal Stem Cells and Villous Atrophy

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BET domain epigenetic reader proteins play a role in controlling the expression of genes that regulate cellular differentiation and proliferation and phenotype maintenance (e.g., cMyc) and are frequently modified in cancer; hence, BET modulators are being explored as potential therapeutics in a number of tumour settings. In addition to regulating tumour cell viability, BET inhibitors are also associated with gastrointestinal toxicity, though the molecular and cellular mechanisms behind this toxicity have yet to be elucidated. We have found that BET inhibitors induce a dose-limiting duodenal villous atrophy in vivo, accompanied by inappetence and body weight loss. Ex-vivo cultures of intestinal organoids confirm that villous atrophy occurs with multiple chemical classes of BET domain inhibitors and that their toxicity is driven by their primary pharmacology. Intriguingly, the intestinal atrophy occurs in the absence of a proliferative block, and in the presence of cMyc, suggesting that the intestinal effects are not mediated by cMyc inhibition as may have been assumed. We find instead that BET domain inhibitors induce a rapid loss of fast cycling intestinal stem cells, suggesting that it is a loss of stem-cell self renewal leading to crypt loss and dose-limiting duodenal toxicity.

633 Safety Study in Mice of an Agent with Potential As an Anti-Infective

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In vitro experiments resulted in the discovery that a series of iridium (Ir) ethylene diamine complexes exhibited antimicrobial activity against Staphylococcus aureus and methicillin-resistant S. aureus at 1-10 µg/mL. For the initial toxicity screening, we examined one of the most active compounds; 3-Ir. The safety study was conducted in groups of adult male mice given a single dose of 5 mg/kg by intravenous (IV) administration. Mice were examined for a series of behavioral indices using a modified functional observational battery at 1, 4 and 6 hr, and 1, 2, 7, 9, 12 and 14 days post-dosing (Tegeria and Balster, Toxicol Sci 22:240). Body weight was measured and recorded at the time of each assessment. Blood and tissue samples were collected at 24 hr, 4 days and 14 days for clinical pathology and histopathological assessment. Of the animals receiving 3-Ir, 5/19 animals showed immediate signs of distress following dosing. Signs of distress ceased within one minute and animals appeared to have normal appearance, activity and locomotion within 5 minutes. No subsequent evidence of neurobehavioral, clinical or histopathological detriment was noted. The doses tested in this IV safety study are within the mg/kg range of currently used drugs. In circumstances such as used here, neurobehavioral or pathological detriments after single administration would be unlikely. These compounds, therefore, have potential to be a useful new class of antimicrobial agents. (Supported by the Virginia Tech Foundation)
634 Acute and Repeated-Dose Toxicity Studies of Synthetic Derivatives of Triazole-Incorporated Pyridazinone As New Class of Antihypertensive Agent

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The pharmacological activity of 4,5-dihydro-6-phenyl-3(2H)-pyridazinones has been extensively studied and it is known for its cardiovascular effects. To support the safety of synthetic compound 6-(4-ethylphenyl)-2-(4-(4-chlorophenyl)-5-thi oxo-4, 5-dihydro-1H-1,2,4-triazol-3-yl)-4,5-dihydropyridazin-3(2H)-one, it has been examined in an acute and in a 4-week repeated dose toxicity study in rats. Animals were divided into groups of 5 animals each. The compound (20 mg/kg body weight and 40 mg/kg body weight) was injected intraperitoneally after suspending in 1% carboxymethylcellulose (CMC) solution in single dose resulted in no adverse events or mortality. Also, the compound administered as a daily dose of 40 mg/kg for 4 weeks by gavage resulted in no adverse events or mortality. No evidence or treatment-related toxicity was detected during both studies. Data analysis of body weight gain, food consumption, clinical observations, blood biochemical, haematology, organ weight ratios and histopathological findings did not show significant differences between control and treated groups. It is concluded that the synthetic compound orally administered to rats was safe and that no treatment-related toxicity was detected in both acute and in repeated exposure and repeated dose (4 weeks) oral route of exposure (40 mg/kg of body weight) toxicity studies.

635 Differential Hepatotoxicity of Liver X Receptor Agonist in Male and Female Rat Hepatocytes

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BMS-852927 is a liver X receptor (LXR) agonist that was being evaluated as a treatment for coronary heart disease. Its mechanism of action involves stimulation of reverse cholesterol transport. In preclinical safety studies, BMS-852927 caused hepatotoxicity in male but not in female rats. We proposed that this difference in toxicity was due to metabolic differences mediated by CYP3A, the highly expressed isozyme in male rats. In vitro studies were conducted to examine the relative toxicity of BMS-852927 and two BMS-852927 downstream P450 metabolites, metabolite A and metabolite B, in cultured Sprague-Dawley rat male and female hepatocytes. Hepatocytes were incubated with the LXR compounds and/or CYP3A1 or CYP3A2 inducers, 5-pregnen-3α- and/or CYP3A2 or CYP3A1 inducers, 5-pregnen-3α-carbonitrile (PCN) or dexamethasone (Dex) for 4 and 24 hours and evaluated for cytotoxicity. CYP3A1 and CYP3A2 mRNA transcript levels and CYP3A activity levels were measured in male induced and non-induced hepatocytes. None of the compounds exhibited significant toxicity in either male or female non-induced rat hepatocytes at concentrations ≤ 100 μM. Metabolite A toxicity was substantially increased in male rat hepatocytes when incubated with PCN (CYP3A2 inducer), but no increase in LXR compound toxicity was observed following incubations with Dex (CYP3A1 inducer). In contrast, induction with Dex or PCN did not increase LXR compound toxicity in female rat hepatocytes. Increased CYP gene expression levels and CYP3A enzyme activity in male rats confirmed that CYP3A12 genes were differentially responsive to inducers. In these in vitro experiments support the hypothesis that the metabolite A is hepatotoxic in male rats through a downstream pathway involving activation by CYP3A2.

636 In Vitro Human Developmental Neurotoxicity Screening Using Multiple Cell Types

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There is a need for rapid and cost effective methods to identify chemical hazards to human brain development. Brain development follows a fairly long but defined timeline; comprised of developmental windows each dominated by specific cell types and processes, potentially presenting unique susceptibility. To accurately assess risk it is thus critical to address developmental neurotoxicity (DNT) in the context of progressive windows of susceptibility (WOS) as well as different cell types, alone or in tissue representative combinations. Here we present an in vitro system; using human pluripotent stem cell (hPSC) derived neural progenitors to model an early proliferative phase, neuronal cells at partial and advanced stages of differentiation to model later stages, and neurons combined with astrocytes to assess toxic outcomes in a multi-cellular environment. Along with acute studies, we perform differentiation on assay plates to allow longitudinal studies on an early and a late WOS. Here we used Bis1, a known neurotoxin, to demonstrate cell and WOS specific differences in response, using high content imaging to quantify various parameters of neurite outgrowth, a well established DNT endpoint. Cell viability assays were included to ensure endpoint specificity. We show that Bis1 effects viability of neural progenitors, neurons and astrocytes to different degrees, while also reducing neurite outgrowth in neurons. Further, the toxic effect of Bis1 in neurons was significantly lowered when neurons were challenged with Bis1 in presence of astrocytes in multi-cellular assays. In longitudinal assays, no differences were observed in neurite outgrowth between the early and late WOS. However, neuron viability was significantly lower in the late WOS. The system described above provides a more complete and robust way to address DNT and combined with suitable compound libraries, will provide valuable inputs for adverse outcome pathway modeling and large scale screening.

637 Stem Cell-Derived Human Sensory Neurons for Toxicity Testing of Drugs/Chemicals and for Identifying Countermeasures

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Human embryonic stem cell (hESC) technology provides a tool to generate any cell type. In vitro. At present, little is known about how the developing peripheral nervous system (PNS) is affected by toxicants, and how toxicity and its countermeasure may be assessed for human peripheral neurons. Therefore, we setup a model of peripheral neurogenesis to assess neurite growth and disturbed calcium signaling as functional endpoints. Based on the combination of small molecule inhibitors that induce PCN differentiation of hESC into sensory neurons (Chambers et al., 2012), we generated a population of peripheral neuronal progenitor cells within 8 days of differentiation. After this stage, cells were cryopreserved. Freshly thawed cells developed neurites and formed a dense neurite network, which was quantified by live cell imaging. Moreover, hESC-derived sensory neurons showed strong calcium signaling upon depolarization. Quantification of neurite growth and of calcium responses on single cell level were optimized as endpoints to assess peripheral neurotoxicity. In this system we identified compounds with several biological activities that had an inhibiting (classical chemotherapeutics, proteasome inhibitors, environmental toxicants) as well as an accelerating (Rho kinase inhibitors) effect on neurite growth. The system reacted differently from central neurons tested in parallel, and the findings correlated with clinical findings on peripheral neurotoxicity. Neutralization of some toxic effects by Rho kinase inhibitors suggests that pharmaceutical rescue strategies may be tested in the model. Our data indicates that we have established a model for human sensory neuron development and toxicity, which is capable of identifying adverse effects of different chemical classes on functional endpoints. Reference: Chambers et al. (2012) Combined small-molecule inhibition accelerates developmental timing and converts human pluripotent stem cells into nociceptors. Nat Biotechnol 30: 715-720.

638 Human iPSC Neurons: An In Vitro Model to Predict Clinical Neurotoxicity

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A major cause of drug attrition from clinical trials is neurotoxicity, a problem likely due to current preclinical models not adequately predicting human safety. One of the most common drug-induced neurotoxicities observed clinically is peripheral neuropathy. Existing in vitro animal models are time-consuming, expensive, and lack a direct species relationship to humans. Current in vitro models consist of immortalized cell lines and primary rodent cultures, which show variable correlation to human physiology. There is an unmet need for high throughput, in vitro, human-based assays that can identify compounds with neurotoxic and neuropathic potential. These in vitro assays can precede in vivo animal studies, and augment those studies with data in the most relevant species before clinical trials begin. We chose to establish such assays with human induced pluripotent stem cells (iPSCs) that have been differentiated into mature neurons. These iPSC neurons offer a renewable source of mature cells that may be used to investigate human drug safety. Our characterization of these neurons shows that they express mature markers at both the transcript and protein levels. Using kinetic and high-throughput imaging we evaluated a large panel of test compounds known to cause clinical neuropathy for their ability to affect neurite outgrowth and viability in these neurons. Results from our assays show that these compounds exert neurotoxicity in a class specific manner and that our assessment correlates with clinical findings. We propose the use of these in vitro human assays to evaluate the potential for neurotoxicity and to provide utility in drug safety applications.
New standards are needed to address the drug development for neurological degenerative diseases (e.g., Parkinson’s disease, Alzheimer’s disease) and neurotoxicological liability screens. Current drug development and toxicology screens employ classical animal derived in vivo and in vitro models, but lack human cell models until clinical trials. To meet this need Axiogenesis AG developed different types of human induced pluripotent stem cell (iPSC) derived neurons (dopaminergic and peripheral neurons) to cover central nervous system as well as peripheral nervous system assays. Here we show that Axiogenesis neurons are a suitable model to assess different neurotoxicological assays, such as 1) mitochondrial toxicity that has been assessed with the Seahorse technology, using a high throughput 96well plate. 2) Functional synaptic activity, measured using dopaminergic neurons plated in 12 well Multi Electrode Arrays, showing a functional network after 7 days in culture. Additionally, dopaminergic burst patterns showed similarities to primary mouse midbrain neurons. 3) Neurite outgrowth analysis using peripheral neurons treated with Angiotensin II and dib-cAMP. The data show that Axiogenesis human iPSC-derived neuronal subtypes display a suitable and physiological relevant human cell model that can be employed for a variety of different already established animal based toxicological assays.

**640 NeuroSafe: A Human Integrated In Vitro Neurotoxicity Safety Platform Using hiPS Neurons (Peri.4U Neurons)**


In vitro pharmacology profiling of new chemical entities during early phases of drug discovery has recently become an essential tool to detect clinical adverse effects. While for cardiac safety testing high technology platform are available, no specific in vitro neurotoxic panels are available. In most of the cases, in vivo models are used. We developed a high throughput platform based on the usage of human stem-cell derived neurons (Peri.4U neurons from Axiogenesis) to be able to directly address human targets. The electrical activity of the Peri.4U neurons and compound related effects was assessed by field potential recordings using the MEA technology (Multi Channel Systems). The MEA system is particularly suitable for measuring neuronal network activity. The MEA system is dimensioned to record from four 6-well MEAs (with 9 electrodes per well) simultaneously to perform 24 experiments in parallel. At first, Peri.4U neurons were electrically characterised by manual patch clamp recordings. The presence of neuron-characteristic currents, sodium and potassium, as well as typical synaptic receptors, glutamate and GABA, was verified. The neurons with physiological values of their resting membrane potential (~50 mV) were able to generate action potentials with a neuron typical waveform. In a subset of the neurons, spontaneous activity with a tendency to a burst-like distribution of the action potentials was observed that provided evidence for synaptic input. When cultivated on the MEA chips, spontaneous activity of the Peri.4U neurons was recorded in at least one electrode in almost all wells (70%). The activity was burst-like patterned in 77% of all recordings indicating the presence and establishment of a functional neuronal network. In this configuration, we validated the assay by application of reference compounds and thereby demonstrated the potency of this in vitro Neurotoxicity Safety Platform to detect and quantify neurotoxic impact of test compounds.

**641 MR Imaging of Human Neural Progenitor Stem Cells: An In Vivo Longitudinal Model**

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Human stem cell sourced neural progenitor (hNP) cells offer a representative cell type for developmental neural toxicity (DNT) in vivo high content and throughput screening. Our overall goal is to establish a novel in vivo toxicological model system using hNP cells and eventually provide additional advanced key event analysis in DNT adverse outcome pathways. The integration of hNP cells into the central nervous system of the chick embryo is the basis for this new model, and the first objective was to establish a longitudinal in vivo tracking of transplanted hNP cells within the chick embryo using magnetic resonance imaging (MRI). The chicken embryo provides an easily accessed and well-characterized model for embryonic development and neurogenesis. MRI allows for noninvasive and repeated imaging of the chick embryos throughout development, and with the aid of magnetic contrast agents, cell migration and fate tracking is possible. We demonstrate hNP cells are efficiently labeled with the super-paramagnetic iron nanoparticle, Moldayon Rhodamine B (MIRB), without adversely affecting hNP cell viability and differentiation potential. Using a combination of mechanical stress and 48 hr exposure to MIRB, hNP cells accumulated 12.45 pg iron/cell and MIRB labeled cells while maintaining the hNP associated expression of NESTIN and SOX1 and were capable of differentiating in post mitotic neurons (MAP2 and Hu/C/D). After transplantation into the neural tube of the stage 15 chick, hNP cells are non-viably documented with MD-1 cell tracking. Neural migration, integration, and maturation can be accessed within a embryo by MRI analysis. These results suggest that future studies should be conducted to determine whether MIRB labeled and neurotoxicant exposed hNP cells migrate and integrate into the chicken embryo and potentially provide a human in vivo relevant longitudinal DNT adverse outcome pathway key event data for human neurogenesis.

**642 Predicting Neurotoxicity in Human-Derived iPSC 3D Mini-Brains**

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The "human-on-a-chip" program is one of the most ambitious initiatives in toxicology initiated by NIH, FDA and DARPA. Our project funded by NCIATS, NIH (U11TR000547) is part of this program and aims to establish and characterize an in vitro model of the developing human brain for the purpose of testing drugs and chemicals. To accurately assess risk, a brain model needs to recapitulate the complex interactions between different types of glial cells and neurons in a 3D platform. Moreover, human cells are preferred over cells from rodents. The use of iPSC allows us to address gene environment interactions of different donors and makes it possible to evaluate inter-individual sensitivities to chemical exposure. The 3D model has shown to recapitulate early in vivo human neurodevelopment. Showing the emergence of different kinds of neurons and glial cells, induction of genes that play important roles in neurodevelopment as well as presence of active glutamate receptors. We have used Rotenone, a pesticide known to induce neurotoxicity by inhibition of mitochondrial complex I, in order to test the relevance of the model to predict toxicity. The model shows increased ROS production and decreased mitochondrial function after exposure to Rotenone. In addition, the model shows a decrease of sensitivity to rotenone exposures with increasing maturation. Eight week mini-brains exposed for 48 hours to 10μM Rotenone showed 25% decrease of mitochondrial activity while two week mini-brains exposed to the same concentration showed 80% decrease of mitochondrial activity. Notably, such human brain models will represent a versatile tool for more complex testing platforms and strategies as well as research into (developmental) neurotoxicity as well as CNS physiology and pathology.

**643 A Functional Phenotypic Screen for Synapse Formation in Human iPSC-Derived Neurons**


Low level exposure to some toxicants can affect neuronal structure leading to developmental and cognitive impairment. Synaptic transmission is the fundamental unit of communication between neurons. Here, we used human induced-pluripotent stem cell (hiPSC) neurons grown in 384 well dishes for up to 15 days to examine the effects of toxicant exposure on synapses in a high content screen (HCS). These cells demonstrate action potentials as early as 9 days and sensitivity to chemical inhibitors as early as 12 days. To detect synapses, we used antibodies to the pre- and post-synaptic proteins, Synapsin -1 and post-synaptic density protein 95 (PSD95), respectively. To analyze synapse response to toxicant exposure, we analyzed fixed images using algorithms to mark neurite regions demarked by expression of β-III tubulin and measured colocalized signals for the pre- and post-synaptic markers only in these functionally-relevant regions. Our HCS approach has enabled us to screen a library of EPA ToxCAST compounds, FDA-approved drugs (the NCC2 library), as well as other agents, such as glutamate and glycine, and Brefeldin A, known to modulate neuronal function. Additional information that may be related to the mechanism of action for chemicals that showed an effect in the assay included the amount of neurite outgrowth and alterations in nuclear texture. Using this approach we have developed a robust platform for large-scale screening of chemicals that affect synapse formation, the basic unit of neuronal function in humans.
**644 Developmental Neurotoxicity of Epigallocatechin Gallate (EGCG) Is Triggered by Interference with β1-Integrin Function in Human Neural Progenitor Cells**

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Epigallocatechin gallate (EGCG), the most abundant catechin in green tea, is actually commercialized as a food supplement due to its antioxidant properties. The idea that food supplements based on herbal products are safe and healthy is deeply seated in the general population, and therefore these products are widely used during pregnancy. However, they do not undergo the same strict research safety and effectiveness requirements than medical drugs, and specific toxicities like neurodevelopmental toxicity are unknown for most of them. In this study, we identify that EGCG binds to the extracellular matrix protein laminin and interferes with integrin-β1 receptors function in human neural progenitor cells (NPCs) in vitro. This macromolecular interaction is translated to significantly decreased adhesion and migration of human and rat NPCs, as well as to other cellular responses like significantly decreased cell density and altered GFAP+ processes orientation at relevant concentrations in vivo. To confirm the in vitro relevance of these findings, and to evaluate the organ response within this novel Adverse Outcome Pathway (AOP), we performed neurohistological analyses in rats after developmental exposure to EGCG in vivo. Offspring of exposed animals had a lower density of 5-bromo-2-deoxyuridine positive cells in cortical layers, indicating that EGCG impairs neurodevelopmental differentiation at the organism level. Thus, our results strongly suggest that EGCG disturbs human fetal brain development at concentrations achieved after maternal supplement intake. Identifying new AOPs leading to disturbed human brain development is of utmost importance to prevent neurodevelopmental disorders and help in regulatory risk management.

**645 Effects of Silver Nanoparticles on Human and Rat Embryonic Neural Stem Cells**


In this study, we determined whether Ag-NPs are capable of causing developmental toxicity, which involves loss of myelin, radiation necrosis, and cognitive deficits. To address this, Pdgfrα+mouse embryonic stem cell line (mES) cells were exposed to MS (1, 10, 100 μM) for cell kinetic studies, and at early (Stage 1) or late (Stage 3) stage for neuronal differentiation studies. Our results demonstrate that 100 μM MS for 24 and 48 hours does not alter cell viability or cell cycle kinetics in mES cells. However, MS causes internalization of β-receptors in a concentration-dependent manner. At stage 1 exposure, MOR gene expression and neuroectoderm specific marker expression of nestin were down regulated in both hNPC and rNPC. In addition, the opiate down regulated glial fibrillary acidic protein (GFAP) in differentiated neurons and astrocytes. Late stage treatment with MS resulted in down regulation of microtubule-associated protein 2 (map2-2), and GFAP, in differentiated neurons and astrocytes. Moreover, late stage co-treatment with naltraxone plus MS inhibited the negative effect of MS alone on neuronal differentiation, suggesting that MS treatment interferes with neuronal differentiation via MOR activation. Together, the results show that, in addition to inhibiting neuronal differentiation of NPC after late stage treatment, MS exposure at early stage significantly alters neuronal genotype and phenotype in progenitor and mature neuronal cells. The results of this study have implications regarding the potential effect of opiate on fetal brain development.

**646 Identification of AOPs Involving Disruption of Thyroid Hormone Signaling in Neurodevelopmental Processes by Using 3D Neurospheres**

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Despite the long knowledge on the importance of thyroid hormones (TH) for proper brain development, there is still a profound gap in the understanding of effects of thyroid hormones on developing brain cells. Moreover, species differences are an understudied field of research and fundamental differences in molecular mechanisms of toxicological processes have been observed between different species. The goals of this project are to identify the actions of the TH T3 (triodothyronine) and T4 (thyroxine) on basic processes of brain development (proliferation, migration, differentiation) in a species-specific manner and to elucidate toxicity pathways and chemicals interfere with these thyroid hormone-mediated processes. For these studies human (h) and rodent (r) neural progenitor cells (NPCs) are used. To identify the species differences regarding the actions of TH, hNPC and rNPC grown as neurospheres were exposed to different concentrations of T3 and T4. This resulted in a reduced proliferation of hNPC, an induced oligodendrogenesis in rNPC and stimulated oligodendrocyte maturation in human and rodent neurospheres; neurogenesis was only induced in hNPC by T4. To elucidate the mechanisms behind these effects, a toxicological analysis of the endocrine disrupting and neurodevelopmentally toxic brominated diphenyl ether (BDE)-99. BDE-99 inhibited human (IC50: 2 μM) and rodent (IC50: 14 μM) oligodendrogenesis, and disturbed T3-induced oligodendrocyte maturation in both species. We found that TH guides neurodevelopment key events (KE) in developing neurospheres in a species-specific manner and that BDE-99 inhibits the KE oligodendrocyte maturation due to TH disruption. This work contributes to AOP building by identifying and studying KE involved in TH-dependent neurodevelopment. In the next step, TH receptor-dependent and -independent molecular initiating events will be elucidated.

**647 Morphine Sulfate Concomitantly Alters Neuronal Differentiation and Opioid Receptor Gene Expression in Mouse Stem Cell**

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Opioids have been shown to negatively affect pre- and postnatal neurodevelopment in mammals. The present study investigates the impact of morphine sulfate (MS) exposure on neuronal differentiation as well as µ opioid receptor (MOR) expression and activity in mouse embryonic stem (mES) cells. We manipulated mES cells in culture to differentiate in 3 sequential stages: 1) cell transformation to embryoid bodies (EB); 2) EB cell differentiation to neural progenitor cells (NPC); and, 3) NPC differentiation to neuronal/glial cells. Each cell type was confirmed for expression of cell-specific marker, MOR, µ opioid receptor (KOR) and δ opioid receptor (DOR) using RT-PCR and flow cytometry analysis. Mouse ES cells were exposed to MS (1, 10, 100 μM) for cell kinetic studies, and at early (Stage 1) or late (Stage 3) stage for neuronal differentiation studies. Our results demonstrate that 100 μM MS for 24 and 48 hours does not alter cell viability or cell cycle kinetics in mES cells. However, MS causes internalization of β-receptors in a concentration-dependent manner. At stage 1 exposure, MOR gene expression and neuroectoderm specific marker expression of nestin were down regulated in both EB and NPC. In addition, the opiate down regulated glial fibrillary acidic protein (GFAP) in differentiated neurons and astrocytes. Late stage treatment with MS resulted in down regulation of microtubule-associated protein 2 (map2-2), and GFAP, in differentiated neurons and astrocytes. Moreover, late stage co-treatment with naltraxone plus MS inhibited the negative effect of MS alone on neuronal differentiation, suggesting that MS treatment interferes with neuronal differentiation via MOR activation. Together, the results show that, in addition to inhibiting neuronal differentiation of NPC after late stage treatment, MS exposure at early stage significantly alters neuronal genotype and phenotype in progenitor and mature neuronal cells. The results of this study have implications regarding the potential effect of opiates on fetal brain development.

**648 Increased Susceptibility of Oligodendrocyte Progenitors to Fractionated Radiation**

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Recognition of the effects of ionizing radiation (IR) on the developing brain is now standard practice in preclinical research. However, the effect of fractionated IR on developing brain cells is still not fully elucidated. One of the effects of IR is the induction of apoptosis in immature neural progenitor cells (NPCs). The purpose of this study was to investigate the effects of fractionated IR on human NPC growth. In this study, MS NPC cells in culture were treated with MS (1, 10, 100 μM) for cell kinetic studies, and at early (Stage 1) or late (Stage 3) stage for neuronal differentiation studies. Our results demonstrate that 100 μM MS for 24 and 48 hours does not alter cell viability or cell cycle kinetics in mES cells. However, MS causes internalization of β-receptors in a concentration-dependent manner. At stage 1 exposure, MOR gene expression and neuroectoderm specific marker expression of nestin were down regulated in both EB and NPC. In addition, the opiate down regulated glial fibrillary acidic protein (GFAP) in differentiated neurons and astrocytes. Late stage treatment with MS resulted in down regulation of microtubule-associated protein 2 (map2-2), and GFAP, in differentiated neurons and astrocytes. Moreover, late stage co-treatment with naltraxone plus MS inhibited the negative effect of MS alone on neuronal differentiation, suggesting that MS treatment interferes with neuronal differentiation via MOR activation. Together, the results show that, in addition to inhibiting neuronal differentiation of NPC after late stage treatment, MS exposure at early stage significantly alters neuronal genotype and phenotype in progenitor and mature neuronal cells. The results of this study have implications regarding the potential effect of opiates on fetal brain development.
detected 1 month post-IR in animals that received 6 Gy*6, indicating that early loss of OPCs may lead to delayed effects in the mature populations. Ongoing studies are assessing recovery and cell fate of OPCs at 3, 6, and 18 months after exposure, as well as evaluating structural and functional changes in the white matter of these animals. This research was supported by the CMCR Program, U19-AR091036, NIAID, and NIEHS, T32-ES07026.

### 649 Generation of a Porcine Rheumatoid Arthritis Model: Collagen-Induced Arthritis Micropig


Collagen-induced arthritis (CIA) rodent animal models have been widely employed rheumatoid arthritis (RA) disease model due to similar pathological features including the activation of immune related cell types by response of both T-cells and B-cells to type II collagen at disease onset and symptoms presenting hyperplasia of synovial tissues, infiltration of mononuclear cell, local inflammation and cartilage degradation. The CIA rodent models have been useful in drug discovery for RA, however, as many other kinds of rodent disease models, they may lack in translational potential to human and limit the efficacy trials due to its small size. A large animal RA model with similar genetic and immune-modulatory characteristics to human will be useful in the drug discovery for this unmet medical need. We have therefore attempted to generate a porcine CIA model in Micropigs. Three months old Micropigs were immunized by 1ml/10kg administration of a mixture of bovine type II collagen (CII) and complete Freund’s adjuvants on Day 1, followed by 1ml/10kg booster administration of a mixture of CII and incomplete Freund’s adjuvants on Day 21 by intradermal injection. The immunized piglets were examined for incidence and severity of arthritis by evaluation of clinical score, paw thickness, body temperature, histopathology, hematometry, radiology and behavioral monitoring until Day 42. Successful induction of pathogenesis similar to RA was subsequently confirmed through these assessments in comparison with the concurrent PBS control animals. The results demonstrated that an autoimmune arthritis model can be generated in pigs with similar pathogenic and symptomatic characteristics to human. Micropigs are considered most suitable for drug discovery research due to its small size and slow growth rate on top of the advantages of pigs being anatomically and physiologically similar to human. CIA Micropig model can be a useful tool in the drug discovery for rheumatic arthritis. Key words: Collagen-induced arthritis. Rheumatoid arthritis. Micropig. Porcine disease model

### 650 Limitations in Testing and Risk Assessment for Dermal Sensitization


Two dermal sensitization studies were submitted to the California Dept. of Pesticide Regulation to support the registration of a fruit and vegetable wash product containing the active ingredients Lactic acid and Dodecylbenzenesulfonic Acid. The first study was performed in Guinea Pigs using a modified Buehler protocol, resulting in positive sensitization scores after challenge (grade 1 erythema in 1/20 animals at 24 hours, increasing to grade 1 in 1/20 and grade 2 in 4/20 at 48 hours). In contrast, negative sensitization potential was indicated in the same study after rechallenge (grade 1 erythema in 2/20 at 24 hours, decreasing to grade 1 in 1/20 at 48 hours). The second study used the Local Lymph Node Assay (LLNA), resulting in stimulation index (SI) values of 1.1, 3.1 and 2.9 for the 25%, 50% and 100% treatment concentrations, respectively (SI values of 3.0 or greater indicate a positive sensitizer). The registrant asserted that two values in the 50% treatment group were possible "outliers" (2922 and 2460 DPM versus a mean of 888 DPM for remaining 3 values), resulting in an SI modification for this group from 3.1 to 1.7. The "dose-response" (i.e., SI values of 1.1, 1.7 and 2.9) as indicated by the LLNA and the results from the Buehler study suggested that the product was a potential sensitizer. Borderline SI values alone present uncertainty in determining whether a subject product is a potential dermal sensitizer. In the workplace, development of sensitivity to allergens and the severity of irritation are thought to be related to exposure levels. Dose-response relationships and no-effect levels from these studies could be used as a basis for risk assessment and to obtain safe exposure concentrations for this and other potential workplace allergens.

### 651 Risks of Allergic Contact Dermatitis Elicited by Nickel, Chromium, and (Meth)Acrylates: Modeled Comparisons of Published Patch-Test Data on ~6,000 Sensitive Individuals

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Risk of allergic contact dermatitis (ACD) from consumer products intended for extended dermal contact has been regulated since 1994 in Europe by the Nickel Directive (EN 1811, updated in 2012), which limits Ni leached from any such product surface over 1 week to less than L* = 0.5 μg Ni/cm². Efforts to limit ACD risk posed by dermal exposures to other metals (e.g., Cr(VI) in metal alloys) and to semi-volatile organic sensitizers (e.g., acrylate adhesive components of wearable products), are complicated because current methods to estimate ACD risks do not address a wide range of sensitizers. To begin to address this gap, a bi-lognormal model P(L,*) was fit to data on percentage P of subjects with a positive (\( \geq 5 \)) ACD response in relation to dermal nickel load L, obtained from 12 Ni patch-test studies involving 597 Ni-sensitized patients; the model predicts P(L,*) = 5.4% of those studied. A similar, but left-shifted model of more potent ACD-response risk was fit to data on patch tests of Cr(VI) from ≥2,500 Cr(VI)-sensitized patients. Estimated shape parameters of these two models were assessed for homogeneity. Where L is patch-test dermal load of chemical X that elicits ACD response level P X, and L Ni = the specific Ni load at which P(L Ni) = P(L,*) = P X, the hypothesis that relative ACD potency of chemical X can be estimated as the "ACD potency" ratio Q X/Ni = L Ni/L* was tested for X = Cr(VI). By extension, relative-poverty values Q X/Ni for nine (meth)acrylates (acrylate, AC; methacrylate, MAC; and ethylene glycol, EG)—tetraEG dMAC, 2 hydroxyethyl MAC, 2 hydroxypropyl MAC, tetraEG dMAC, diEG dMAC, trimethyl propyl triAC, 2-hydroxyethyl AC, 2-phenoxethyl AC, and tetrahydrofurfuryl AC—were estimated to be 0.0011, 0.0015, 0.0044, 0.015, 0.015, 0.024, 0.060, and 4.7, respectively. The proposed hypothesis, and its initial application reported here, provide a step toward quantitative ACD risk assessment for a wide range of dermal sensitizers.

### 652 Particulate Matter Enhances the Pulmonary Allergic Immune Response to House Dust Mite in a BALB/c Mouse Model

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Ambient particulate matter (PM), a component of air pollution, exacerbates airway inflammation and hyper-reactivity in asthmatic patients. Studies have shown that PM has adjuvant-like properties in enhancing the allergic inflammatory response, however the mechanisms through which PM enhances allergic responses remain elusive. The objective of this study is to assess 1) how ambient PM exposure shapes the allergic airway immune response to house dust mite in BALB/c mice and 2) how PM modulates macrophage and dendritic cell (DC) activation during the allergic response. Eight week old BALB/c mice were exposed to PBS, Sacramento ambient particulate matter (PM, 2.5μm), house dust mite (HDM), or HDM+PM (n=4 for all groups). Lung tissue and bronchoalveolar lavage (BAL) were analyzed for extent of inflammation. Gene expression was analyzed by qPCR using lung homogenate. Administration of PM during allergen sensitization to HDM lead to a significant increase in airway inflammation compared to HDM sensitization only. Total cells, macrophages and eosinophils recovered in BAL fluid were significantly elevated in the HDM+PM group compared to the HDM only group. Histopathological analysis of lung tissue supported these findings. Gene expression analysis of the lung indicates that the dendritic cell markers CD80 and MHC class II were elevated in the HDM+PM treatment groups vs. the HDM only group. Our findings suggest that particulate matter enhances the allergic inflammatory response. The upregulation of dendritic cell markers suggest PM may enhance the activation of DCs during the allergic response.
563 Inhalation of the Reactive Aldehyde Acrolein Promotes Antigen Sensitization and Enhances Allergic Responses to Ovalbumin

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Acrolein (ACR), an α,β-unsaturated aldehyde and a major component of tobacco smoke, is a highly reactive electrophilic respiratory irritant implicated in asthma pathogenesis and severity. However, few studies have directly investigated the influence of ACR exposure on allergen sensitization and pulmonary inflammation. The present study examined the impact of ACR inhalation on allergen sensitization to the inhaled antigen ovalbumin (OVA), as well as pulmonary inflammation during subsequent OVA challenge. Adult male C57BL/6j mice were exposed to inhaled OVA (1%, 30 min/day, 4 days/week) and/or ACR (5 ppm, 4 h/day, 4 days/week) over 2 weeks and subsequently challenged with aerosolized OVA (1%, 30 min/day) over 3 consecutive days. Sera, bronchoalveolar lavage (BAL), fluids, and lung tissues were collected after OVA challenge to assess pulmonary inflammation and antigen sensitization.

Serum anti-OVA IgG1 levels were increased significantly in animals exposed to both OVA and ACR, compared to animals exposed to either OVA or ACR alone. In addition, differential cell counts revealed an increase in BAL neutrophils in animals exposed to both OVA and ACR. Exposure to both OVA and ACR did not influence mRNA expression of iL13, iL5, or TNFα, but significantly increased mRNA expression of cCL20. Additionally, ACR exposure enhanced lung mRNA levels of IL17f. Overall, our findings indicate that ACR inhalation can promote airway-mediated sensitization to otherwise innocuous inhaled antigens such as OVA, and promotes neutrophilic airway inflammation.

565 The Myeloid U937 Skin Sensitization Test (MUSST) to Address the Activation of Dendritic Cell Event in the Adverse Outcome Pathway for Skin Sensitization


The MUSST (Myeloid U937 Skin Sensitization Test) is an in vitro method to assess skin sensitization. It models dendritic cell activation upon exposure to sensitizers by measuring phenotypical changes, i.e. induction of CD86 expression, in the U937 human myeloid cell line. Cell viability is assessed using propidium iodide exclusion. Both parameters are measured by flow cytometry and used to classify a substance as a sensitizer or a non-sensitizer. The predictive capacity of the MUSST was comprehensively explored by comparing results obtained with this method to human and LLNA data of a total of 179 substances. When confronted to the human data set of 101 substances (with sensitizers dispatched from class 1 to 5 and non-sensitizers in class 6) in combination with the additional LLNA data set of 74 substances, the MUSST showed a specificity of 79%, a sensitivity of 89% and an accuracy of 87%. The large applicability domain of the MUSST was demonstrated through the wide diversity of use categories and chemical reactivities of this dataset, including pre or pro-haptens but excluding membranous disrupting substances (like surfactants). In addition, the identification of the potential place and role of the MUSST in an integrated strategy was investigated. The relationship between the 2 quantitative parameters of the MUSST (EC150 and CV70) and the potency classes defined on the basis of LLNA EU-CLP classification or based on clinical data was analysed, showing as an example, that these 2 parameters could be used by applying cut-offs. Therefore the MUSST is promising as a tool to be integrated in a testing strategy supporting skin sensitization risk assessment. Corresponding author: nalepee@rd.loreal.com

564 The Role for TAK1 in the TCE-Induced Contact Hypersensitivity Response

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Trichloroethylene (TCE) is a ubiquitous environment contaminant and occupational exposure to TCE has been associated with severe, generalized contact hypersensitivity (CHS) skin disorder, which is categorized as a delayed-type hypersensitivity reaction. Innate and adaptive immunity are both important for CHS development. Transforming growth factor-β activated kinase-1 (TAK1) (encoded by Map3k7) is essential for controlling the survival, differentiation and function of innate and adaptive immune cells and thus related responses. We hypothesized that TCE-induced CHS response is mediated by TAK1 activity. The local lymph node assay (LLNA) was employed to study the role of TAK1 in the CHS induction by TCE through comparing the effects in local and dendritic cell (DC)-specific TAK1 deletion mouse models and in wild-type (WT) mice. Mice were treated on both ears, daily for 3 consecutive days, with 80% (V/V) TCE. The draining auricular lymph nodes (DLNs) were excised 24 hours after BrdU injection, and the cells were prepared for measurement of BrdU content by ELISA and T-cell phenotype analysis using flow cytometry. In contrast to WT mice, TAK1 locally deficient mice had a much reduced lymphocyte proliferation triggered by TCE and defective T cell expansion was also observed. In addition, when TAK1 is deficient in DC, the mice had a significantly lower stimulation index with TCE treatment compared with WT. Percentage of activated (CD62LlowCD44high) DCs and CD4+ T cells and regulatory T cells (CD25+Foxp3+) in DLNs were also lower after TCE induction in DC-specifically TAK1 deficient mice. This study demonstrates an important role for TAK1 in controlling lymphocyte proliferation and T cell function in response to TCE and suggests that targeting TAK1 might be a viable approach to prevent and treat TCE-induced occupational health hazard.

567 Silica-Induced Lymphocyte Infiltration and Proinflammatory Cytokine Responses in Lung Correspond to Accelerated Onset of Glomerulonephritis and Autoimmunity in Lupus-Prone Female NZBWF1 Mice


Genetic predisposition and environmental factors are known to influence the development of human autoimmune disease. Occupational exposure to crystalline silica (SiO2) has been etiologically linked to increased incidence of autoimmunity, including systemic lupus erythematosus (SLE), but the underlying mechanisms are poorly understood. The purpose of this study was to test the hypothesis that early subacute SiO2 exposure will modulate both latency and severity of autoimmunity in the lupus-prone female NZBWF1 mouse. Weekly intranasal exposure to SiO2 (0.25 and 1.0 mg) for 4 wk beginning at 9 wk of age both reduced latency and increased intensity of glomerulonephritis. SiO2 exposure also elicited robust inflammatory responses in the lungs as evidenced by extensive perivascular lymphoplasmacytic infiltration as well as elevated concentrations of IgG and the proinflammatory cytokines MCP-1, TNF-α and IL-6 in bronchoalveolar lavage fluid. These effects paralleled dose-dependent elevations of IgG, autoantibodies, and proinflammatory cytokines in the plasma. Taken together, SiO2-induced lung injury in the NZBWF1 mouse corresponded closely to systemic inflammatory and autoimmune responses as well as the early initiation of pathological outcomes in the kidney. These findings suggest that following airway exposure to an inflammatory toxicant such as silica, the lung might serve as a platform for early initiation and exacerbation of systemic autoimmunity and glomerulonephritis.
Environmental and occupational exposure to trichloroethene (TCE) has been linked to an autoimmune response. Mechanisms underlying the TCE-mediated autoimmunity remain unclear. Previous studies from our laboratory in MRL+/+ mice suggest that reactive TCE metabolites and oxidative stress contribute to TCE-mediated autoimmunity. The present study was undertaken to further assess the significance of TCE metabolism leading to oxidative stress and autoimmune response by using cytochrome P450 2E1 (CYP2E1)-null MRL+/+ mice. CYP2E1-null MRL+/+ mice were generated by backcrossing CYP2E1-null mice to MRL+/+ mice for 6 generations followed by intercrossing of N6 heterozygous mutants to obtain homozygous mutants. Female MRL+/+ and CYP2E1-null MRL+/+ mice were given TCE (10 mmol/kg, i.p., every 4th day) for 6 weeks; their respective controls received corn oil only. TCE treatment in MRL+/+ mice led to significant increases in serum malondialdehyde (MDA)- and 4-hydroxynonenal (HNE)-protein adducts and their respective antibodies. TCE exposure was also associated with significant increases in serum anti-nuclear antibodies (ANA), anti-double stranded DNA antibodies (anti-dsDNA) and IL-17 levels. TCE treatment in CYP2E1-null MRL+/+ mice also led to increases in serum MDA/HNE-adducts and their respective antibodies along with increases in ANA, anti-dsDNA and IL-17, but interestingly, the increases in the oxidative stress and autoimmunity markers in the CYP2E1-null MRL+/+ mice were significantly less pronounced compared to that in MRL+/+ mice. Attenuation of autoimmune response in CYP2E1-null MRL+/+ mice further supports the contribution of CYP2E1-mediated TCE metabolism in the induction of autoimmunity. Supported by NIH ES016302.

Growing evidence indicates that local inflammatory responses following nanoparticle (NP) exposure result in modified innate immune responses, facilitating the development of respiratory diseases including asthma. Graphene oxide (GO) – having unique physico-chemical properties – is a promising carbonaceous nanomaterial with a multitude of medical and industrial applications. Here we investigated the modulation of antigen-presenting cells in a murine model of ovalbumin (OVA)-induced asthma by pulmonary exposure to GO. The data showed that GO administration at the time of initial allergen sensitization augmented airway hyperresponsiveness and airway remodeling in the form of goblet cell hyperplasia and smooth muscle hypertrophy. The levels of IL-4, IL-5 and IL-13 were reduced in bronchoalveolar lavage (BAL) fluid and serum of GO-treated mice as compared to OVA-only group. Exposure to GO increased macrophage and lymphocytes in BAL fluid, as compared to those in OVA-only treated animals. Similarly, GO administration during sensitization stimulated the production of OVA-specific IgG2a and down-regulated the levels of IgG1 and IgG3. Moreover, exposure to GO increased the macrophage production of the mammalian chitinas, CHI3L1 and AMCase, whose expression is associated with asthma. Conclusively, while GO exposure reduces Th2 immune response in a murine model of asthma, it potentiates airway remodeling and hyperresponsiveness.

Allergic contact dermatitis (ACD) is a common skin disease that is caused by type IV delayed-type hypersensitivity responses to chemicals that come into contact with the skin. It has a high prevalence in Europe (15 to 20%). Among the 3000 known sensitizers, nickel is the most involved in ACD reactions. IL-12p70 (composed of IL-12p40 and IL-12p35) and IL-23 (composed of IL-12p40 and IL-23p19) are two cytokines involved in inflammation and adaptive immunity, from the IL-12 cytokine family and produced by dendritic cells. They are also involved in the amplification of ACD and play a major role in the generation of allergen-specific T cell responses. In this study, we address the question whether the sensitizer nickel sulfate (NiSO4) can induce the secretion of IL-12p70 and IL-23 in humans. Monocyte-derived dendritic cells (MoDC) in response to NiSO4 stimulation. We also showed that NiSO4 induced a novel effect of nickel on IL-12p70 synthesis and IL-23 production. We further showed that NiSO4 induced early expression of IL-23p19 mRNA and IL-23p19 mRNA but mRNA levels of IL-23 were not modified. Furthermore, we showed that NiSO4 required the presence of IFN-γ to induce IL-23p19 mRNA expression. By contrast, this association induced a decrease in IL-23p19 mRNA expression. On the other hand, we found that p38MAPK was involved in the expression of IL-23p19 and IL-12p35 mRNA induced by NiSO4. Finally, our results contribute to the understanding of the mechanisms of nickel-induced ACD and describe a novel effect of nickel on IL-12 cytokine family in human Mo-DC.
all aggregates enhance DC capacity to induce T cell proliferation in an allogenic model of DC-T cell co-culture. Conclusion: Taken together these results suggest that in contrast to BP native counterparts, BP aggregates can influence DC maturation, and that protein formulation may have a modulating role on this activation. This in vitro model is a valuable tool to further understand how the immune system processes BP aggregates.

**Impact of Contamination with Bacterial Products and Aggregation on Immunogenicity**

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There has been a dramatic increase in the use of protein therapeutics over the last 10 years, including antibodies and fragments such as single chain Fv (scFv) fragments that can be produced in *E. coli* vectors, Treatment with such may be associated with the development of anti-drug antibodies that cause adverse events or reduce efficacy of the protein therapeutic. It is important therefore to understand the factors that may impact on immunogenicity. In the current experiments a humanized scFv expressed in *E. coli* was purified by Protein A affinity chromatography and size exclusion chromatography to monomeric (mean diameter 7nm) and dimeric fractions. A single protein band was observed for both fractions on SDS-PAGE analysis, indicating acceptable purity. Reproducible aggregates within the subvisible particle size range (mean diameter 1900nm) were formed by incubation of the dimeric fraction at 40°C for 20 min at pH 7.0. Serum antibody responses (protein specific total IgG and IgG1 and IgG2a subclasses) induced in the BALB/c strain mice by intraperitoneal exposure to the monomeric, dimeric and aggregated forms of the scFv (1 mg/ml) were characterized. Immunization with the dimeric fraction induced considerably more vigorous IgG1, IgG2a and IgG2b antibody responses than did the monomeric fraction, with IgG2a antibody responses further enhanced by aggregation of this fraction. Mass spectrometric analysis revealed the presence of trace contamination with a number of *E. coli* derived proteins including the chaperone protein DnaK in the dimeric, but not the monomeric, fraction. Taken together these results indicate that the presence of small amounts of host cell derived proteins that are not detected by conventional checks for protein purity may enhance the immunogenicity of recombinant proteins. In addition, aggregation augments immune responses further by skewing towards a preferential Th1 type response (IgG2a>IgG1), possibly by mimicking the repetitive structures found in viruses.
The endogenous ligands for the cannabinoid receptors (CBs) are collectively termed endocannabinoids (ECs). The two most commonly researched ECs are N-Arachidonylethanolamine (anandamide, AEA) and 2-Arachidonoylglycerol (2-AG). Both AEA and 2-AG are ligands for the CB1 receptor but only 2-AG is considered a CB2 agonist. While it has been well documented that EC levels are elevated in various inflammatory models, including multiple sclerosis, autoimmunity, hepatitis, and rheumatoid arthritis, the role ECs are playing in the correlated inflammation is harder to ascertain. Recently our lab has set out to establish if these ECs are harmful or beneficial in T cell mediated inflammation. To this end we have found that in vitro lymphocyte activation results in significantly elevated secretion of both AEA and 2-AG. Additionally, delayed type hypersensitivity (DTH), in vivo lymphocyte activation, resulted in significantly increased systemic levels of 2-AG compared to naive control. To further understand the impact 2-AG has in reducing inflammation we next looked at microRNA dysregulation. Using the PI3K/Akt pathway as a focus we observed that miR-21-5p, which targets PTEN, was up-regulated. Interestingly, we found that an injection of Δ9-tetrahydrocannabinol (THC), a known anti-inflammatory molecule, into naive mice also increased systemic levels of 2-AG and low doses have been shown to decrease Akt phosphorylation, so we next used THC treatment in DTH mice and found a reversal of miR-21-5p up-regulation. Together, these data show a beneficial role for 2-AG in DTH mediated inflammation. (This work was supported in part by National Institutes of Health grants P01AT003961, R01AT006888, R01ES019313, R01MH094755, and P20GM103641 as well as by Veterans Affairs Merit Award BX001357).

An estimated fifty million Americans, mostly women, suffer from a debilitating and often life-threatening autoimmune disorder. There is no cure for most autoimmune diseases and better treatment is desperately needed. Impaired regulatory T cell (Treg) function has been implicated in the progression of autoimmune disease and thus offers an exciting alternative strategy for autoimmune disease treatment. The capacity of Tregs to suppress immune responses is dependent on their ability to accumulate at sites of inflammation and adapt to the local microenvironment. The factors involved in the control of these aspects of Treg physiology remain enigmatic. The transcription factor aryl hydrocarbon receptor (AhR) has been shown to induce Treg differentiation and provide protective benefit in models of autoimmunity including multiple sclerosis (MS), diabetes, and colitis. Hence, AhR ligands are environmental factors that regulate Treg differentiation. Considering the sex-bias nature of autoimmunity and the importance of estrogen receptor (ER)-AhR crosstalk, there is a significant knowledge gap regarding a possible sex-bias generation of AhR-Tregs. To address this we compared males and females in an in vitro FoxP3 Treg induction assay. In these studies, CD4+FoxP3NEG cells were purified from male or female MOG-specific CD2 T cell receptor (TCR) transgenic FoxP3GFP reporter mice and stimulated in vitro with cognate antigen and irradiated syngeneic splenocytes as antigen presenting cells (APCs). The cultures were co-treated with either transforming growth factor (TGF)-β1 or 2-(1H-indolo-3-carboxylic acid methyl ester) (ITE), an AhR agonist, and harvested 4 days later. Our results demonstrated increased generation of FoxP3+ Tregs in response to TGF-β1 or ITE treatment in cells obtained from female mice. Collectively, our data suggest that ER may influence the ability of AhR to control T cell differentiation including iTregs. (This work is supported by NIH R01 ES022966).

The FDA is re-evaluating the safety and efficacy of triclosan, an antimicrobial chemical used in numerous products including soaps, dental and first aid supplies, toys, kitchenware, medical devices and clothing. The CDC reports that 75% of Americans have detectable levels of triclosan in their urine, and epidemiological studies have found a positive correlation between triclosan burden and diagnosis of allergic diseases. Mice exposed dermally to triclosan have also shown augmented allergic responses, however the mechanisms behind these effects are unknown. To further elucidate these mechanisms, we exposed both ear skin and draining lymph nodes at the site of dermal exposure were examined in BALB/c mice to identify early immunological changes induced by triclosan (0, 0.75% and 3% w/v). We discovered significant increases in thymic stromal lymphopoietin (TSLP) expression at the transcript protection against external pathogens. However, in the context of a susceptible genetic background, TSLP has the potential to augment the development of autoimmunity, including multiple sclerosis (MS). We have previously demonstrated that greater than 90% of female, but few male, tumor necrosis alpha receptor type 2 (TNFR2)-deficient mice crossed onto the myelin oligodendrocyte glycoprotein peptide fragment 35-55 (MOG35–55)-specific T cell receptor (TCR) transgenic background rapidly develop spontaneous experimental autoimmune encephalomyelitis (EAE), a pre-clinical model for human multiple sclerosis. We now report that when these TNFR2-deficient TCR transgenic mice receive oral administration of trimethoprim/sulfamethoxazole, a non-steroidal anti-inflammatory drug and MSxN sequencing and RT-PCR to demonstrate distinct sex-biased microbiome profiling in male and female TNFR2-deficient MOG35–55-specific TCR mice, including increased SFB expression in the females relative to their male cohorts. Collectively, these findings suggest a role for TNFR2 in regulating commensal microbiota in the context of myelin autoantigen to control autoreactive T cells and autoimmune demyelination (This work is supported by NIH R01 ES022966).
and protein levels in the ear, but not in the lymph nodes or blood serum. In vivo administration of neutralizing anti-TSLP antibody impaired allergic responses augmented by dermal exposure to 3% triclocan during sensitization to ovalbumin. These effects include a significant decrease in skin pathology with a reduction in skin hyperplasia, redness, and oedema. This also reduced cellular infiltration into the skin draining lymph nodes, decreases in B cell frequencies, and reduced cytokine and GATA-3 transcription factor protein expression in Th2 CD4+ T cells. These observations were further extended to human skin cultures where we found that in vitro application of triclocan also induced TSLP expression. To our knowledge, this is the first report that triclocan can induce TSLP expression as a possible mechanism for augmenting allergic diseases.

673 Differential Analysis of Protein Expression in RNA Binding Protein-Transgenic and Parental Rice Seeds Cultivated under Salt Stress and Allergenicity Test of the Rice Extracts

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Transgenic plants tolerant to various environmental stresses are being developed to ensure a consistent food supply. We used a transgenic rice cultivar with high salinity tolerance by introducing an RNA-binding protein (RBP) from the ice plant (Mesembryanthemum crystallinum); differences in salt-soluble protein expression were similar and included cholesterol biosynthesis, TREM1 and GM-CSF signalling. These results suggest that triclocan can induce TSLP expression as a possible mechanism for augmenting allergic diseases.

674 Lung Toxicity and Allergy Responses in Mice Exposed to Nanoparticle Silver


With expasive use of silver nanoparticles (AgNP) in medical applications and consumer products, potential for worker exposure during manufacturing has become a concern. The goal of the current study was to characterize the potential effects of AgNP in an ovalbumin (OVA)-induced allergy model in BALB/c mice. To characterize the effects of AgNP alone, mice were exposed via pharyngeal aspiration (PA) to physiological dispersion medium (DM), 6.1 µg, 18.2 µg, or 73 µg AgNP. Twenty nm diameter AgNP with 0.3% wt polyvinilpyrrolidone coating (NanoAmor, Inc.) were suspended in DM and sonicated before exposures. For all studies lung function was assessed using enhanced pause (Penh); bronchoalveolar lavage (BAL) was performed on the whole lung; BAL cell counts and fluid were retained for analysis of lung-associated injury, inflammation, phenotyping and lymph nodes (LN) were harvested for enumeration and immune cell phenotyping. AgNP alone did not result in changes in Penh, while cellular responses in the lung indicated a dose-dependent injury and inflammation by post-exposure day 10, which began to resolve by day 29. Our previous studies have shown that exposure to AgNP prior to OVA-sensitization results in a trend for the development of airway reactivity. In this study, effects of AgNP on the elicitation phase were examined. Animals were sensitized with i.p. injections of OVA (dose) + aluminum hydroxide gel on days 1 and 10. To elicit an OVA-specific response, two PA challenges with OVA were given on days 19 and 28. AgNP were administered by PA on day 27. AgNP did not appear to significantly enhance Penh, and lung-associated LN total cell numbers, BAL cell numbers and IgE levels in serum were not increased above those of the allergy model control (OVA). The results indicate that although AgNP may have a moderate effect on airway resistance in the lung when administered before sensitization, they do not significantly alter the course of allergy development when given either prior to sensitization or during the elicitation phase.

675 Gene Expression Changes Induced by Skin Sensitizers in THP-1 Cells: Possible Relationship to Protein Binding Domains

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The objective of our study is to generate a global view of transcriptional changes induced in target cells exposed to sensitizing chemicals with the goal of identifying common functional and regulatory pathways/molecules. Six replicate wells of THP-1 cells (human monocytic cell line) were exposed for 24h to single concentrations of 9 skin sensitizers (SS), 8 low molecular weight respiratory sensitizers (RS), and 9 non-sensitizers (NS) along with 11 vehicle control replicates. Cells were harvested for transcript profiling using the Affymetrix GeneTitan® U219 array plates. Statistical analyses at the individual gene level using cut-off values of a 1.5 fold-change with a false discovery rate <0.05 identified 181, 72, and 273 unique gene expression changes for the SS, RS and NS, respectively. The 181 unique genes for the SS were analyzed by hierarchical clustering. Examination of the SS heat map revealed possible clustering by protein reactivity domains. Enrichment analysis using MetaCore™ software (Thomson Reuters) was conducted on all of the SS and individual SS grouped by domain using genes common to all in the group. The most significant pathways for all SS grouped together and the SN2 electrophiles were similar and included cholesterol biosynthesis, TREM1 and GM-CSF signalling. The top pathways for the MA electrophiles were related to oxidative stress and the activation of antioxidant defense system. Pathways activated by the one SS classified as an acylating agent included immune response pathways associated with antigen presentation, complement, and histamine signalling. These results suggest that the nature of the hapten-protein binding chemistry may influence activation of specific cellular pathways in immune cells.

676 Increased Expression and Immunoregulatory Potential of microRNA 210 in a Murine Model of TDI Sensitization

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MicroRNAs are single-stranded RNAs that exhibit functional significance through the regulation of gene expression; however, their roles in chemical sensitization have not been elucidated. Toluene 2,4-diisocyanate (TDI) is a low molecular weight chemical sensitizer that causes occupational asthma. In order to investigate
677 A Weight-of-Evidence Investigation of Novel Amino Alcohols for Skin Sensitization Potential Using In Silico, In Vitro, In Vivo, and Genomic Approaches

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Assessment of the skin sensitization potential of chemicals is an important component of the safety evaluation process. Key chemical and biological events underlying the skin sensitization process have been outlined, facilitating a weight of evidence (WoE) approach which aligns with an adverse outcome pathway. In a previous study, we evaluated the sensitization potential of four amino alcohols (aminocyclohexane (ACyHM), 2-aminopropanol (2-AP), aminomethylcyclohex- anol (AMCyHOL) and 2-aminopropanol (2-AP)) using in silico (DEREK, TIMES 384-well TaqMan Array micro fluidic cards pre-plated with primers and probes for 38 prior generated gene signatures (Adenuga et al., 2012). Therefore in the present study, a modified LLNA approach which aligns with an adverse outcome pathway. In this study, we assessed the functional role of miR-210 during TDI sensitization, BALBc mice were dermally exposed to TDI (0.5–4% v/v) and gene and protein expression were evaluated in the draining lymph nodes (dLN) and ears using RT-PCR and Western blot/flow cytometry, respectively. Increased total serum IgE levels confirmed sensitization in these mice. Augmented miR-210 expression was observed in the dLN and ears following exposure to both irritant and non-irritating TDI concentrations. Increased expression of a potential miR-210 inducer, hif1α, was observed in the ears following TDI exposure. Alterations in expression of confirmed miR-210 transcription factor target foxp3 were observed in the dLN and ears and decreases in predicted targets (foxp3, runx1, runx3) and smad4 mRNA expression were observed in the dLN of TDI-exposed mice. These transcription factors are involved in regulatory T cell (T-reg) expansion and function; therefore, miR-210 may play a functional role in TDI sensitization by potentially inhibiting T-reg differentiation and function. This hypothesis is supported by the T-reg population’s kinetics and the presence of miR-210 in CD4+ T cells during TDI sensitization. Because the roles of T-reg and miR-210 in chemical sensitization have not been elucidated these data contribute to the understanding of the immunologic mechanisms of chemical induced allergic disease and are critical for the development of preventative and therapeutic strategies. This work was supported by internal funds from NIOSH/HELD.

680 Anti-Mesothelial Cell Autoantibodies Upregulate Transcription Factors Involved in Collagen Pathways

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Amphibole asbestos exposure leads to autoantibody production in both mice and humans. These autoantibodies have been linked with pleural disease in the asbestos contaminated vermiculite mining community of Libby, Montana. However, the exact intracellular mechanism of how these autoantibodies cause an increase in collagen deposition remains unknown. This study sought to gain insight into the signal transduction linking autoantibody binding to collagen production in human mesothelial cells. In this study, transcription factor activation profiles were generated from human mesothelial cells treated with sera from the patients of Libby. Analysis of these profiles indicated differential expression in 31 of the 48 transcription factors analyzed compared to the untreated control. Thirteen of these transcription factors are associated with type 1 collagen deposition. These data suggest autoantibodies are directly involved in type 1 collagen deposition and may elucidate potential therapeutic targets for auto antibody mediated fibrosis.

679 Asbestos-Induced Pleural Fibrosis Involves Autoantibody-Mediated Protein Tyrosine Phosphorylation Pathway

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Asbestos induced Lamellar Pleural Fibrosis (LPF) is an emerging disease with the potential to affect thousands of lives through environmental exposures. Unlike typical pleural plaques, LPF is progressive and results in severe limitations in pulmonary function. Many details of the mechanism of pathology remain unknown, but a key factor in the pathology is the presence of mesothelial cell autoantibodies (MCAA) that have been generated in response to asbestos exposure. Excessive collagen deposition, a key event in the development of fibrosis, has been observed in cell cultures exposed to these antibodies, along with activation of matrix metalloproteinases (MMP). The focus of this study is to identify the cellular signaling pathways affected by these antibodies. Using a variety of methods including flow cytometry and ELISA, we have demonstrated that increased tyrosine phosphorylation occurs in mesothelial cells exposed to amphibole asbestos induced MCAA. This is consistent with other studies demonstrating a role for tyrosine phosphorylation in fibrogenic pathways and MMP activation. Serine and threonine phosphorylation were also examined, but statistically significant changes were not observed. This data will influence future efforts in characterizing the signaling pathways and identifying the target receptor for MCAA.

678 An Integrated In Vitro Approach to Identify and Characterize Respiratory Sensitizers


Currently, there is no fully accepted approach to identify chemicals as respiratory sensitizers. This study was designed to provide an integrated exposure-response profile to identify low molecular weight respiratory sensitizers and differentiate them from dermal sensitizers. Brown Norwegian rats received 2 equipotent dermal applications of 2,4-dinitrochlorobenzene (DNCB), hexyl cinnamaldehyde (HCA), trimellitic anhydride (TMA) or orthopthalaldehyde (OPA) in methylethyl ketone (MCAA) that have been generated in response to asbestos exposure. Excessive collagen deposition, a key event in the development of fibrosis, has been observed in cell cultures exposed to these antibodies, along with activation of matrix metalloproteinases (MMP). The focus of this study is to identify the cellular signaling pathways affected by these antibodies. Using a variety of methods including flow cytometry and ELISA, we have demonstrated that increased tyrosine phosphorylation occurs in mesothelial cells exposed to amphibole asbestos induced MCAA. This is consistent with other studies demonstrating a role for tyrosine phosphorylation in fibrogenic pathways and MMP activation. Serine and threonine phosphorylation were also examined, but statistically significant changes were not observed. This data will influence future efforts in characterizing the signaling pathways and identifying the target receptor for MCAA.

681 A Retrospective on Drug Allergy in Dogs at a US Veterinary Teaching Hospital

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Adverse drug reactions affect approximately 7% of the general population, represent the 4th-6th cause of death. A 1/3 of these reactions are immune-mediated (drug hypersensitivity [HS] or allergy). These events can be immediate (IgE-mediated: e.g. anaphylaxis) or delayed (IgG- or T cell-mediated: e.g. toxic epidermal necrolysis). The dog has been proposed as a potential animal model to study drug HS. Indeed, canine patients experience similar drug HS as observed in humans. However, the incidence of drug HS in dogs remains unknown. This retrospective study aimed to estimate this incidence and further characterize drug HS reactions in dogs seen between 01/01/2003 and 04/28/2014 at the veterinary teaching hospital (University of Illinois, USA). We identified 99 cases (12 immediate and 87 delayed reactions). Our primary results suggest an overall incidence of 0.33%. The suspected drug was an antibiotic for 63 cases (72.4%); 22 penicillins; 25 cephalosporins; 11 fluo-
Selective Deletion of Estrogen Receptor α in FoxP3+ CD4+ T Cells Influences Peripheral T Cell Homeostasis

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Being female confers a greater risk of developing autoimmune disease than any single genetic or environmental risk factor known. It is unclear how differences between sexes impact environmental factors but a predominance of females with an autoimmune disorder has given rise to an interest in estrogens in the immune system. Given that a loss of regulatory T cell (Treg) function contributes to autoimmune disease and estrogen receptor alpha (ERα) induces Tregs and suppresses experimental autoimmune encephalomyelitis, an animal model of multiple sclerosis, ERα-expressing Tregs may contribute to the pathogenesis of autoimmunity.

FoxP3+ Treg-restricted ERα in females harboring a loxP-flanked 'floxed' Esr1 gene (ERα flox/flox) were bred to mice expressing a yellow fluorescent protein (YFP)-Cre fusion protein driven by an IRES reporter. Deletion of ERα in CD4+FoxP3-YFP pos but not CD4+FoxP3-YFP neg cells was validated. The frequency of thymic, lymphoid, and splenic CD4+ and CD8+ T cells did not differ between WT and ERα-flox/flox FoxP3-cx mice, except for fewer thymic FoxP3+ Tregs in female ERα-flox/flox FoxP3-cx mice. The frequencies of peripheral CD4+ and CD8+ T cell subsets were also unaffected in ERα-flox/flox FoxP3-cx mice except for a sex-bias reduction of FoxP3+ Tregs in female ERα-flox/flox FoxP3-cx mice. This coincided with a 62% increase in the total number of splenocytes in ERα-flox/flox FoxP3-cx mice compared to WT controls. Collectively, these results demonstrate that deletion of FoxP3+ Treg-specific ERα influences peripheral T cell homeostasis.

Examining the Role for Off-Target MDR3 Inhibition in the Hepatotoxicity of Therapeutics


MDR3/ABCB4 is an ABC transporter located at the canalicular domain of hepatocytes. MDR3 is responsible for the secretion of phosphatidylcholine (PC) into bile, where it protects cholangiocytes from the detergent properties of bile acids. Mutations in ABCB4 are associated with PFIC3, or MDR3-deficiency. A hallmark of MDR3-deficiency is the accumulation of cholesteryl esters in hepatocytes. MDR3 inhibition may provide value in early decision making for small molecule drug candidates.

Human Liver Response to Clinical Doses of Compounds That Can Cause Liver Injury

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To characterize human response to clinical drugs associated with liver adverse effects, via diverse mechanisms, an ex vivo human liver slice model was dosed daily for 3 days. Human liver tissue was obtained from procurement agencies, according to accepted medical and ethical standards, as outlined by the Uniform Anatomical
Concordance in RNA-Seq Profiles between Formalin-Fixed Paraffin-Embedded and Frozen Liver Samples

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Biorepositories have immense potential value in characterizing molecular targets of environmental chemicals. Use of archival resources has been limited to date by inconsistent methods for transcriptomic profiling from formalin-fixed paraffin-embedded (FFPE) samples. RNA-Seq offers a novel way to address this problem. The goal of this study was to use RNA-Seq to evaluate mRNA responses in paired FFPE and frozen (FROZ) liver samples. We analyzed 16 pairs of FFPE and FROZ liver samples from adult B6C3F1 mice treated for 7 days with control diet or feed containing 36.1 ± 1.2 for FFPE compared to 37.0 ± 1.6 for FROZ. Analysis of the first 3 principal components showed intact relative profiles (consistent bias) between FFPE and FROZ sample pairs. The number of statistically-filtered differentially expressed genes (DEGs) on RNA-Seq for low, mid, and high DEHP doses was 436, 689, and 829, respectively, for FFPE samples and 274, 589, and 539 for FROZ samples. Across all DEHP dose groups, 75% (587/779) of FROZ DEGS were comparable to FROZ samples in sequencing quality metrics, labeling, read-outs, and DEG profiles using ribo-depletion-based RNA-Seq analysis. With further development, these methods should broaden the use of archival resources in both preclinical and clinical studies.

Detecting the Diclofenac-Induced Liver Injury Using TNF-Alpha Exposure Mouse Model and Candidate for Its Mechanism

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[Introduction] In recent years, innate immune mediated drug-induced liver injury (DILI) in humans has been focused as one of the mechanisms of idiosyncratic DILI, and especially the role of tumor necrosis factor (TNF-α) in the innate immune-mediated DILI is of current interest. In the present study, we used diclofenac, as a tool compound for DILI, then tried to detect its liver injury using TNF-α-exposure mouse model and investigate the potential mechanism. [Methods] Diclofenac (100 mg/kg) was orally administered in a single dose to mice, and TNF-α (50 μg/kg) was injected intraperitoneally 3hr after that. On the following day, mice were necropsied and blood and tissue samples were collected. Plasma liver enzymes (ALT, AST) and hepatic mRNA of cytokines and chemokines were measured and histopathological changes in the liver were also examined. [Results and Discussion] Although neither diclofenac nor TNF-α alone caused liver injury, co-treatment of diclofenac and TNF-α induced an elevation of ALT and AST, and hepatic focal necrosis. In monitoring of inflammatory mediators, mRNA expression of interleukin (IL) (IL-1β which is known as one of the critical molecules for DILI was elevated in diclofenac alone, and this elevation was suppressed in co-treatment mice. Based on the results, this model could detect the diclofenac-induced liver injury and one of the mechanisms could be down-regulation of IL-6 expression.

MicroRNA Involvement in Drug-Induced Hepatotoxicity: A Multi-Omics Approach

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Drug-induced liver injury is a frequently reported reason for project termination during drug development and withdrawal of drugs already available on the market. Therefore, early detection of drug-induced toxicity is important. Liver specific microRNAs have been proposed as early biomarkers of hepatotoxicity; however their role in drug-induced toxicity is largely unknown. We investigated the microRNA and mRNA expression changes induced in vitro by three well known hepatotoxics. HepG2 cells and primary mouse hepatocytes were treated with acetaminophen, amiodarone and cyclosporin A and RNA was isolated. Microarrays were performed to investigate the hepatotoxicant-induced changes. Different significantly expressed microRNAs are observed in HepG2 cells and primary mouse hepatocytes. Mir-2110 and mir-3152 are differentially expressed in HepG2 cells after treatment with all three compounds for 24 hours. In primary mouse hepatocytes mir-212 and mir-3470a are overlapping after treatment for 24 hours with these three compounds. Investigation of these microRNAs and their targets indicates a candidate role in regeneration and proliferation, which may induce cell repair and inhibit apoptosis. Furthermore, drug-specific changes in microRNA expression levels are observed in both cell models. Investigation of these drug-specific differentially expressed microRNAs suggests a possible role in the processes involved in liver injury. MicroRNAs targeting genes involved in lipid metabolism are differentially expressed in multiple treatments. Furthermore, changed microRNAs in in vitro cholestasis are differentially expressed after CaS treatment in primary mouse hepatocytes. Targeted research of these drug-specific microRNAs including their target genes and the proteins they code for may further unravel the mechanisms underlying drug-induced hepatotoxicity.
Induced Pluripotent Stem Cell (iPSC) derived hepatocytes (iHeps) offer the promise of an unlimited supply of cells from single donors and specific disease populations with genetic polymorphisms that may contribute to intrasomatic drug induced liver injury. However, though these cells express various liver specific features and functions, it is well documented that they are immature compared to human primary hepatocytes, expressing fetal-like proteins, and possess lower levels of activity for certain CYP450 metabolic enzymes, that play a crucial role in drug metabolism and toxicity. In this study, we exposed fresh iHeps to hemodynamic, blood flow and transport parameters that recreate the physiological microenvironment of the sinusoidal space. Under these same conditions, it has been previously shown that cryopreserved human adult primary hepatocytes restore morphology, biology, and demonstrate efficacy or toxicity responses to drugs at concentrations that approximate in vivo exposure levels. Microarray analysis of RNA was performed comparing iHeps in a static cell culture collagen gel sandwich and iHeps exposed to liver physiological parameters. We show that the following pathways are enhanced when iHeps are exposed to physiological parameters relative to static conditions: Key nuclear factors driving hepatocyte differentiation like HNF4α, Nrf2, NF112 (PXR) and NR1I3 (CAR), and their downstream pathways - Phase 1 (cytochrome p450), Phase II, and transporters. Peroxisome proliferator-activated receptor α (PPAR-α) was activated with enhanced mitochondrial beta oxidation, energy expenditure. Similarly to PGC1α, PPRC is a highly unstable protein, but has previously been shown to be induced by stabilization during cellular stress and energy deprivation. Expression of PPRC was also observed with metformin treatment, suggesting that also non-pathological metabolic stress is associated with PPRC induction. We also employed db/db mice as a model of fatty liver and also demonstrated significantly higher levels PPRC protein in liver of db/db mice with severe steatosis. These data suggest that PPRC integrates signals of metabolic and xenobiotic stress in the liver and may provide a novel mechanism mediating adaptive and pathological responses.

**PS 692 Activation of PGC-1-Related Coactivator during Metabolic and Xenobiotic Stress in the Liver Is Associated with Adaptive and Pathological Responses**


The PGC-1-related Coactivator (PPRC) belongs to a small family of transcriptional co-activators that have been shown to control mitochondrial biogenesis and energy expenditure. Similarly to PGC1α, PPRC is a highly unstable protein, but has previously been shown to be induced by stabilization during cellular stress and is linked to induction of inflammatory gene expression in U2O2 cells. In order to investigate whether PPRC also mediates adaptive response to toxicants in liver, we have analyzed PPRC protein using Western blot in HepG2 cells, rat primary hepatocytes and in mouse and rat liver. PPRC protein is highly expressed in rodent liver and in primary human hepatocytes, relative to other tissues tested. In vitro experiments in HepG2 cells and rat primary hepatocytes indicate that PPRC protein is induced by a variety of liver toxicants. Transient transfection of PPRC in these cells leads to increased ATP/ADP ratio and oxygen consumption. In agreement with previous studies, PPRC induction was also associated with expression of pro-inflammatory cytokines. In vivo, PPRC mRNA was also shown to be rapidly induced in mouse liver following concavatin A treatment. Similar results were also observed with metformin treatment, suggesting that also non-pathological metabolic stress is associated with PPRC induction. We also employed db/db mice as a model of fatty liver and also demonstrated significantly higher levels PPRC protein in liver of db/db mice with severe steatosis. These data suggest that PPRC integrates signals of metabolic and xenobiotic stress in the liver and may provide a novel mechanism mediating adaptive and pathological responses.

**PS 693 The Hepatic “Matrisome” Responds Dynamically to Toxic Stress: Novel Proteomic Characterization of the Hepatic ECM**


Background. There are no therapies to halt or reverse chronic liver disease (e.g., ALD), which follows a common natural history leading to end-stage liver disease. Outside the context of fibrosis, the nature and impact of the dynamic responses of the hepatic ECM protein (i.e., matrisomes) to stress are poorly understood. The goal of this work was to develop a proteomic method to characterize the hepatic matrisome and compare the impact of ethanol and lipopolysaccharide (LPS) on this compartment. Methods. Mice were exposed to liver toxins (e.g., CCl4, ethanol and LPS). Liver sections were processed in a series of increasingly rigorous extraction buffers to capture proper proteomic signals. Extracted proteins were identified using liquid chromatography-tandem mass spectrometry (LC-MS/MS). ECM proteins were mined, categorized by primary function and compared between experimental groups. Immunoblotting was performed to validate changes and for proteins previously found to be significant in hepatic ECM remodeling. Results. The extraction method separated distinct pools of ECM proteins that were identifiable by LC-MS/MS. The matrisome responded dynamically to stress and all exposures caused a dramatic change. The enhancement of LPS-induced liver damage by ethanol preexposure was associated with unique protein changes. (Supported by NIEHS and NIAAA).

**PS 694 Development of an In Vitro High-Content Imaging Assay for Quantitative Assessment of Mouse Hepatocyte Proliferation**


The two-year rodent bioassay is the standard metric for determination of carcinogenicity from exposure to synthetic or natural chemicals. This approach is labor- and cost-intensive with limited throughput for assessing hepatocarcinogenesis of novel chemicals with different modes of action. Transition to an in vitro model has been hampered by challenges with reproducible proliferative responses in cultured primary hepatocytes. Using an array of recombinant growth factors, cytokines, and model CAR activators, an effort was made to develop an in vitro high-content imaging based assay for quantitative assessment of nascent DNA synthesis in primary cultures of CD-1 mouse hepatocytes. Detection of DNA synthesis was performed using click chemistry labeling of the nucleoside analog 5-ethyl-2'-deoxyuridine (EdU). Optimization of the DNA labeling index revealed time- and seeding density-dependent effects of growth factor induced DNA synthesis. Hepatocytes were responsive to the CYP induction properties of known CAR activators such as T3CPOBOP, but were unresponsive for DNA synthesis. Subsequently, the proliferative responses to growth factors EGF, HGF, and TGF-β were evaluated and optimized. Additional supplementation with the cytokine IL-6 significantly increased DNA synthesis by each of these growth factors. The assay was multiplexed to enable direct quantitation of DNA synthesis, cytocotoxicity, and cell count endpoints. Using the optimized defined media cocktail, the EdU labeling response was enhanced in T3CPOBOP treated hepatocytes in a concentration-dependent manner. The results demonstrate that hepatocytes normally available to hepatocytes via non-parenchymal cells enhance the induced DNA synthesis response to a CAR activator.

**PS 695 Plateable Cryopreserved Human Hepatocytes Pooled from Multiple Donors for In Vitro Evaluation of Adverse Drug Properties**

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Primary human hepatocytes represent the gold standard for in vitro drug metabolism and hepatotoxicity experimentation. A major challenge of the use of primary human hepatocytes is the known individual differences, thereby necessitating the use of hepatocytes from multiple donors for investigation. One practice to overcome this challenge is to use cryopreserved human hepatocytes pooled from multiple donors. Currently, pre-pooled cryopreserved human hepatocytes are known to lose their ability to be cultured (non-plateable), presumably due to additional cellular damage upon a second cryopreservation, and are used as suspension cultures for relative short-term (maximum duration of 4 h) studies. We present here our success in the preparation of cryopreserved human hepatocytes pooled from multiple donors while retaining their ability to be cultured (plateable). Plateable cryopreserved human hepatocytes from 5 individual donors were thawed and re-cryopreserved using an optimized procedure. The cells were re-cryopreserved as individual donors or as a pool of the 5 donors. The post-thaw viability of the re-cryopreserved human hepatocytes were consistently >85%, and formed monolayer culture with the typical epithelial morphology. P450 induction studies performed with the single donor and pooled donor re-cryopreserved human hepatocytes showed robust response to the model inducers for CYP1A2 (omeprazole), CYP2B6 (phenobarbitals) and CYP3A4 (rifampin). The fold induction of the pooled cryopreserved hepatocytes was similar to the mathematical average of that for the individual donors. An in vitro hepatotoxicity assay was developed with the plateable pooled human hepatocytes using cellular ATP and reactive oxygen species (ROS) as endpoints.
Drug-induced liver injury (DILI) is a leading cause of failed drug development and accounts for one-third of drug withdrawals from the market. The prediction and prevention of DILI have been hampered by limited knowledge of the underlying molecular mechanisms. The development of DILI could involve perturbations of multiple pathways. In this study, we assessed the in vitro cytotoxicity of a library of DILI-associated drugs and no-DILI-concern drugs from FDA’s Liver Toxicity Knowledge Base (LTKB) using a panel of cell-based assays. Multiple endpoints were measured, including cell proliferation (MTT assay), apoptosis (caspase-3 activation), necroptosis (cyclophilin A release), cellular stress (HSP70/72 expression), oxidative stress (carboxy-H2DCFDA probe, ABE reporter), endoplasmic reticulum stress (CHOP/GADD153 expression, Gaussia luciferase reporter), mitochondrial dysfunction (JC-1 probe), and lipid peroxidation (MDA assay). Drugs were clustered according to the similarity of assay profiles. The predictive power of each measure as well as a combination of measures for DILI potential was evaluated. The preliminary results would help define a more comprehensive picture of toxicity mechanisms of DILI-associated drugs and provide basis for further mechanistic studies.

Analysis of Acetaminophen-Induced Hepatotoxicity in Female C57BL/6J Mice Lacking the ATP-Binding Cassette Subfamily C Member 4 (Abcc4, Mrp4)

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Acetaminophen (APAP) overdose accounts for up to half of the cases of acute liver failure in most developed countries. Approximately half of these cases are accidental, a result of APAP being a commonly used singular and combinational analgesic and antiprhythmic. Our previous work led us to hypothesize that increases in the expression of liver Mrp4 (Abcc4) confers resistance to APAP-induced hepatotoxicity and also plays a role in APAP autoprotection, a form of tolerance to APAP hepatotoxicity. In this study, we used female C57BL/6J Mrp4 knockout (KO) mice to determine the role of Mrp4 in protecting against APAP hepatotoxicity. Notably, Mrp4 KO female mice receiving 500 mg/kg APAP exhibited significantly greater hepatotoxicity compared to wild-type (WT) mice, as evidenced by plasma ALT values and histopathology. Furthermore, Mrp4 KO female mice receiving APAP pretreatment developed tolerance to subsequent APAP challenge, a response also observed in WT mice. We also observed that Mrp4 gene expression in WT female mice does not change with APAP pretreatment. This is in contrast to WT males, where APAP pretreatment resulting in autoprotection is accompanied by Mrp4 induction. To identify additional factors that may contribute to the altered susceptibility of female Mrp4 KO mice to APAP toxicity and their retained ability to autoprotect, we examined other efflux transporters, such as Mrp3 and Mrp6, and stress genes such as NAD(P)H dehydrogenase quinone 1 (Nqo1) and hemoxigenase-1 (Hoe-1). In addition to lack of Mrp4 induction, we also observed that female Mrp4 KO mice, unlike WT female mice, do not exhibit induction of Nqo1 upon APAP dosing. No noticeable differences in expression of other transporters were also observed. Overall, this study supports the notion that factors other than Mrp4 are responsible for APAP autoprotection.

Increased Hepatic O-GlcNAcylation Aggravates Acetaminophen-Induced Liver Injury


Overdose of acetaminophen (APAP), the most widely used analgesic, results in acute liver failure. We have investigated the role of the increase in a post-translational modification of proteins called O-GlcNAcylation, where a single β-D-N-acetylglucosamine (O-GlcNAc) moiety is added to the protein, in pathogenesis of APAP-induced liver injury. Hepatic O-GlcNAc levels were increased by inhibition of O-GlcNAcase, the enzyme that removes O-GlcNAc from proteins using a specific inhibitor, Thiame-G (TGM). Male C57BL/6J mice were treated with 30 mg/kg APAP followed by 400 mg/kg TGM 1.5 hr after APAP administration and studied over a time course of 0 to 24 hr. TGM treatment resulted in significant increase in hepatic O-GlcNAc. The mice treated with TGM exhibited significantly higher APAP-induced liver injury as indicated by increase in serum transaminase levels and histopathological analysis. Treatment with TGM did not affect hepatic Cyp2e1 levels. APAP protein adduct formation, APAP induced mitochondrial damage, hepatic GSH, or depletion of GSH after APAP treatment. However, a significantly higher and prolonged JNK activation was observed in TGM and APAP-treated mice. Interestingly, the increased injury secondary to increased O-GlcNAcylation did not affect liver regeneration. In conclusion, these data indicate that increase in O-GlcNAcylation of proteins can exacerbate APAP-induced liver injury via modulating JNK activation. These data are of significance because O-GlcNAcylation is a common effect of dietary supplements such as Glucosamine and TGM is being developed as a drug for Alzheimer’s and questions the safety of APAP in these settings.

Role of Mitochondrial ATP Binding Cassette Transporter Abcb6 in Acetaminophen Hepatotoxicity

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Acetaminophen (APAP) overdose results in acute liver failure and has limited treatment options. APAP hepatotoxicity is strongly associated with decreased propensity for liver regeneration. Previous studies have shown that promoting liver regeneration is critical for survival after APAP overdose. However, the mechanism of liver regeneration following APAP injury is not clearly understood. In this study, we used Abcb6-/- mice as an in vivo model to explore the association between liver regeneration and APAP hepatotoxicity. Abcb6 is a mitochondrial ATP binding cassette transporter which has been shown to play a role in cell proliferation. Abcb6 expression is induced during hepatic carcinogenesis and promotes cell growth and proliferation, while loss of Abcb6 leads to delayed progression through the cell cycle. Interestingly we found that Abcb6-/- mice were protected against APAP (300 mg/kg/i.p.)-induced early liver injury. Liver regeneration in Abcb6-/- mice after APAP treatment was significantly increased as measured by increased expression of PCNA secondary to Cyclin D1 expression. In addition Abcb6-/- mice also demonstrated rapid recovery of hepatic glutathione, and decreased activation of c-Jun N-terminal kinase. Overall these results demonstrate role of Abcb6 in APAP hepatotoxicity.

L-Phenylalanine-Derived Rhodanine Analog (LPAR)-Induced Diverse Cytoprotective Mechanisms in Cultured Mouse and Human Hepatoma Cells


Rhodanine class of compounds has attracted enormous attention due to their emerging biological activities. However, some of rhodanine analogs are known to be cytotoxic. A toxic chemical often can induce cytoprotective mechanisms, such as activation of nuclear factor erythroid-derived 2-related factor 2 (Nrf2) and fibroblast growth factor (Fgf) 21, to ameliorate its generated toxicity. It is unknown whether rhodanine or its analogs can induce cytoprotective mechanisms. To test this, we treated L-phenylalanine-derived rhodanine (LPAR) in mouse Hepa1c1c7 and human Hep3B hepatoma cells. Two-day treatment of 30μM of LPAR suppressed cell proliferation in both Hepa1c1c7 and Hep3B cells, as evident from decreased cell number and more cell detachment compared to the control group. Morphologically, LPAR caused apparent cell shrinking or stretching, and even lead to spindle-like appearance. In Hepa1c1c7 cells, LPAR decreased Fgf21 mRNA expression, but induced mRNA expression of heme oxygenase-1 (HO-1) and NADPH quinone oxidoeductase-1 (Nqo-1), indicating that Nrf2 is activated. In contrast, in Hep3B cells, LPAR induced Fgf21 mRNA expression, but did not alter the mRNA expression of Nrf2 target genes HO-1 and Nqo-1. It has been previously reported that Fgf21 expression can be induced via activation ofaryl hydrocarbon receptor (AhR) or peroxisome proliferator-activated receptor alpha (PPARα) in mouse liver. We next determined whether activation of AhR or PPARα is required for the induction of Fgf21 by LPAR in Hep3B cells. Surprisingly, our data showed that LPAR did not induce the mRNA expression of either AhR-target gene Cyp1a2 or PPARα-target gene Cyp4a14 in Hep3B cells. Thus, the mechanism other than AhR or PPARα activation is responsible for the induction of Fgf21 by LPAR in human hepatoma cells. In conclusion, LPAR induced Fgf21 expression in human hepatocytes, whereas it activated Nrf2 signaling in mouse hepatocytes.
Uric Acid Inhibits Lipid Metabolism through Suppression of Lipophagy

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Fructose which is reported to be lipogenic is rapidly phosphorylated to fructose-1-phosphate by fructosekinase using ATP and it results in AMP accumulation leading to activation of AMP deaminase and generation of uric acid. Patients with hyperuricemia or gout usually accompany hypertriglyceridaemia and hyperuricemia is also prevalent in patients presenting nonalcoholic fatty liver disease (NAFLD) that develops hepatic steatosis in the lack of alcohol abuse. In this context, we hypothesized that uric acid which comes from the fructose metabolism promotes triglyceride accumulation and development of fatty liver. Fatty liver results from increased de novo lipogenesis, increased transport of triglyceride into the liver and decreased lipolysis. In this paper, we tried to characterize the role of uric acid in lipolysis. A newly found function of autophagy is the degradation of lipid droplets. Accordingly, we hypothesized that uric acid contributes to the pathogenesis of development of fatty liver by regulation of autophagy. We found that uric acid inhibits autophagy in concentration- and time-dependent manner in HepG2 cell line. Although uric acid did not suppress the expression of autophagy related proteins but activated Akt followed by inhibitory phosphorylation of TSC2, subsequently increased phosphorylation of mTOR and decreased autophagy. It is well known that starvation-induced autophagy degrades lipid droplet and releases fatty acids for mitochondrial β-oxidation. Pretreatment of uric acid suppressed starvation-induced autophagy and decreased intracellular lipid utilization by starvation. In addition, long-term treatment of uric acid itself increased intracellular lipid content and cells incubated with oleate in the presence of uric acid deposited more lipid than those only treated with oleate. In conclusion, this study first demonstrated a novel mechanism that uric acid regulates lipid metabolism through suppression of autophagy.

The Role of the Adaptive Immune System in a Mouse Model of Halothane-Induced Liver Injury

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Idiosyncratic drug-induced liver injury remains a significant public health concern and in many cases is believed to result from an aberrant immune response. In halothane-induced liver injury (HILI) clinical features including: delayed onset of injury, elevated serum immunoglobulin against trifluoroacetylated (TFA) liver proteins and increased risk of hepatotoxicity with subsequent exposures are indicative of a role of the adaptive immune system. However, until recently animal models of HILI have not included a significant contribution of the adaptive immune system. The goal of the present study was to investigate whether two ubiquitous PBDEs, 2,2',4,4'-Tetrabromodiphenyl ether (BDE-47) and 2,2',4,4,5-pentabromodiphenyl ether (BDE-99) could affect hepatic lipid accumulation in cultured liver cells. HepG2 cells were treated with TCDD for 1-4 hours. Next, cells were treated with MDMO vehicle (0.01% DMSO), BDE-47, or BDE-99 (0.1 nm-25 μm) alone or in combination with oleic acid for 48 hours in a low glucose phenol-red free media. Cells were collected after treatment and lipids were extracted with phenol-chloroform. Triglycerides were quantified from the lipid extracts. As anticipated, oleic acid increased hepatocyte lipid content. At the concentrations evaluated, BDE-99 did not significantly increase total lipid content or triglycerides. However, BDE-47 (0.1 nm) approximately doubled total cellular lipid and triglyceride content. Neither BDE-47 nor -99 treatment affected OA-induced cellular lipid accumulation. These preliminary data suggest that BDE-47 might possess a lipid modulating effect in hepatocytes.

Progression of Liver Injury Results from Increased Levels of Lipid Peroxidation Products in Male GSTA4-4 Knockout Mice Fed Alcohol Chronically

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Alcoholic liver injury (ALD) results from a mechanism in which the first hit, reversible development of fatty liver, is followed in susceptible individuals by several other hits, including various forms of oxidative stress and endotoxemia, ultimately resulting in progression of pathology to inflammatory and hepatic necrosis and hepatocellular carcinoma. In the current study we examined the potential role of reactive short chain aldehyde (SCA) products of lipid peroxidation in progression of ALD by examining the development of ALD in male wild type SVJ mice (WT) and SV/J mice lacking the enzyme glutathione-S-transferase (GSTA4-4 GST KO) which is responsible for detoxification of SCAs such as 4-hydroxynonenal (4-HNE), Mice (n = 10/group), 13 wk old, were pair-fed (PF) high fat Lieber DeCarli liquid diets containing 28% calories as ethanol (EtOH) or added dextrose for 120 d. Both PF and EtOH treated mice developed steatosis, inflammation, necrosis and fibrosis and had elevated serum ALT values relative to Chow-fed controls (P<0.05). However, whereas pathology and gene expression in PF mice did not differ significantly between genotypes, in the EtOH-treated groups, expression of inflammatory markers TNF alpha and IFN gamma mRNA were elevated in the GST KO mice compared to WT (P<0.05). In addition, matrix remodeling genes MMP9, MMP13 and the fibrosis marker collagen 1α mRNA were higher in EtOH GST KO compared to EtOH WT mice (P<0.05) in the absence of increased expression of TGF beta. mRNA encoding the B cell marker B220 was decreased while mRNA for CD138 a marker of fully differentiated antibody producing plasma cells was elevated by EtOH treatment compared to PF mice independent of genotype (P<0.05). These data suggest that SCA play a role in progression of ALD and not nonalcoholic fatty liver injury but that effects of EtOH on B-cell differentiation are not related to SCA. Supported in part by 801 AA009300 (DRP).

Exposure to TCDD Increases Fibrogenesis during Experimental Liver Fibrosis

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The environmental contaminant 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) elicits toxicity through the aryl hydrocarbon receptor. It was recently shown that exposure to TCDD enhances gross markers of liver damage during experimental liver fibrosis. Fibrosis is a common pathophysiological response to chronic liver injury and is mediated by the activation of myofibroblast precursors, namely hepatic stellate cells (HSC). Previous studies in our laboratory revealed that TCDD treatment enhances activation of a human HSC line in vitro. The goal of the present study was to test the hypothesis that TCDD treatment increases HSC activation in vivo. To test this, we measured the consequences of TCDD treatment on fibrosis-related endpoints that are typically attributed to activated HSCs, such as the production of TGFβ1 and collagen type I. To induce liver fibrosis, mice were treated with carbon tetrachloride (CCL4; 5 ml/kg diluted 1:10 in corn oil) twice a week for eight weeks. During the final two weeks, mice were treated with TCDD (20 μg/kg) or vehicle (peanut oil). Results indicate that TCDD treatment enhanced collagen deposition based on increased Sirius red staining in liver sections, as well as mRNA levels of Colα1 that were fold higher than in mice treated with CCl4 alone. TCDD treatment also elicited an approximate 3-fold increase in mRNA levels of the pro-fibrogenic mediator TGFβ1 and increased levels of hydroxyproline in liver homogenates. Finally, TCDD treatment increased expres-
The liver is the main site for drug and environmental contaminants metabolism, and as such is one of the most important organs when it comes to predictive evaluation of compounds for efficacy and toxicity, including inactivation of the compound or bioactivation to a toxic metabolite. In order to improve the predictability of drug safety and efficacy in clinical development, and to have a clearer perspective of the potential human health effects from exposure to environmental contaminants, there is a critical need to accurately model human organ systems such as the liver in vitro. We are developing a 3-dimensional (3D) microphysiological system based on a new commercial (Nortis Inc.) microfluidic platform that can utilize primary liver cells from any species (e.g., rat and human). Compared to the standard monolayer cell culture (sandwich culture) that typically survive for 5–7 days, primary rat hepatocytes and cryopreserved human hepatocytes in Nortis devices (3D) exhibited higher viability (measured by LDH/AALT release and vital staining), improved hepatic functions, such as albumin production (measured by ELISA), and expression of hepatocyte marker-HNF4 alpha and rifampin-responsive xenobiotic sensing nuclear receptor- PXR (measured by immunocytochemistry) up to 14+ days. Additionally, dose-related induction of Cytochrome P450 via ethoxyresorufin-O-deethylase (EROD) assay in cryopreserved human hepatocytes was observed in Nortis devices over 14 days. These results indicate that hepatocytes grown in the Nortis microphysiological system provide a promising approach for evaluating drugs or xenobiotics in vitro, where testing over extended periods of time is needed, such as enzyme induction studies.

**709 Dynamic Flow Models of Tissue Bioreactors for 3D Hepatocyte Culture**


Dynamic flow devices for 3D cell cultures are increasingly used to better recapitulate the *in vivo* microenvironment. In this investigation 3D flow dynamics were used to determine the optimal design of a system for metabolism assays. One commercially available flow device from RealBio™ (RB) and one custom developed flat bed device (FB) were modeled with regard to turbulence, 3D flow characteristics, compound diffusion, oxygen distribution and consumption. The results showed limited turbulent flow patterns in both devices; however, 3D flow analysis demonstrated areas of low or no flow in the RB device. The geometric design for the FB device was optimized to ensure homogenous flow within the chamber. In both devices, compound distribution was shown to be dependent on flow convection with increased impact of diffusion at lower flow rates. The RB device was seeded with hepatocyte suspensions, whereas beads of polymerized alginate that encapsulate hepatocytes were used for the FB device. Thus, simulations were performed in the heads alone before the FB bioreactor simulations. Oxygen permeation in alginate beads up to 1000 um in diameter was investigated. The results show that oxygen permeates the alginate polymer well and no size limitation due to low oxygen permeation was observed in this size range. This simulation was supported by imaging analysis of cell viability within the heads. Based on the simulated oxygen consumption in the system, oxygen distribution appears to be the limiting factor in relation to the number of cells that can be sustained in the RB device. Our computational results indicate that both devices could be used for long term hepatocyte culture with careful consideration of limiting factors, i.e., flow (RB) or oxygen distribution (FB). Our work demonstrated the value of fluid dynamic modeling in designing a 3D device for *in vitro* metabolism and toxicity studies (Supported by ACC-LRI).

**708 Effect of Gut Microbiota Depletion on the Ontogeny of Drug-Processing Genes in Mouse Liver**

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Profound changes occur in the ontogeny of drug metabolizing enzymes and transporters (together called “drug-processing genes” [DPGs]) in liver. Our RNA-Seq data in germ-free (GF) and Conventional (CV) mice have shown that gut microbiota is a regulator of many DPGs in adult liver. However, the effect of gut microbiota on the ontogeny of DPGs is unknown. In this study, livers were harvested from age-matched CV and GF male mice at 1, 3, 5, 15, 30 and 90-days of age (n=4 per group). The mRNAs of 67 critical DPGs were quantified by qPCR. Protein patterns of the highest expressed hepatic transporter Ntcp was quantified by LC-MS/MS. The mRNAs of the DPGs partitioned into 4 developmental patterns: Pattern 1 has 14 DPGs of which the mRNAs gradually increased to adult levels during development in CV livers; whereas in GF mice, most of these genes were down-regulated between 15 and 90-days of age, exemplified by Cyp3a11, which encodes the major drug-metabolizing enzyme in liver. Pattern 2 has 32 DPGs and contains many Cyp2 and Cyp4 family members, as well as the major hepatic uptake transporter Oatp1b2, which were up-regulated in GF livers at multiple ages. Pattern 3 has 4 DPGs that were neonatal-enriched in both CV and GF livers, among which the hepatic carnitine transporter Octn1 was markedly up-regulated in livers of 90-day old GF mice. Pattern 4 has 17 DPGs that were enriched predominantly at 15-days of age in both CV and GF mice and were regulated by gut microbiota in an age-specific pattern. For example, the sterol efflux transporters Abcg5 and Abcg8 were both down-regulated at 15-days of age, but were up-regulated thereafter compared to CV mice. There was a 50% increase of Ntcp protein from 15 to 30-days age in CV livers; whereas in GF livers, most of these genes were down-regulated between 15 and 90-days of age, exemplified by Cyp3a11, which encodes the major drug-metabolizing enzyme in liver. Pattern 2 has 32 DPGs and contains many Cyp2 and Cyp4 family members, as well as the major hepatic uptake transporter Oatp1b2, which were up-regulated in GF livers at multiple ages. Pattern 3 has 4 DPGs that were neonatal-enriched in both CV and GF livers, among which the hepatic carnitine transporter Octn1 was markedly up-regulated in livers of 90-day old GF mice. Pattern 4 has 17 DPGs that were enriched predominantly at 15-days of age in both CV and GF mice and were regulated by gut microbiota in an age-specific pattern. For example, the sterol efflux transporters Abcg5 and Abcg8 were both down-regulated at 15-days of age, but were up-regulated thereafter compared to CV mice. There was a 50% increase of Ntcp protein from 15 to 30-days age in CV livers; whereas, the absence of gut bacteria blunted this ontogenic increase. In conclusion, gut microbiota markedly impacts the ontogeny of many hepatic DPGs in an age-specific manner. (supported by R01 grants ES019487 and GM111381)

**707 A Tissue-Engineered Rat/Human Liver Microphysiological System for Drug and Chemical Testing**

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The liver is the main site for drug and environmental contaminants metabolism, and as such is one of the most important organs when it comes to predictive evaluation of compounds for efficacy and toxicity, including inactivation of the compound or bioactivation to a toxic metabolite. In order to improve the predictability of drug safety and efficacy in clinical development, and to have a clearer perspective of the potential human health effects from exposure to environmental contaminants, there is a critical need to accurately model human organ systems such as the liver in vitro. We are developing a 3-dimensional (3D) microphysiological system based on a new commercial (Nortis Inc.) microfluidic platform that can utilize primary liver cells from any species (e.g., rat and human). Compared to the standard monolayer cell culture (sandwich culture) that typically survive for 5–7 days, primary rat hepatocytes and cryopreserved human hepatocytes in Nortis devices (3D) exhibited higher viability (measured by LDH/AALT release and vital staining), improved hepatic functions, such as albumin production (measured by ELISA), and expression of hepatocyte marker-HNF4 alpha and rifampin-responsive xenobiotic sensing nuclear receptor- PXR (measured by immunocytochemistry) up to 14+ days. Additionally, dose-related induction of Cytochrome P450 via ethoxyresorufin-O-deethylase (EROD) assay in cryopreserved human hepatocytes was observed in Nortis devices over 14 days. These results indicate that hepatocytes grown in the Nortis microphysiological system provide a promising approach for evaluating drugs or xenobiotics in vitro, where testing over extended periods of time is needed, such as enzyme induction studies.
Numerous modes-of-action (MOAs) underlie chemical-induced liver injury. An MOA of particular concern is altered lipid metabolism, which can progress to serious injury. Steatosis is marked by excess neutral lipid accumulation; phospholipidosis (PLD) is marked by excess phospholipid accumulation. Methods to detect these conditions during chemical development and testing include traditional primary hepatocytes and liver cell lines. However, these models are neither amenable to long-term study nor have the full complement of biochemical functions present in vivo. Micropatterned co-cultures of human and rat hepatocytes surrounded by mouse fibroblasts (STJ3-J2; MPCC; HepatoPac™) maintain morphologic and biochemical characteristics of hepatocytes in vivo long term. The objective of this study was to develop a higher-throughput, robust method using high-content imaging (HCI) analysis of MPCC to assess the steatosis and/or PLD-inducing potential of chemicals. MPCC and stromal cell-only plates were optimized using the prototypic hyperlipidemic reference agents cyclosporin A (steatosis) and propranolol (PLD) and five different staining methods. A single multiplexed method using LipidTOX Red (PLD) and LipidTOX Green (neutral lipid) was chosen and used to examine 20 selected hepatotoxins from the EU Joint Research Centre (JRC) and ToxCast hepatotoxicant lists. Cells were stained with high-content imaging (HCI) probes to examine hepatocyte-specific cell loss, membrane permeability, oxidative stress, and apoptosis endpoints. In conclusion, the MPCC combined with HCl-based multiplexed endpoint assays is a promising new tool to conduct MOA-based screening for chemical toxicity testing.

The current suite of in vitro ToxCast assays may not represent complex biochemical and multiple-cellular responses observed in vivo with adequate fidelity. We have recently shown that human hepatocytes in a hepatic culture model in which cryopreserved primary human hepatocytes are seeded onto micropatterned 96-well plates and co-cultured with murine embryonic fibroblasts (MPCC; HepatoPac™) retains key biochemical functions of the liver in vivo, including metabolic capacity. However, the retention of functional nuclear receptor pathways in this model has not yet been determined. MPCCs with human hepatocytes were treated with 17 compounds, including prototypical nuclear receptor activating compounds rifampin (RIF), phenobarbital (PB) and GW7667, along with additional compounds from the ToxCast dataset (e.g., PF0A and haloperidol), using an eight-point concentration range (0-100 μM). Changes in the expression of eight different genes, including a house-keeping gene (ACOX1, CYP4A11, HMGC52, CYP1A2, CYP2B6, CYP2C9, CYP3A4, HPR), were determined following 24 and 72 hr exposures. Results show concentration-dependent induction of the majority of target genes by the prototypical inducers. However, we found that hepatocytes in this model are more sensitive to induction by some compounds, such as RIF and CITCO, compared to the standard sandwich-culture model. A minimum three-fold induction was observed in at least one target gene at a single time point following treatment with each compound, except for the negative control caffeine. A 50% suppression of at least one target gene at a single time point also was observed for the majority of compounds tested. Human MPCCs recapitulate outcomes between known agonists and their respective receptor/target gene pathways with high fidelity, representing a sensitive, robust and reproducible model for the measurement of changes in nuclear receptor-mediated gene expression.

A recent analysis of results from ToxCast’s Phase I studies, which utilized mainly cell lines supplemented with conventional primary hepatocyte monocultures, indicated that high-throughput screening assays have limited ability to predict in vivo toxicity. Improved in vivo models that maintain in vivo biochemical functions and cell-cell interactions are needed. We previously showed that primary rat and human hepatocytes seeded as micropatterned co-cultures (MPCC; HepatoPac™) in 96-well plates surrounded by stromal cells (mouse 3T3-J2 fibroblasts) maintain long term morphologic characteristics and biochemical functions similar to those in vivo. The objective of this study was to determine the fidelity of human MPCC combined with multiplexed endpoint assays for mode-of-action (MOA)-based screening. MPCC and corresponding stromal cell-only plates were treated (0-100 μM) for 24 and 72 hr with the prototypic compounds valinomycin (apoptosis) and menadione (oxidative stress) and 20 select compounds from the EU Joint Research Centre (JRC) and ToxCast hepatotoxicant lists. Cells were stained with high-content imaging (HCI) analysis to assess cell loss, membrane permeability, oxidative stress, and apoptosis endpoints. In conclusion, the human MPCC combined with HCl-based multiplexed endpoint assays is a promising new tool to conduct MOA-based screening for chemical toxicity testing.

Drug induced liver injury (DILI) is a major health problem in the United States and accounts for the majority of clinical holds and postmarketing use restrictions by the FDA. The majority of adverse liver reactions are idiosyncratic and their underlying mechanisms are still not well understood. Better predictive models for DILI would enable the preclinical elimination of drug candidates with hepatotoxic liabilities. We have previously developed a model in which primary hepatocytes (rat, human, dog, or monkey) are seeded onto ECM-coated domains of optimized dimensions and subsequently co-cultivated with murine embryonic fibroblasts (HepatoPac®). Hepatocytes in HepatoPac retain their in vivo-like morphology, express a complete complement of liver-specific genes, metabolize compounds using active Phase I/II drug metabolism enzymes, secrete diverse liver-specific products, and display functional bile canaliculi for several weeks in vitro. Here, we supplement the HepatoPac co-cultures with primary Kupffer macrophages for use in evaluating inflammation-drug interactions. Kupffer cells were added to human HepatoPac cultures at a precise hepatocyte:Kupffer cell ratio of 10:4 to generate HepatoMuneTM co-cultures. We then assessed whether stimulation of HepatoMune co-cultures with 50ng/ml LPS sensitizes the cultures to trovafloxacin (TVX), clopant (CLP) or chlorpromazine (CZM) toxicities. Human HepatoMune co-cultures were treated with increasing concentrations of drugs (+/- LPS) and assessed for changes in hepatic ATP content. TVX, CLP and CZM caused concentration dependent depletion of cellular ATP in HepatoMune cultures which was exacerbated by addition of LPS to the cultures (TC50 357 vs. 94μM for TVX, 68 vs. 39μM for CLP, and 29 vs. 17μM for CZM respectively). The potentiation of CLP and CZM toxicities were more pronounced after repeated co-administration of drug/LPS over 6 days. In conclusion, human HepatoMune co-cultures may be used to predict drug induced liver injury mediated by inflammatory stress.
Tolvaptan (TVP) is a vasopressin receptor antagonist under investigation as a treatment for patients with autosomal dominant polycystic kidney disease (ADPKD). FDA approval has not been received due, in part, to reports of idiosyncratic drug-induced liver injury (DILI) associated with tolvaptan use in ADPKD patients. The objective of this study was to determine whether a new mouse genetic resource, the Collaborative Cross (CC), could be used to 1) identify mouse strains sensitive to the tolvaptan liver injury and 2) identify risk factors associated with susceptibility to tolvaptan DILI in humans. Eight (8) mice in each of 45 CC strains were treated with a single dose of either tolvaptan (100 mg/kg) or vehicle. Significant elevations in serum alanine aminotransferase (ALT) levels in tolvaptan-treated animals relative to vehicle-treated controls were observed in 3 of the 45 strains (p<0.05, Bonferroni post test). No histological changes in the liver were associated with tolvaptan treatment in any strain, but ALT fold change was significantly correlated with changes in serum aspartate aminotransferase and miR-122 (Pearson correlation). The mechanism(s) responsible for DILI, however, have yet to be elucidated. Tolvaptan undergoes extensive hepatic metabolism with <1% of the parent drug excreted in urine. Two major circulating metabolites, DM4103 and DM4107, may accumulate in the liver. Inhibition of hepatic bile acid transport may be one mechanism of tolvaptan-associated liver injury. This study was designed to determine the inhibitory potency of tolvaptan, DM4103 and DM4107 on [3H]taurocholate (TCA) transport by the bile salt bile salt export pump (BSEP) and sodium taurocholate cotransporting polypeptide (NTCP) using inverted membrane vesicles from S9 cells and stably-transfected CHO cells, respectively. The IC50 for [3H] TCA transport by BSEP (2μM;2min) and NTCP (2μM;5min) was estimated by nonlinear regression. TCV (IC50:25.0±μM) and DM4103 (IC50:41.5±μM) were potent BSEP inhibitors, but DM4107 (IC50:119±μM) was not. Inhibition of BSEP-mediated TCA (2-30μM) transport by TCV (0-50 μM) was noncompetitive (Ki=14.2±μM), while DM4103 (0-20μM) exhibited competitive inhibition (Ki=3.7±μM). TCV and DM4103 were weak NTCP inhibitors (IC50:41.6±μM and 14.9±μM, respectively); the IC50 for DM4107 was 100±μM. The ratio of the steady-state maximum total plasma concentration (Csp) to the IC50 for TCV, DM4103 and DM4107 was calculated for NTCP and BSEP to provide an estimate of the potential clinical relevance of inhibition as determined by a Csp/IC50 ratio ≥0.1. These data support the hypothesis that TCV and DM4103 are relevant inhibitors of hepatic transporters, which may be useful in elucidating the mechanism(s) responsible for liver injury in TVP-treated ADPKD patients.

**Effect of Tolvaptan on the Hepatobiliary Disposition of Bile Acids in Human B-CLEAR® Hepatocytes**

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Bile acid homeostasis in the liver is tightly controlled through various regulatory pathways including multiple transport proteins that remove bile acids from the blood and excrete them either back into the blood via basolateral efflux transporters or into the bile by canalicular efflux transporters. Most drugs that inhibit the efflux of bile acids also inhibit their uptake to some extent. The relative extent of inhibition of both uptake and efflux determines the net effect on the biliary clearance and intracellular accumulation of bile acids. The effect varying concentrations of tolvaptan (0.15 μM to 50 μM) on the hepatobiliary disposition of a model bile acid, taurocholate (TCA), were evaluated in sandwich-cultured hepatocytes using B-CLEAR® technology. To mimic physiologic conditions and account for non-linear protein effects, experiments were performed in the presence of a physiologic concentration of protein (4% BSA). The intracellular concentrations of tolvaptan in the hepatocyte increased in a dose dependent manner over the dose range evaluated. Following tolvaptan exposure at a concentration of 15 μM in the presence of protein, total concentrations of tolvaptan inside the hepatocyte were approximately 10X greater (129 μM). This extent of accumulation was similar throughout the dose range. Increasing concentrations of tolvaptan decreased the uptake of TCA to 62% of control and the canalicular efflux of TCA to 67% of control in a dose dependent manner. The net effect of these inhibitions on both the uptake and efflux of TCA yielded a decrease in the biliary clearance of TCA to 42% of control. The intracellular concentration of TCA remained constant except at the higher exposures where the intracellular concentration of TCA increased to 130% of control. Under in vivo relevant conditions, tolvaptan accumulated in the hepatocyte, and inhibited both the uptake and efflux of a model bile acid, resulting in an overall decrease in the biliary clearance.
719 Nonlinearities in Cellular Dose-Response Behaviors Can Be Enhanced by Protein Stabilization

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Chemical safety assessment requires a quantitative understanding of cellular dose-response behaviors. A common form of nonlinear dose response is a sigmoidal curve which has a steeper slope than the usual Michaelis-Menten curve. Many cellular pathways have sigmoidal responses to chemical stressors. These observed nonlinearities arise from a suite of intracellular network motifs called ultra-sensitive response motifs. Zero-order ultra-sensitivity via post-translational covalent modification cycle, exemplified by the activation of MAPK, is a key signaling motif for these responses. Sigmoidal curves arise when the modifying enzymes (kinases and phosphatases in the case of MAPK) operate near saturation for their protein substrates. Conventionally models of these motifs keep the total amount of the protein substrates constant. However, covalent modification of signaling proteins often changes protein stability. For example, phosphorylation of p53 by ATM in the DNA damage response leads to stabilization. Here we use mathematical models to explore nonlinear dose-response properties of posttranslational modification leading to protein stabilization. Our simulations show that protein stabilization through increasing steady-state protein substrate concentrations moves modifying enzymes closer to saturation. As a result the nonlinearity of the process increases, leading to more steeply sigmoidal responses with higher effective Hill coefficient (n-value) and higher magnitude. As toxicity testing shifts toward a cell-based approach, the quantitative mechanism for dose-response as studied here will be key tools for interpreting mechanisms of chemical perturbations and predicting in vitro dose-response behaviors. This is an abstract or a proposed presentation and does not necessarily reflect USEPA policy. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

720 Applying the Skin Sensitisation AOP to Human Health Risk Assessment


Despite our understanding of the key events that drive skin sensitisation (recently documented as an AOP) our ability to combine non-animal hazard data with exposure information to establish a safe level of human exposure for a sensitising chemical remains a key gap. Our aim is to apply mechanistic understanding of skin sensitisation to improve our ability to make risk assessment decisions. Central to our approach is mathematical modelling of the response and evaluation of model output against available clinical data on sensitisation. Our current model outputs naïve CD8+ T cell activation as a surrogate measure for sensitisation induction in humans. Ordinary differential equations are used to model key events of the AOP: skin penetration (chemical diffusion and partitioning), haptenation of protein nucleophiles and antigen processing and presentation by skin dendritic cells. Biological parameters are taken from the immunological literature with human data used where possible. Bioavailability and chemical-specific parameters are derived from bespoke in vitro experiments and from sensitiser-specific literature. The model has been used to simulate a study published previously by Friedmann et al. in which 152 healthy volunteers were exposed to one of five doses of the contact allergen 2,4-dinitrochlorobenzene. As a significant proportion of each dose cohort were sensitised to DNCB within this study, comparison of model simulation results to these clinical data have provided an opportunity to explore the relationship between naïve CD8+ T cell activation and clinical sensitisation. This analysis has enabled selection of an optimal model output parameter (T cell receptor trigger rate) for risk assessment decision-making and demonstrated the inherent difficulty in extrapolating from this cellular event to predict the extent of clinical sensitisation. To address this finding, immune characterisation of allergic contact dermatitis patients is underway to enable mathematical modelling of the sensitiser-induced memory T cell response.

721 Semi-Mechanistic Systems Pharmacology Modeling of High-Dose Baclofen for Assessment of Cardiovascular Site of Action


In homeostatic systems such as the cardiovascular system (CVS), assessing mechanism of action can be difficult as perturbations to the system can lead to both primary and secondary effects. While PK/PD models can quantify the exposure response relationship of these endpoints individually, they often lack information about interplay between observations in the system. Semi-mechanistic models hold promise for greater interpretation of data by accounting for interactions between elements of the system, and by separation of the data into drug specific and system specific parameters. These additional parameters can be difficult to inform independently from experiments on a single drug, especially when mechanism is unknown. Here we attempt to resolve this by testing systems model of the CVS in rat which incorporate HR and MABP, as well as Total Peripheral Resistance (TPR), Cardiac Output (CO) and Stroke Volume (SV). To assess direction and magnitude of effect. Specifically, these models were fit to Heart Rate (HR) and Mean Arterial Blood Pressure (MABP) observations in rats following oral administration of baclofen. The models were unable to adequately describe the observed responses when baclofen effect was incorporated as a single perturbation on HR or TPR alone. On the other hand, a model which accounted for drug effect on both HR and TPR simultaneously was able to explain both the magnitude and direction of the observed CVS changes in response to treatment. These results suggest that baclofen is exerting its effect with similar magnitude on both endpoints in concert. Reference(s): 1. Snelder, N., Ploeger, B.A., Luttringer, O., Rigil, D.F., Webb, R.L., Feldman, D., Fu, F., Beil, M., Jin, L., Stanski, D.R. and Danhof, M. (2013) BJP. 169(7):1510-1524. 2. Snelder, N., Ploeger, B.A., Luttringer, O., Rigil, D.F., Webb, D., Fu, F., Beil, M., Stanski, D.R. and Danhof, M. (2014) BJP. doi: 10.1111/bph.12824.

722 Mathematical Models Predicting the Effects of Generic Medicinal Countermeasures on Hematopoiesis following Radiation Exposure


Mechanistic models of hematopoiesis following acute radiation exposure which incorporate the effects of a generic medicinal countermeasure (MCM) are presented. These models simulate the process of lymphocyte, granulocyte, and platelet blood cell formation using a series of ordinary differential equations. Cells are divided into compartments based on their maturation level and location in the body. Biologically-based feedback mechanisms within the model allow for the system to maintain and, if perturbed, return to healthy states. Radiation exposure leads to a dose-dependent proportion of cell-killing, and cells transition between compartments at specific rates. Hematopoietic models were first developed for rhesus macaques using well-controlled experimental data following radiation and/or MCM exposure. A human model was then developed using the same model structure, but the model was re-parameterized using hematological and observational data following MCM treatment or radiation exposure in humans. Then by combining both radiation exposure and the MCM as model inputs, the potential mitigating and/or toxic effects of the MCM are predicted which may not be apparent from clinical trials. For example, an MCM that decreases the rate of megakaryocyte maturation leads to a slight thrombocytopenia in healthy humans; however, when radiation exposure is included, the decreased maturation rate causes the thrombocyte nadir to be higher and leads to a faster recovery. These models provide a tool for MCM development and for predicting MCM effects. These models allow for the integration of treatment timing relative to pre- or post-radiation exposure. If it is desirable to alter specific qualities of the blood cell time-profiles (i.e. nadir depth, recovery time), model simulations can be performed to quantify alterations to kinetic rates or repair processes that provide the desired results. In turn MCMs with that desired effects can be engineered. Thus, these models provide a tool for MCM development and the subsequent assessment of their effectiveness.

723 Computational Modeling of the HPG Axis and Toxicity Equivalent Calculations to Predict Effects of Endocrine Disruptors on Estradiol Levels in Fathead Minnows


Aromatase (CYP19A) converts testosterone to estradiol (E2). Some endocrine disruptors inhibit aromatase, potentially impacting fish reproduction. We previously developed a computational model of the fathead minnow hypothalamic pituitary gonadal (HPG) axis. In the present study, the reference aromatase inhibitor fadrozole (F) and 7 other chemicals with varying potencies as aromatase inhibitors were used with the HPG model to predict effects on plasma E2. The toxicity equivalent (TEQ) approach was used, with a concentration of 25 μg/L for each of the 7 chemicals converted into equivalent concentrations of F. Aromatase inhibition data from the ToxCast® database, using cell-based (Tox21 Aromatase Inhibition) and cell free (NVS ADME hCYP19A1) assays, were used. Letrozole and fadrozole, high-potency aromatase inhibitors, were predicted by the HPG axis model to depress plasma E2 by at least 70% at 25 μg/L. For the remaining 5 compounds,
which are less potent aromatase inhibitors, TEF calculations were inconsistent between the two assays. For example, plasma E2 was predicted to be reduced by 47% using the TEF for propiconazole based on the NYS assay but by 0.4% based on the TEF from the Tox21 assay. In laboratory experiments, fathead minnows will be exposed to the 8 compounds at 25 μg/L and 720 μg/L to evaluate the accuracy of the TEF approach for assessing the endocrine disrupting effects of aromatase inhibitors. Aromatase inhibition is a molecular initiating event (MIE) in the adverse outcome pathway leading to effects on reproduction. This work illustrates the potential for in vitro assessment of effects on the MIE with follow-on computational modeling to predict an in vivo adverse outcome. This is an abstract of a proposed presentation and does not necessarily reflect EPA policy. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

**724 A Quantitative Adverse Outcome Pathway Linking Aromatase Inhibition in Fathead Minnows with Population Dynamics**


An adverse outcome pathway (AOP) is a qualitative description linking a molecular initiating event (MIE) with measurable key events leading to an adverse outcome (AO). Given an established AOP, identification of a toxicant that participates in an MIE is analogous to hazard identification – the linkage to the adverse outcome is implicit, but the quantitative dose response and time course of the toxicant to AO relationship are not characterized. Development of quantitative AOPs (QAOP) is intended to provide this predictive capability. We linked three computational models that represent different levels of biological organization: the hypothalamic-pituitary-gonadal (HPG) axis; oocyte growth dynamics; and a population dynamics model. We simulated aromatase inhibition in fathead minnows exposed to 0, 2, 10, or 50 μg/L laddrozole, a model aromatase inhibitor with an HPG axis model to obtain predictions of plasma vitellogenin concentrations. The time course of predicted plasma vitellogenin concentrations was input into the oocyte growth dynamics model to predict clutch sizes and spawning intervals which were then input to the population dynamics model. Mild to severe reductions in fecundity and associated reductions in population size over an interval of 10 years were predicted, from 80% of normal at 2 μg/L to 0% at 10 μg/L and above. Linking the three models to construct a QAOP creates a structure that can be iteratively refined as new, relevant data are acquired. Thus, prediction accuracy can be expected to increase with time and to provide support for regulatory decision making. This is an abstract or a proposed presentation and does not necessarily reflect EPA policy. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

**725 Predicting Serum Thyroid Hormones Changes in the Bottle-Fed Infant Ingesting Dietary Iodine and the Environmental Contaminant Perchlorate**


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The first step in predicting chemically induced perturbations in serum thyroid hormones of an infant and the potential for subsequent neurodevelopmental toxicity is to gain a quantitative understanding of the maturation of the hypothalamic-pituitary-thyroid (HPT) axis. A biologically based HPT axis model was constructed to predict the temporal changes in serum thyroid hormones of an infant as a function of maturation and iodine intake from formula. The model was calibrated using literature derived data and fitting to serum data. A perchlorate sub-model for the bottle-fed infant was subsequently added to the HPT axis model to predict changes in free and total thyroid hormones in serum caused by competitive inhibition between iodide and perchlorate at the sodium iodide symporter protein in the basolateral membrane of the thyroid follicular cells. Preliminary simulations suggest that for a 3-month old bottle-fed infant, the first month of life was the most sensitive to decreases in iodine intake and exposure to perchlorate in the formula. The bottle-fed infant model will serve as the basis for a nursing infant model. The development of pharmacodynamic models for endocrine disruption, such as thyroid hormones, offers a transformative approach for regulatory science and safety assessments of chemicals. The views in this abstract are those of the authors and do not reflect views of the FDA, EPA, or ATSDR.

**726 A Computational Approach for a Quantitative and Mechanistic Understanding of Thiocyanate Kinetics and Dose Response**

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Thiocyanate (SCN-), a common contaminant found in food, water, and tobacco smoke, is a potent inhibitor of thyroidal uptake of iodide. Co-exposure to a mixture of thyroid-active environmental chemicals, such as perchlorate and nitrate, which share a common mode of action of uptake inhibition, could lead to depletion in iodide stores and thyroid hormone insufficiencies affecting sensitive sub-populations. Thiocyanate, unlike perchlorate, interacts with several intra-thyroidal mechanisms crucial for thyroid hormone production. The complex dose-response relationship of thiocyanate is not well characterized and is the focus of our current work. This study initially focused on the lack of proportionality in thyroid hormone perturbations as would be expected following thiocyanate-mediated competitive inhibition of iodide uptake alone. The relative inhibitory potency of thiocyanate is 15-fold lower than that of perchlorate. In comparison, the exposure based mean serum concentrations of thiocyanate is several hundred folds higher. We hypothesized that interplay between multiple mechanisms contributes to the apparent disproportionality, and sought to survey and use available data on SCN- across platforms, in vitro and in vivo, with modeling as a tool for hypothesis generation and testing. A minimalistic physiologically based pharmacokinetic model was developed for describing the intra-thyroidal and whole body disposition kinetics of radiolabeled iodide and SCN-, using rats as the animal model. An evaluation of observed data using model simulations helped discern the potential role of SCN- in altering thyroid follicle permeability to iodide increasing the thyroidal influx. Depending on the degree of perturbation, this could provide means to balance the demands in thyroidal iodide stores and hormone production in the occurrence of uptake inhibition by thiocyanate. Our ongoing efforts have extrapolation potential to humans for evaluating the non-additive cumulative risk from co-exposure to thyroid-active chemical mixtures.

**727 Using PBPK Modeling to Evaluate the Concurrent Effects of Perchlorate, Other Goitrogens, and Iodine on Thyroid Status**

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Perchlorate, a chemical found widely in drinking water and food, is currently the focus of regulation by environmental and food safety authorities. However, the basis for determining perchlorate exposure limits is often a simplistic single-chemical risk assessment that ignores the contributory effects of other environmental goitrogens and dietary iodide intake. Using a previously published PBPK model (Lumen et al., 2014) that predicts maternal and fetal responses to perchlorate and iodide exposure, we evaluated the impact of a variety of exposure scenarios for these chemicals and other goitrogens. In the case of foods that contain perchlorate, we also used data from the literature on iodide content and that of other potential goitrogens (nitrate, thiocyanate) to model exposure. Our intent was to use the results of our analysis to evaluate regulatory limits on perchlorate in context. Our analysis produced several pertinent results: (1) the model-predicted level of thyroid hormone perturbation at current regulatory drinking water limits is extremely small (typically less than 1%) and would be overwhelmed by other goitrogen exposures, e.g., from typical consumption of nitrate- and thiocyanate-containing produce; (2) only minimal iodide supplementation (approximately 2 μg/d) would be necessary to counteract exposure to perchlorate in drinking water, and (3) in almost all cases, there would be no impact from consuming produce containing perchlorate because produce iodide content would ameliorate any perturbation of thyroid status. Our analysis also indicated that while an individual consuming a vegan diet may experience slightly greater depression of thyroid hormones levels from perchlorate than a typical diet (again, less than 1% at current regulatory limits), vegetarian diets result in higher predicted thyroid hormone levels relative to typical diets for a given perchlorate exposure. In context, current regulatory limits for perchlorate in water and food are very conservative, and more than sufficient to protect sensitive populations.
A Multiscale Virtual Tissue Model of the Liver Lobule to Assess Zonal Heterogeneity in AhR Adverse Outcome Pathway-Induced Biological Responses

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Multi-scale spatial "virtual tissue" models provide a computational framework to link early initiating events in adverse outcome pathways (AOP), e.g., receptor activation or tissue reactivity, to tissue-level phenotypic outcomes, e.g., cell proliferation or stress pathway activation. We have developed a multicellular virtual tissue model of the liver lobule to predict biological responses to dioxin (TCDD)-induced aryl hydrocarbon receptor (AhR) activation. A decreasing linear gradient of Wnt signaling was applied across the lobule model from the centrilobular (CL) to the periporal (PP) regions. Ultrasensitive interactions reproduced sharply non-linear expression of APC and beta-catenin across the lobule, in accordance with experimental observations. Beta-catenin in turn induces preferential centrilobular expression of AhR and activation of downstream batteries of genes, including cytochromes 1A1, 1A2 and 1B1. Our multi-scale model recapitulated observed progression of gene induction patterns from CL to panlobular with increasing dose of TCDD. At higher doses of TCDD, we hypothesize that Wnt-beta-catenin signaling is strongly activated in precursor cell populations proximal to the PP end of the lobule, leading to induction of genes associated with proliferation and cell-cycling. A combination of published TCDD-induced gene expression data and genome-wide AhR location analysis was used to derive a transcriptional regulatory network underlying the AhR AOP, and examine differential network activation in CL and PP areas. A simplified version of this data-derived network was incorporated into each hepatocyte in the virtual lobule model to be consistent with preferential proliferative responses in the PP region. Overall, this multi-scale computational model was successful in reproducing experimentally observed dose-response behaviors for TCDD-induced AhR activation and could support modeling of key events, i.e., proliferation, in the AhR AOP.

Agent-Based Computational Modeling of Cell Culture: Variation in Dose with Degree of Confluence


Variability of in vitro data can reflect, in part, cell-to-cell variability in dose absorbed from the culture medium. We developed a 3-dimensional computational model of cells growing in a culture dish using the stochastic, agent-based software CompuCell3D. The model was configured so that, when plated at low density, individual cells adhered to the culture dish and assumed a "fried-egg" shape. Cells were allowed to divide, forming clones that gradually coalesced to full confluency. During this process, individual cells transitioned from the fried egg shape to more cuboidal forms. Attendant was a corresponding change in the surface area that cells presented to the overlying medium. This surface area is a determinant of the dose to toxicant received over any given time interval. We calibrated the model with data from the human bronchial epithelial cell line (BEAS-2B). With initial plating at about 4% confluency and growth until fully confluent, the average surface area that cells present to the overlying medium decreased 20-30%. These model-predicted changes in surface area were then incorporated into a separate, differential equation-based computational model describing the degree of oxidative stress in BEAS-2B cells exposed to H2O2 in vitro. The resulting simulations showed that changes in surface area are associated with corresponding changes in the oxidative stress response. This result suggests that a quantitative understanding of cellular morphology, with attendant computational modeling, can provide insight into the role of dosimetry as a source of variability in in vitro toxicity assays. This is an abstract or a proposed presentation and does not necessarily reflect EPA policy. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

How Much Are the Cells Exposed to Nanoparticles versus Ions after Exposure to Silver Nanoparticles in Vitro?

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Silver (Ag) nanoparticles (NPs) have been used in a wide variety of consumer products for antimicrobial effects. The effects are likely due to released Ag ions, which may also be responsible for potential toxic effects of Ag NPs. However, only limited information is available on the fate of Ag NPs or release of Ag ions in the cells. We developed an in vitro kinetic model to describe disposition of Ag, both in NP and ions, in an in vitro assay system. The model was structured and parameterized using data from in vitro kinetic experiments conducted with various cell types from mouse macrophages (RAW264.7), rat lung epithelial cells (RLE) and rat aortic endothelial cells (RAEC) using Ag NPs of varying physicochemical properties including two different sizes (20 and 110 nm) and two different coating materials (citrate and polyvinylpyrrolidone). The model described behaviors of NP in the media including dissolution as well as cellular kinetics including cellular uptake of NP and ions, intracellular dissolution of NPs, intracellular trafficking of NPs and ions, oxidation and sulfidation of ions, and clearance of NP and/or ions from cells. The model was able to simulate cell type-specific uptake (RAEC>RLE>RLE) and clearance of Ag (RAEC>RLE>RWE). The parameters in the model, which were estimated for each of the specific Ag NP properties (e.g., size and coating), experimental conditions (medium composition), and different cell types, provided useful insights into the key properties that determine the potential activity of different Ag NPs as a function of the experimental situations. Our model will contribute to extrapolating in vitro effects data to predict in vivo effects using the cellular exposure to the active form(s) of Ag from Ag NP exposure as the basis of extrapolation (NIOSH Award U19ES019752).

Utilizing a Tetraphasic-Logistic Growth Model to Assess Developmental and Reproductive Effects in F344/N Rats Exposed Two Years to Butyl Benzyl Phthalate


Butyl Benzyl Phthalate (BBP) is a plasticizer used to impart flexibility and durability in plastics such as polyvinyl chloride. Growth data from a 1997 National Toxicology Program (NTP) chronic bioassay in F344/N rats was utilized to retrospectively determine whether exposure to BBP leads to dose-related changes in growth rates and age at peak growth velocity (APVG), in addition to the observed reductions in mean body weights at high doses. NTP exposed male and female rats to 0, 3,000, 6,000, and 12,000 ppm or 0, 6,000, 12,000, or 24, 000 ppm BBP in the diet for two years. A tetraphasic logistic growth model was fitted to mean body weight growth curves, assuming that during a two-year study period the animals undergo four major phases of growth. Specifically, a peri-pubertal phase (APGV occurs during the period from PND 30-55), a post-pubertal phase (APGV occurs during the period from PND 56-200), an early senescence phase (APGV occurs during the period from PND 200-500), and a late-senescence phase (APGV occurs during the period from PND 501-1000). The results of the analysis showed an increased growth rate in peri-pubertal and post-pubertal male rats compared to controls, while the rates of the early- and late-senescence phases were not significantly changed. In females, the peri-pubertal and post-pubertal growth rates were significantly increased at the highest dose, while the rates of the early and late senescence phases were significantly decreased. Additionally, with the exception of the peri-pubertal phase, BBP advanced the timing of the APGV of the other three phases in males and females. These results suggest that two year exposure to BBP may cause both developmental and reproductive effects in rats. The views expressed are those of the authors and do not necessarily reflect the views or policies of the U.S. Environmental Protection Agency.
Acetaminophen (paracetamol, APAP) is one of the most widely used analgesic and antipyretics in the world. It is a well-known hepatotoxicant and, owing to its ubiquitous usage, is the principal cause of acute liver failure in both the United States and the United Kingdom. APAP is metabolized primarily by sulfation and glucuronidation. However, it can also be oxidized by CYP isozymes to form the active metabolite, and putative mediator of toxicity, N-acetyl-p-benzoquinonimine (NAPQI). Current predictions of APAP pharmacokinetics are based on models developed using limited experimental data in humans, especially for doses encountered following self-harm ingestion (> 7000 mg or > 90 mg/kg of APAP over 24 hours). In addition, although clinical treatment for APAP overdose often requires the emergency care worker to estimate the administered dose to prescribe an ameliorative therapy, no computational tools are available to aid in this assessment. To fill this gap, we developed a PBPK model for APAP, derived from, and validated by, multiple datasets at widely varying doses, and through the use of Bayesian inference, applied this model to predict administered APAP dose under various clinically-relevant scenarios. Here, we detail the structure and formulation of the PBPK model, demonstrate the ability of the model to accurately predict APAP pharmacokinetics within therapeutic and overdose scenarios, and illustrate the effects of plasma sampling time on the uncertainty of the computed administered dose. We expect that in the future, this framework and methodology will help to inform the clinical assessment of APAP overdose patients.

**734 Understanding the Kinetics of Amiodarone and Its Metabolite Desethylamiodarone in Rats Using a Physiologically Based Pharmacokinetic Model**

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Amiodarone (AMD) is an effective antiarrhythmic for atrial fibrillation, but it has been associated with adverse effects in multiple tissues. Both the parent compound and its major metabolite desethylamiodarone (DEA) share similar pharmacological and toxicological properties. The present study developed a physiologically-based pharmacokinetic (PBPK) model for AMD and DEA in rats to predict tissue dosimetry and provide insight into their adverse effects. Pharmacokinetic data published in 10 intravenous studies were pooled for both model construction and evaluation. Metabolic parameters were obtained and extrapolated based on in vitro measurements. This model consisted of 11 tissue compartments, Heart and blood are the therapeutic targets, liver, kidney, skin, thyroid, and brain are the toxicological targets. Validation was performed in 15 different dosing regimens, including single bonus dosing (10 to 200 mg/kg), repeated bonus dosing (4x daily) and single slow infusion (0.257 to 10 hour). Model simulations were in good agreement with the observed time courses of the drug-metabolite pair in tissues. The linear regression for observed data versus corresponding simulation showed a slope of 1.106, cut-off of 0.018 and R2 of 0.921. The key pharmacokinetic properties of AMD, such as extensive tissue distribution, substantial storage in fat, and long half-lives in several tissues were appropriately reflected in the simulations. The PBPK model provides a quantitative understanding of the dose-response relationship of AMD and DEA in rats. This model also provided a good basis for extrapolation to humans, allowing one to gain insight into adverse AMD and DEA effects in humans. This abstract has been cleared by the EPA but solely expresses the view of the authors.

**735 Assessment of Nicotine Uptake and Particle Deposition following a Single-Puff Inhalation of Cigarette Smoke**


The doses and sites of uptake for constituents of smoke in the lung following inhalation of a cigarette puff are needed to assess the health effects associated with smoking. Following generation, constituents of smoke are dispersed into aerosols. However, medium and high vapor constituents evaporate rapidly when mixed with dilution air. Model predictions give faster release of nicotine than observed experimentally. The time difference is attributed to partition of the particle constituents in the cigarette particles. Protonation limits the amount of a constituent available for phase change and depends on particle temperature, relative humidity, and particle acidity. Models of particle evaporation are needed to predict local and regional deposition of cigarette particles and their components in the respiratory tract. Uptake predictions of a bolus of cigarette smoke traveling through a denuder was matched with reported uptake measurements by selecting an adjustment factor in the particle size change model. The particle size change model was then used in the deposition model for cigarette particles to predict particle deposition and nicotine uptake fraction in the lung. The degree of protonation was found to depend on particle temperature. Model calculations gave similar predictions for particle deposition between the new and existing models. However, site of deposition for nicotine and other components did not necessarily coincide with that of particle deposition. While existing models predicted nicotine uptake to occur in the first few upper airways of the lung, the improved model predicted nicotine vapor to be released from particles and travel deep into the lung to be taken up by the airway walls in the alveolar region where convective flow has subsided and there is ample time for vapor diffusion to the walls. This study was funded by British American Tobacco.

**736 Evaluation of Oral, Inhalation, and Dermal Exposure to Tebuthiuron in Rats by the Application of Predictive Pharmacokinetic Modeling Tools**

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The use of QSAR and pharmacokinetic modeling may reduce the need for further animal testing by enabling route-to-route extrapolation of oral toxicity data to other exposure scenarios. To this end, the fractions of Tebuthiuron, a pesticide, absorbed following oral and inhalation exposure were predicted, using the software GastroPlus (v8.5, Simulations Plus Inc, Lancaster, CA), to be 99.9% for oral exposure, 97.2% for acute inhalation exposure, and 72.3% for subchronic inhalation exposure. However, the amount of Tebuthiuron predicted to be absorbed in the lung was very low, 6.4% and 9.0% for single and subchronic inhalation exposures, respectively. The remainder of the absorbed dose from inhalation exposure (90.8% and 63.3%, respectively) is predicted to be transferred to the GI tract via swallowing and absorbed. Therefore, absorption by the oral and inhalation routes can be considered similar. A large portion of the inhaled dose is predicted to be absorbed by the GI tract, incorporating oral dose first-pass metabolism, and therefore can be considered to have been well evaluated with existing oral toxicity data, negating the need for additional animal studies. An assessment of Tebuthiuron dermal absorption was made utilizing another program, Finite Dose Skin Permeation Calculator (NISOSH/CDC) and was predicted to be only 5.5% in humans. Therefore, absorption by the dermal route is predicted to be much lower
Acrolein and diacetyl, highly water soluble irritant gases, are absorbed predominantly in the upper respiratory tract and rapidly metabolized in the respiratory epithelium. Acetaldehyde, a component of butter flavoring, is more water soluble than acrolein and diacetyl, but more resistant to mass transfer into the epithelial tissue. Studies of diacetyl inhalation in rodents demonstrate that epithelial tissue damage occurs predominantly in the upper respiratory tract. To determine whether mouth-breathing and light exercise conditions affect relative bronchiolar doses of diacetyl, acrolein, and acetaldehyde in humans, we applied a computational fluid dynamic-physiologically based pharmacokinetic model under these conditions. At inhaled concentrations of 1 ppm, comparable low bronchiolar tissue concentrations (<0.01 ppm) were found for diacetyl and acrolein, but the predicted tissue concentrations of acetaldehyde were nearly 4-orders of magnitude higher due to its much slower metabolism and resistance to mass transfer in the tissue, leading to more distal absorption in the respiratory tract. Occupational exposure limits for 8-h exposures to acetaldehyde (200 ppm) and acrolein (0.1 ppm) are roughly proportional to sensory irritation potencies in rodents (RD50 ~3000 ppm and 200 ppm, respectively), but they do not account for the greater bronchiolar accumulation predicted for acetaldehyde. Diacetyl has an intermediate sensory irritant potency (RD50 ~900 ppm) between acetaldehyde and acrolein and a low predicted bronchiolar concentration consistent with acrolein, yet proposed OELs for diacetyl are much lower (e.g., 0.005-0.06 ppm). Given these irritant potencies and our prediction of bronchiolar doses for these compounds, acetaldehyde may present a greater risk of bronchiolar tissue damage compared to acrolein and diacetyl under conditions of mouth breathing with light exercise.

## In Vivo Efﬁcacies of Hexavalent Chromium Reduction in the Gastric Environments of Mice, Rats, and Humans

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Hexavalent chromium (Cr(VI)) is a known human carcinogen via inhalation, but less is known about human risks via ingestion. Increased incidences of neoplasms in the oral cavity of rats and in the small intestine of mice have been observed in long-term drinking water bioassays (NTP, 2008). When ingested, Cr(VI) can be reduced to trivalent chromium (Cr(III)) within the gastrointestinal (GI) tract. Cr(III) is thought to pose little or no carcinogenic risk. Interspecies differences in GI tract physiology are known to significantly impact the reduction (and thus the detoxification) of ingested Cr(VI). Therefore, an understanding of GI tract reduction is important in evaluating the NTP cancer findings in the context of human health risk assessment. Several *ex vivo* models describing reduction of Cr(VI) in gastric juice were evaluated by simulating *in vitro* stomach conditions for mice, rats, and humans. The models were tested under a variety of dietary or physiologic assumptions, including frequency of exposure, fed or fasted status, and gastric pH. These models indicate that reduction capacity is not fully saturated or depleted in the rat or mouse stomach within the range of doses examined by NTP (2008). While mice exhibit less efficient Cr(VI) stomach reduction than rats, this is primarily due to physiological factors and not due to loss of the reducing agent. At low concentrations, humans are estimated to reduce Cr(VI) more efficiently in the stomach than rodents due to lower pH and slower gastric emptying. Variations in assumptions related to GI physiology impact the estimated reduction efficiency more significantly than changes in the reduction model. Therefore, characterization of GI parameters in rodents and humans is important for cross-species extrapolation. The views expressed are those of the authors, and do not necessarily represent the views or policies of the U.S. EPA.

## Evaluating Epidemiological Associations between High Blood Concentrations of Poly Brominated Diphenyl Ether (PBDEs) and Altered Timing of Menarche: Are They Meaningful?

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Reported associations between blood PBDEs and altered time of menarche in the general population has raised concerns for potential health effects of PBDE exposure in early ages. However, individual differences in growth patterns and the resulting body types are often associated with their timing of puberty, which can also affect the chemical disposition substantially. In this study we evaluated the impact of kinetic variability during puberty on the reported epidemiological association of BDE-47, one of the most abundant PBDE congeners, with earlier menarche. A probabilistic PBPK modeling approach was used to simulate a hypothetical population that resembles that of NHANES. The simulated average blood concentrations of BDE-47 and its distribution were comparable to those in NHANES and the simulated population was matched to the study population in terms of growth physiology and markers of pubertal events. Model sensitivity analysis revealed that variability of individual exposure as a function of age and body weight and natural correlations between physiological body type and an individual’s lipid metabolism status are the two most influential factors affecting the strength and direction of blood BDE-47 associations with age at menarche; these two factors were also identified as the major data gaps in the current analysis. Our study demonstrates that a Monte Carlo PBPK modeling approach can provide a tool to critically assess the role of pharmacokinetic variation in chemical-health effect associations in epidemiological studies (supported by the ACC-LRI).
reduction period. Thereafter, a dual-spike approach was used to help characterize the concentration-dependence of the 2nd order reduction rate constants: 1) spike 1 was added at the start of the incubation using low CrVI concentrations (0.04-0.3 mg/L); 2) after 30 minutes, spike 2 was added to provide a characterization of higher concentrations (1-3 mg/L), and the reaction was allowed to continue for a total of 150 minutes. To improve our understanding of CrVI reduction at higher pH levels, additional data were collected for gastric contents from proton-pump inhibitor users (3 individual samples, fasted, pH 5.7-5.5) and by artificially increasing pH from one fed individual (pH=2 adjusted to pH 4.7 and 5.8). Time-course data were analyzed using specialized isotope dilution mass spectrometry (SIDMS) and 2-pool model (pool 1 =fast rate with low capacity; pool 2 = slower rate with higher capacity). These data support conclusions that: 1) human gastric samples, like those collected from rats and mice, contain multiple pools of reducing agents; and 2) long concentrations of CrVI are reduced more rapidly than at high concentrations of CrVI. With the combined data set, several pH-dependent forms of the model were considered and will be discussed.

**742 Comparison of Phthalate Biomonitoring and High-Throughput Screening Data Using Pharmacokinetic Modeling**
M. Moreau and A. Nong, Environmental Health Sciences and Research Bureau, Health Canada, Ottawa, ON, Canada. Advances in toxicity testing approaches have brought new ways to measure for a large numbers of biological endpoints. The leading challenge is now interpreting these results in terms that are applicable for dose exposure relevant in human or animal. High throughput screening (HTS) of chemicals is a rapid method to help prioritize research targets for health evaluation. This study integrates pharmacokinetic models to estimate these HTS biological responses into blood or urine levels of phthalates. These results can then be compared and related to population biomonitoring surveys. Screening data was collected from the US EPA ToxCast™ database. Phthalates with available metabolite data were selected: mono-2(ethylhexyl) phthalate (MEHP), mono-n-butyphthalate (MnP), mono-benzyl phthalate (MnBP) and mono-methyl phthalate (MMP). A steady-state pharmacokinetic relationship was used to extrapolate the adverse concentration (AC50) from a series of assays used for screening. This relationship was then used to estimate urine concentrations of their respective major mono-ester metabolites in urine. The AC50 ranged from 5.22-62.5 μM, 1.48-53.9 μM and 5.12-34.9 μM for MEHP, MnBP, and MMP respectively. MnBP had no significant response. Pharmacokinetic parameters were obtained from chemical structure-properties equations. Once extrapolated as urine concentrations, the AC50 assay results overlapped the range of different Biomonitoring Equivalents based on animal point-of-departure. These urine concentrations ranged from 202-1930 μg/L for MEHP, 1600-2550 μg/L for MnBP and 878-2210 μg/L for MMP. The in-vitro and in-vivo generated screening values were greater than the population urine levels from the Canadian Health Measures Survey (CHMS); thus, indicating that levels of these phthalates are below guidelines values. This research demonstrates the use of a scientific tool that can help interpret and integrate different types of biological data, such as high throughput toxicity screen and biomonitoring surveys, to provide necessary weight-of-evidence for the assessment.

**743 A Mechanism-Based Translational Mathematical Model for Gastrointestinal Toxicity**
H. Shankaran1, P. Jasper1, M. Wagena1, T. Toloma2 and J. Mettee1, 1Drug Safety and Metabolism, AstaZeneca, Wellesley, MA and 2RES Group Inc, Needham, MA. The gastrointestinal (GI) epithelium undergoes rapid cell proliferation and self-renewal, and is hence a frequent target for chemotherapy-induced toxicity. The ability to quantitatively predict the extent of GI damage and the kinetics of recovery in humans would enable the adjustment of doses and schedules for chemotherapeutic agents. Towards this end we have built a mechanism-based model of GI toxicity with the objective of quantitatively translating preclinical observations to man. The ordinary differential equation-based model includes the proliferating stem, and transit amplifying cells in the intestinal crypt, the differentiated enterocytes in the villi, and citrulline, a validated plasma biomarker for GI damage. In the model, stem cells divide asymmetrically to generate transit amplifying cells, which undergo multiple rounds of cell division before differentiating into enterocytes. Enterocytes then pass through a series of transit compartments before being shed from the tips of the villi. The citrulline submodel includes synthesis that is proportional to the enterocyte mass, and the first order elimination of this amino acid. Predictions for steady-state cell numbers and enterocyte shedding fluxes were in agreement with additional literature data. A key aspect captured by the model is the slower cell division and transit kinetics in humans that is predicted to contribute to slower recovery in humans following damage to the proliferating cells. Importantly, model predictions for citrulline kinetics in humans were in agreement with data from several studies involving myeloablative treatments that induce significant GI toxicity. In addition to enabling quantitative translation, the model can be used to understand the mechanisms of GI toxicity, and to link biomarker dynamics to intestinal histopathology.

**744 INTEGRA: Advancing Risk Assessment Using Internal Dosimetry Metrics**
D. A. Sarigiannidou1, S. P. Karakitsios2, A. Gorti1, V. Handakas1 and K. Papadaki1, 1Chemical Engineering, Aristotle University of Thessaloniki, Thessaloniki, Greece and 2Chemical Process and Energy Resources Institute, Centre for Research and Technology Hellas, Thess., Greece. The objective of the INTEGRA project is to bring together all information necessary for assessing the source-to-dose exposure continuum over the entire life cycle of substances covering an extensive chemical space through the use of QSARs. The major outcome of INTEGRA is a computational platform that integrates multimedia environmental and micro-environmental fate, (external) exposure and internal dose within a dynamic framework in time. Coupling seamlessly exposure models with refined computational tools for internal dosimetry transforms exposure/risk assessment of environmental chemicals since it allows risk characterization to be based on internal dosimetry metrics. In this way high throughput system data such as the ones generated by Tox21 in vitro testing can be used, towards the nowadays need of exposure based risk assessment. This opens the way towards a higher level of assessment that incorporates reduced exposure (tissue dosimetry) and toxicity testing (Biological Pathway Altering Dose – BPAD). The applicability of INTEGRA was tested on bisphenol-A. Several exposure scenarios were investigated, incorporating data from external exposure assessment (food residues, food consumption patterns), as well as from human biomonitoring data using exposure reconstruction algorithms. Internal exposure metrics were used to evaluate the associated risk, based i) by translating into equivalent tissue dose the Tolerable Daily Intake of 50μg/kg bw/d using the ToxCast 21 related assays. The assessment indicated that specific exposure scenarios (i.e. bottle fed neonates and premature infants hosted in intensive care units) are close to the legislative thresholds. The refined analysis, using BPAD as an internal exposure risk characterization metric, resulted in increased margins of safety compared to conventional exposure/risk characterization.

**745 Predicting Oral Bioavailability of Parabens Using Biokinetic Absorption Model Together with a New In Vitro Intestinal Absorption Model of Carboxylesterase-2 Expressing Caco-2 Cells**
M. Yoon, X. Zhao, X. Sun, J. Dong and H. J. Clewell, The Hamner Institutes for Health Sciences, Research Triangle Park, NC. Oral bioavailability and hence potential toxicity of environmental esters such as parabens can be greatly affected by intestinal hydrolysis. Conventional Caco-2 cells are not a good model for presystemic hydrolysis due to the striking difference in carboxylesterase (CES) expression patterns: Caco-2 cells primarily express CES-1, whereas human enterocytes predominantly express CES-2. In this study, we stably expressed CES-2 in Caco-2 cells to a level comparable to human enterocytes, as a first step to develop an appropriate in vitro tool to evaluate the impact of hydrolysis on the absorption of parabens. Studies on transport and metabolism of methyl- and butylparabens performed with the Caco-2/CES-2 cells showed that butylparaben is rapidly hydrolyzed to p-hydroxybenzoic acid (pHBA) while being absorbed across the Caco-2/CES-2 monolayer, while the hydrolysis of methylparaben was slower than that of butylparaben, as expected from substrate specificity of CES-2. After hydrolysis, pHBA was mainly found in the apical side (i.e., luminal side of gut) indicating that hydrolysis occurs at or closer to the apical membrane. Our results indicated that the apparent permeability coefficient (Papp) measured with conventional Caco-2 would have incorrectly estimated oral bioavailability of both longer and shorter alkyl-chain parabens due to lack of CES-2 mediated hydrolysis and presence of CES-1 mediated hydrolysis, respectively. We developed an in vitro biokinetic model that describes the kinetic behavior of different alkyl-chain parabens and pHBA in Caco-2/CES-2. The model estimated permeability and hydrolysis parameters were extrapolated to humans and were in agreement with in vivo and in vitro results. The model accurately predicted oral bioavailability of parent parabens and their metabolite, pHBA, under different exposure conditions, including mixtures of methyl- and butylparabens, indicating the value of our work for assessing human exposures to parabens (supported by ACC-LRI).
746 Development of a Physiologically Based Pharmacokinetic Model for Bisphenol A in Humans to Address the Uncertainties Surrounding the Risk Assessment of BPA

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A previously developed physiologically based pharmacokinetic (PBPK) model for bisphenol A (BPA) in monkeys was extended to humans based upon clinical studies with deuterated BPA (d6-BPA), where serum concentration profiles and urinary excretion data for d6-BPA and its phase II conjugates were collected in adult humans after oral bolus dosing with d6-BPA. Hepatic and gastrointestinal phase II metabolic constants for d6-BPA were derived from published in vitro metabolism studies with BPA using the in vitro-in vivo extrapolation (IVIVE) approach. In order to describe the slowed systemic clearance of d6-BPA glucuronide, it was necessary to assign approximately ten percent of d6-BPA glucuronide in the liver to be excreted into the bile and undergo enterohepatic recirculation. This PBPK model was adequately simulated newly reported serum concentrations of unconjugated d6-BPA and its phase II conjugates after oral administration of d6-BPA. The current model will more confidently predict serum concentrations of BPA based on either dietary intake assessments or urinary spot measurements of total excreted BPA, thus helping the interpretation of human biomonitoring data and providing guidance on the extrapolation of experimental animal toxicity findings to humans.

747 Quantitative In Vitro to In Vivo Extrapolation for Environmental Esters: PBPK Model for Methyl-, Propyl-, and Butylparaben in Rat and Human


Parabens are widely used in consumer products including cosmetics, foods and pharmaceuticals. Recent evidence indicates that parabens may be endocrine active at high doses in rodents. In order to understand the potential risk to humans from exposure, a rat and human PBPK model for methyl-, propyl-, and butylparaben was developed. The model parameterized through a combination of Qsar for tissue solubility and quantitative in vitro to in vivo extrapolation (QIVIVE) for metabolism in portals of entry including intestine and skin as well as in the primary site of metabolism, the liver. Overall, the model provided very good agreement with published time-course data in blood and urine from controlled dosing studies in rat and human, and demonstrates the potential value of QIVIVE in expanding the incorporation of human biomonitoring data in risk assessment. An in vitro based cumulative margin of safety (MOS) was calculated by comparing the effective concentrations from an in vitro assay of estrogenicity to the free paraben concentrations predicted by the model to be associated with the 95th percentile urine concentrations reported in NHANES IV. The calculated cumulative MOS for the parabens was approximately 100 for the adult female and over 400 for the adult male.

748 Development of a Toxicokinetic Model for the Insensitive Munitions (IMX) Component 2, 4-Dintronisoilne

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The Armed Forces have an interest in developing new explosives that are less susceptible to unintentional detonation (insensitive munitions—IMX). It is important that toxicological impacts of new munitions be assessed in a proactive manner to minimize risks to military personnel and the community. 2,4-Dintronisoilne (DNAN) is a component of IMX formulations. Toxicokinetic data for DNAN are required to extend the understanding of how DNAN is distributed, metabolized, and eliminated in rats and provide insights into the possible fate of DNAN in exposed humans. Male Sprague-Dawley rats were exposed to DNAN by gavage (5, 20, or 80 mg/kg in corn oil) and blood and tissues were collected from 0.5 to 24 hrs post dosing. Blood and tissue samples were analyzed to determine levels of DNAN and its metabolite 2,4-dinitrophenol (DNP). These data and data from a previous study of DNAN in rats and macaques were used for calibration and validation of a preliminary physiologically based pharmacokinetic (PBPK) model. On average, the model simulations were within less than a factor of 2 of the experimental data. The model simulations indicated saturable metabolism of DNAN in rats accompanied by prolonged DNP clearance at higher doses. Macaques appeared to have elevated levels of DNP relative to rats receiving similar doses of DNAN. The PBPK model was extrapolated to estimate the toxicokinetics of DNAN and DNP in humans, allowing the estimation of human-equivalent no-effect levels of DNAN exposure from no-observed adverse effect levels determined in laboratory animals, which may guide the selection of exposure limits for DNAN.

749 PBPK Modeling Describes Route-Specific Kinetics of Cyclic Volatile Methyl Siloxanes

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The cyclic volatile methyl siloxanes (cVMS), octamethylcyclotetrasiloxane (D4) and decamethylcyclopentasiloxane (D5), may be present in a variety of consumer products. The aim of this project was to incorporate the oral route of exposure into an existing physiologically based pharmacokinetic (PBPK) model of inhalation and dermal exposure to cVMS in the rat and human. The oral component of this model was built using kinetic data on D4, D5 and total metabolites following oral gavage of 30mg D4/kg body weight in liquid rodent diet vehicle. Oral uptake of cVMS was initially described with a simple, first-order oral uptake compartment while all other model compartments and parameters were fixed at the previous values used to describe inhalation and dermal exposures. This description under-predicted plasma concentration of D4 and D5 and over-predicted the rate of exhalation of these materials, indicating that cVMS in the plasma were not freely available for exhalation following oral exposure compared to their distribution following inhalation and dermal exposures. The existing model descriptions of the kinetic behavior of cVMS following inhalation and dermal exposure required a sequestered pool of cVMS that was associated with endogenous blood lipids available for distribution in the blood but not available for exhalation. Consistent with this behavior of cVMS, the oral absorption model was modified to include two pathways of cVMS absorption, direct absorption of freely available dietary cVMS and cVMS that is bound to the dietary lipids and absorbed via the lymphatic system. The kinetic data on the early phase distribution to tissue were better described with this updated and physiologically relevant model construct. The inclusion of an oral route of exposure to this existing model offers a single, unified PBPK model to describe the dose-response behavior of cVMS materials for use in various risk assessment applications.

750 Application of Local and Global Sensitivity Analysis to a Human Multiroute PBPK Model for Bromodichloromethane


Due to its skin permeability and presence in water as a volatile disinfection byproduct, BDCM exposure via multiple routes contributes significantly to internal dose. Mechanistic data suggest target tissue metabolism may be important for BDCM-induced carcinogenicity. We refined our multi-route PBPK model for BDCM such that all data used for parameter estimation were derived from studies using human cells and tissues. Compartments in the model were skin, liver, gut, fat, kidney, and slowly and rapidly cleared tissue groups. Metabolism occurred in the liver via two pathways (cytochrome P450, glutathione S-transferase). The model adequately predicted data from the published literature for oral, dermal and showering/bathing exposures. Because variation in influential parameters such as metabolic rate constants could be an important determinant for toxicologically-relevant dose metrics, we chose to employ global sensitivity analysis (GSA) in addition to local sensitivity analysis (LSA). A screening level GSA method (Morris) was used to prioritize parameters for the more computationally intensive extended Fourier Amplitude method (eFAST). Dose metrics (responses) evaluated were BDCM blood concentration (CV) and its area under the curve (AUC), exhaled breath, and amount metabolized in liver per hour (AML) for both oral and showering exposures (10 ppb BDCM in water). While both LSA and Morris GSA methods tended to identify the same sets of parameters as being influential, the Morris GSA method consistently ranked the chemical-specific parameters as being most influential compared to LSA. This is most likely because GSA accounts for parameter variability whereas LSA does not and known variation for metabolic rate and oral absorption constants are typically larger than those for most physiological parameters. Our results illustrate the value of global sensitivity analysis to more thoroughly characterize the influence of parameter uncertainty and variability on toxicologically-relevant dose metrics. (This abstract does not reflect Agency policy).
Pharmacokinetics of ethanol and propylene glycol (PG) following infusion of a new formulation of the anticancer drug, docetaxel, were predicted to evaluate the safety of the formulation and address questions from the US Food and Drug Administration (USFDA). Docetaxel is given to patients as a one hour infusion followed by a three week interval prior to a second dose. Plasma concentrations were predicted using standard one compartment equations. Published literature provided human plasma concentration data in three studies for ethanol infusion pharmacokinetics and in one study for PG. Metabolic clearance of both is predominately through alcohol dehydrogenase. Propylene glycol has 25–45% renal clearance; for ethanol it is minimal. Variations in clearances were evaluated using literature data. The 20-fold lower Km reported for ethanol compared to PG indicated there could be inhibition of PG metabolism by ethanol, so this was evaluated semi-quantitatively. End of infusion concentrations for ethanol (6.4 g total dose) were predicted to be approximately 15 mg/dL with less than 15% variation from the mean assuming half lives from 1–4 hr. End of infusion concentrations for PG (7.5 g total) were predicted to vary between 10 and 14 mg/dL under different assumptions. Peak concentrations predicted at end of infusion do not vary substantially because as clearance gets slower, the infusion duration represents a smaller fraction of the steady state concentration. The plasma values are well below those associated with intoxication or other toxicity endpoints. This analysis was taken into consideration by the USFDA when they approved the formulation.

Physiologically Based Pharmacokinetic Modeling for 1-Bromopropane in Rat Using Gas Uptake Inhalation Studies

1-Bromopropane (1-BP) was introduced into the workplace as an ozone depleting alternative solvent (ODA). The potential for human exposure to 1-BP and the reports of adverse effects associated with occupational exposure to high levels of 1-BP have increased the need to understand the mechanism of these adverse effects in animal models as a means of understanding human risk in workers. The objectives of the current studies were to develop a physiologically based pharmacokinetic (PBPK) model for 1-BP. The development of the PBPK model for the 1-BP was accomplished by simulating the closed chamber concentration data from the gas-uptake experiments in F-344 rats. We tested different metabolic hypotheses, including two-pathway hypothesis in which metabolism responsibilities are shared by the p450 CYP2E1 and glutathione transferase pathways (GST). The results showed that two metabolic pathways hypotheses generated the best predicted values for the actual chamber concentrations. Furthermore, the above model was tested by simulating the gas-uptake data of rats with pre-treated with 1-amino-benzotriazole (ABT), a potent general P450 suicide inhibitor, or DL-buthionine (S,R)-sulfoximine (BSO), an inhibitor of glutathione (GSH) synthesis, prior to exposure to 1-BP. The comparative investigation on metabolic pathway of 1-BP through the PBPK modeling in both gender provided critical information in understanding the role of p450 and GST pathways and eventually help to quantitatively extrapolate these animal studies to human (Supported by R21 OH010473).

In Silico Modeling Can Predict the Unforeseen Renal Failure Caused by SGX523, a c-MET Kinase Inhibitor

SGX523 is a quinoline-containing molecule that was a promising c-MET kinase inhibitor with an IC50 of 4 nM and >1,000-fold selectivity over other protein kinases. It is orally bioavailable and inhibited the growth of human glioblastoma lung and gastric cancer xenografts in mice. However, in a phase 1 clinical trial, the six patients that received ≤80 mg daily doses all developed renal failure as confirmed by a rise in serum creatinine and blood urea nitrogen. Hydration therapy returned these levels to baseline after 1 to 4 weeks. Follow-up studies revealed that the cause of renal toxicity was drug-induced nephropathy due to a metabolite of SGX523 that was not detected in preclinical studies. Aldehyde oxidase transforms the quinoline ring into a quinolinone. Generation of the quinolinone metabolite is species-dependent; it is formed in human and monkey liver S-9 but not in dog S-9 treatments. Here, we demonstrate the use of predicted physicochemical and biochemical properties to predict the toxicokinetics of SGX523 and its oxidized quinolinone metabolite. These predicted properties were then used in a mechanistic oral absorption and physiologically based pharmacokinetic (PBPK) simulation of the plasma and renal concentrations versus time. Oxidative metabolism results in conversion of the basic quinoline (predicted pKa of 4.2) group in SGX523 to an acidic lactam ring with a predicted pKa of 11.0. This also results in decreased solubility; the predicted aqueous solubility drops from 2.4 µg/mL in SGX523 to 0.56 µg/mL in its quinolinone metabolite. Our PBPK simulations show high concentrations of the quinolinone metabolite in the kidney; beyond its solubility, creating the probability of precipitation. Thus, our in silico analysis predicts the observed renal toxicity in humans and monkeys due to crystallization of the metabolite in the kidney.

Application of the All-Ages Lead Model to Improve Evaluation of Variable Exposures

Childhood lead (Pb) exposure is associated with neurological consequences. Removal of Pb from gasoline and paint and exposure mitigation education has resulted in a drop in blood Pb levels (BLLs) in the US. However, approximately half a million US children have BLLs ≥5 µg/dL, the level at which CDC recommends public health action be initiated. A better understanding of intermittent Pb exposures is needed to improve mitigation recommendations. The EPA is developing an All-Ages Lead Model (AALM) that implements exposure model averaging times as short as one day and allows for prediction of BLLs arising from variable exposure frequencies and durations. As a part of an interagency test group, we explored the utility of a beta version of the AALM to assess the impact of exposure frequency on BLL, and evaluate exposures that might occur during daycare or for a child exhibiting pica behavior. We simulated exposure from birth to age 7, varying exposure frequency (1–7 days/week) and soil Pb concentration. Results indicated that exposures to soil Pb concentrations of 100–200 ppm did not produce BLLs ≥5 µg/dL; regardless of exposure frequency. Exposures to soil Pb concentrations of 200 ppm ≥5 days per week resulted in a ≥5% probability of exceeding 5 µg/dL. Soil Pb one study ≥300 ppm resulted in predicted BLLs with a ≥5% probability of exceeding 5 µg/dL if exposures occurred only one or two days per week. Model simulations of exposures 5 days/week at daycare from birth to age 5 predicted that soil Pb concentrations ≥200 ppm would result in BLLs with a ≥5% risk of BLL exceeding 5 µg/dL from ages 1-3. Model simulations of exposures from pica events (ingestion rate = 5000 mg soil ingested/event) once every two weeks from 6 months to 1 year predicted BLLs exceeding 5 µg/dL more than one year following the end of pica behavior for soil Pb concentrations ≥300 ppm. Thus, the shorter averaging times implemented by the AALM allow for evaluation of short-term lead exposures occurring at contaminated sites.

A Multiobjective PBPK-PD Approach for Dose Schedule Optimization of Brain Tumour Treatments Using Myelosuppressive Drugs

Frequently, compounds that exhibit significant promise for the treatment of brain tumours possess the undesirable liability of being myelosuppressive – i.e. toxic to bone marrow function. Maximizing the therapeutic index for these compounds through dose schedule optimization can significantly enhance the chances of clinical success in drug discovery. The process of identifying the most effective schedule is complicated however by the need to simultaneously manage two competing objectives – maximizing efficacy through dose-coverage in the tumour while minimizing hematological toxicity. Formal multi-objective algorithms provide a means to deal with these complexities, and serve as frameworks to integrate the distinct, but coupled models required to optimize dosing schedules. Care must be taken to select appropriate models for each component. Efficacy of anti-neoplastic drugs in the treatment of brain tumours is highly dependent on the concentration profile of the drug in the tumour tissue. Physiologically-based Pharmacokinetic (PBPK) models are well suited for the prediction of tissue concentrations, and algorithms anchored on tissue concentration data from mouse xenograft experiments enable us to link plasma and tumor concentrations. Similarly, with respect to chemotherapy-induced myelosuppression, semi-mechanistic models based on the work of Friberg can be used to combine system parameters with drug-specific parameters to model the
depletion and recovery of blood cells via the processes of proliferation and differentiation in the bone marrow. We have developed an integrated multi-objective platform for simultaneously optimizing efficacy and toxicity for the treatment of brain tumours using the models described above. Upon completion of an optimization, the platform delivers a Pareto front of potential dosing paradigms which identify the most efficacious dosing strategies available for all tolerable toxicity scenarios.

**756 Analysis of Measured Serum-PFOA Data Using a PBPK Model Predicting Drinking-Water Exposure Concentrations in a Contaminated Community**

R. R. Worley1,2 and J. Fisher3, 2

Exposure to perfluorooctanoic acid (PFOA) is widespread - PFOA is routinely found in human blood as reported by the National Health and Nutrition Examination Survey (NHANES). Here, we apply a physiologically-based pharmacokinetic (PBPK) model to elucidate human pharmacokinetic behavior of PFOA. We modified an earlier version of our PBPK model to include a composite value for urinary PFOA clearance based on measured isomer specific clearances reported in the literature and partition coefficients for fat, liver, and kidney derived from measured PFOA concentrations in human cadavers. Bodyweight and drinking water intake parameters were modified from prior versions to reflect values reported in the 2011 EPA Exposure Factors Handbook. Four published biomonitoring datasets from the Little Hocking, OH community were used to evaluate the model’s ability to predict PFOA-serum concentrations given a known exposure scenario. This analysis revealed that our model accurately predicts serum concentrations resulting from exposure to PFOA in drinking water. We then used the model to conduct reverse dosimetry to estimate PFOA concentrations in a public water supply in a community with contaminated drinking water based on the measured serum-PFOA concentrations (unpublished data). Assuming no non-drinking water exposure sources, we estimate drinking water concentration necessary to result in the measured serum-PFOA concentrations would be 0.29 – 0.39 ppb, and not expected to exceed the EPA’s provisional health guideline of 0.4 ppb. This research sheds light on the pharmacokinetic behavior of PFOA in humans, provides a mechanism for site-specific assessment of PFOA, and gives scientists a tool to interpret biomonitoring data for public health applications. Toxicity studies indicate that the liver is a possible target organ for PFOA-induced toxicity in humans. Further research will attempt to describe dosimetry in the liver to improve the understanding of observed liver endpoints following PFOA exposure.

**757 Application of Reverse Dosimetry to Compare In Vitro and In Vivo Estrogen Receptor Activity**

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High-throughput screening (HTS) assays provide an efficient way to identify endocrine-active chemicals. However, nominal in vitro assay concentrations of a chemical do not reflect the blood or tissue tissue levels that cause in vivo effects, mostly due to differences in bioavailability and clearance between the two systems. In this study, we developed and applied physiological based pharmacokinetic (PBPK) models to quantitatively correlate in vitro and in vivo dosimetry for a list of estrogen receptor (ER) reference chemicals. All the chemicals were tested in a HTS estrogen receptor transactivation assay, BG1Luc, from which we derived the point-of-departure (POD) values for each chemical. Using PBPK models built using GastroPlus software, we estimated the daily oral equivalent doses (OEDs) that would result in a Cmax value equivalent to the POD values. Critical model parameters (e.g. metabolic clearance, fraction of plasma protein binding) were derived from published experimental data or predicted from quantitative structure-activity relationship models. Where available, the daily OEDs were compared to the lowest effective doses (LEDs) in rat uterotrophic assays, rat multigenerational studies, or human exposure values. Our preliminary results showed that OED estimated using BG1Luc HTS assay data for bisphenol A, a highly studied and environmentally relevant ER reference chemical, was far lower than the oral LED for this chemical in rat uterotrophic assays, suggesting that the BG1Luc HTS assay may provide a more conservative hazard estimate for use in risk assessment. Our modeling approach highlights the importance of pharmacokinetic considerations in assessing and ranking endocrine-active chemicals based on in vitro HTS assays. This project was funded in whole or in part with Federal funds from the NIEHS, NIH under Contract No.HHSN27220140006C.

**758 Evaluation of Physiologically Based Toxicokinetic (PBTK) Modelling for Reverse Dosimetry Approaches**

E. Fabian, K. Guth, R. Zbranek, B. van Ravenzaaw and B. Landsiedel

Information on toxicokinetics of substances is essential for the risk assessment without in vivo data. In this strategy, PBTK models are potential in silico tools to translate effect concentrations e.g. in cell culture to external doses, e.g. for rats. Although selected input parameters for PBTK models like physiological and physicochemical data, partition coefficients or information on hepatic clearance are well established and easy to achieve, gaps exist for other parameters, e.g. established in vitro systems to address renal clearance or information on applicability domains for intestinal permeability assays. For the evaluation of PBTK results, we modelled kinetics of 14 registered pesticides (MW: 222 – 594 g/mol; logP: -1.5 to 4.3) using a PBTK model for rats consisting of compartments, including fat and liver. Beside the distribution of the test substance between the compartments (basic model), in vivo data on hepatic clearance from rat liver S9-fraction as well as on intestinal permeability from Caco-2 or PAMPA-assays were included. The mathematical model was solved with Berkeley Madonna software for different oral dose levels. For each dataset, the calculated maximum plasma concentration (Cmax) was compared to existing in vivo data. Modelled Cmax-values in the same order of magnitude than the Cmax-values determined in vivo were assessed to be correctly predicted. The predictivity of the basic model was 36% “correctly predicted”. When hepatic clearance was included, correct predictions increased to 43%. When additionally permeability data were included up to 50 % of the modelled datasets were in the same order of magnitude as in vivo data. Taken together, plasma concentrations calculated based on in silico in vivo data by PBTK modelling differed for at least 50 % of datasets from in vivo results by factors > 10. Therefore the accuracy of PBTK modelling should be carefully taken into consideration, when these data are e.g. intended for in silico in vitro based risk assessments.

**759 Advanced QSAR Models for Use in Toxicokinetic Modelling**

D. A. Sarigiannis, K. Papadaki and S. P. Karakitsios

A current limitation to further introducing PBPK models in the risk assessment arena is the lack of generic character of these models. A critical limiting factor of describing the ADMET process for a large chemical space is the proper parameterization for “data poor” compounds. In order to expand the applicability of PBPK models to cover as much as possible the chemical space, model parameterization for data poor chemicals is done using advanced quantitative structure-activity relationships (QSARs). Several QSAR modeling approaches have been investigated, including (a) an algorithm based on fractional content of and the lipophilicity of the compound of interest; (b) the molecular fractions algorithm that takes into account the frequency of occurrence of the several molecular fragments of the compounds and (c) Abraham’s solvation equation for estimating biological properties, which takes into account the polarizability of the compound dipolarity/polarizability, the solute effective or summation hydrogen-bond acidity, the solute effective or summation hydrogen-bond basicity and the McGowan characteristic volume. Up to now, these QSARs seem to perform adequately for a limited number of chemical families. A major breakthrough came from the use of Artificial Neural Networks coupled to Abraham’s solvation equation parameters for predicting chemical-specific biological/biochemical properties such as blood-tissue partition coefficients, maximal velocity (V_max) and the Michaelis - Menten constant. This was a remarkable advance, since the prediction capability of the Michaelis - Menten constant in the existing studies to date was rather poor (R^2 up to 0.3). With our coupled ANN - Abraham’s solvation equation model for the investigated group of 55 chemicals, R^2 rose to 0.8. For the rest of the parameters (partition coefficients, V_max), performance of prediction against experimental values was consistently high (R^2 always above 0.9), outperforming any other existing methodology.

**760 PAHs and PM2.5 Emissions and Female Breast Cancer Incidence in Metro Atlanta and Rural Georgia**

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Geographic variations in breast cancer incidence occur nationally and internationally, with a high incidence rate in more industrialized regions. Exposure to higher levels of hazardous chemical substances in the environment could be an important etiologic factor. In this study, we examined emissions of polycyclic aromatic hydrocarbons (PAHs) and particulate matter below 2.5 μm in diameters (PM2.5) to the ambient air in relation to incidence rates of female breast cancer in metro Atlanta.
and rural Georgia. Age-adjusted incidence rates of female breast cancer for the years of 1992-2011 were analyzed using the Surveillance, Epidemiology, and End Results (SEER) Program of the U.S. National Cancer Institute. The annual emissions of both air pollutants were collected at the county level for metro Atlanta and rural Georgia areas as designated by the SEER program. The results showed that metro Atlanta region had a significantly higher average annual incidence rate of female breast cancer (132.6 per 100,000; 95% CI: 131.2, 134.0) than rural Georgia (113.7 per 100,000; 95% CI: 108.2, 119.5). In relation to this, metro Atlanta area had a 2.7-fold and 4.6-fold higher emission density (emissions per square mile) of PAHs and PM2.5, respectively, compared to the rural Georgia area. Pearson product-moment correlation analysis revealed that emission density of PM2.5 (r = 0.479; p = 0.038), but not PAHs (r = 0.367; p = 0.122), was significantly positively correlated with breast cancer incidence in metro Atlanta counties. This study suggests that ambient air pollution, especially PM2.5, could have a significant effect on the increased incidence rate of female breast cancer in urban areas.

671 A Systematic Review of the Association between Pleural Plaques and Changes in Lung Function

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Pleural plaques are one of the earliest and most common manifestations of asbestos-related disease. There are differing opinions in the scientific literature as to whether pleural plaques are associated with changes in lung function. We conducted a systematic review and meta-analysis of changes in lung function in relation to presence of pleural plaques in asbestos-exposed populations. Database searches of PubMed and Web of Science were supplemented by review of papers' reference lists and journals' tables of contents. Methodological features (e.g., consideration of potential confounding by smoking) of identified articles were reviewed by at least two reviewers using a set of criteria defined a priori. Meta-analyses of 20 studies estimated a summary effect of the decrements in percent predicted (%pred) forced vital capacity (FVC) and forced expiratory volume in one second (FEV1) associated with presence of pleural plaques. The presence of pleural plaques was associated with statistically significant decrements in FVC 4.09 %pred, 95% CI: 2.31, 5.86, and FEV1 (1.99 %pred, 95% CI: 0.22, 3.77) compared to asbestos-exposed workers without abnormalities. Effects of similar magnitude were seen when stratifying by imaging type (x-ray or high resolution computed tomography) and when excluding studies with potential methodological limitations. Thus, undetected asbestos was considered an unlikely explanation of the observed decrements. In addition, studies provided evidence of an association between size of pleural plaques and degree of lung function decrease, and presence of pleural plaques and increased rate or degree of pulmonary impairment. The presence of pleural plaques is associated with a small, but statistically significant mean difference in FVC and FEV1, in comparison to asbestos-exposed individuals without abnormalities. From a public health or population perspective, small group mean decrements in lung function coupled with an increased rate of decline in lung function of the exposed population may be consequential. Disclaimer: This abstract does not represent views or policies of US EPA.

672 Interactive Effects of N6AMT1 and AS3MT in Arsenic Biomethylation and the Resulting Cytotoxic Outcomes

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High levels of arsenic are naturally present in drinking water and have become a public health concern in some areas of the world. While the modes of action for arsenic are not fully elucidated, it is well accepted that arsenic metabolism plays a public health concern in some areas of the world. While the modes of action for arsenic are not fully elucidated, it is well accepted that arsenic metabolism plays a role in the inter-individual susceptibility and target specificity for arsenic toxicity. (This work was supported by NIEHS grant R21ES02329 to X.R.)

673 Forest-Fire Fine Particulate Matter and Daily Mortality in Greater Boston and New York City


During July 2002, forest fires in Quebec, Canada blanketed the US East Coast with a plume of wood smoke. This "natural experiment" exposed large populations in northeastern US cities to significantly elevated concentrations of fine particulate matter (PM2.5), providing a unique opportunity to test the association between daily mortality and ambient PM2.5 levels that are uncorrelated with societal activity rhythms. We obtained PM2.5 measurement data and daily mortality counts for a four-week period in July 2002, for the Greater Boston metropolitan area (population of over 1.7 million) and New York City (population of over 8 million). Daily-average PM2.5 concentrations were markedly increased for three days over this period, reaching as high as 63 μg/m3 for Greater Boston and 86 μg/m3 for New York City, from background ambient levels of 4-48 μg/m3 in the non-smoke days. We examined temporal patterns of natural-cause deaths and 24-hour ambient PM2.5 concentrations in July 2002 and did not observe any discernable increase in mortality subsequent to the dramatic elevation in ambient PM2.5 levels. Comparison to mortality rates over the same time periods in 2001 and 2003 also showed no evidence of impact. Results from Poisson regression analyses likewise showed no associations of daily mortality with 24-hour ambient PM2.5 concentrations. In conclusion, substantial short-term elevations in PM2.5 concentrations from forest fire smoke were not followed by increased daily mortality in Greater Boston area and New York City.

674 Elderly Health and Indoor Environment

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Rationale and Scope: This article focus on respiratory symptoms of older people living in elderly care centers (ECC). Results have been produced by measuring and characterizing indoor air quality, thermal comfort and respiratory health in 21 ECC in Porto. Experimental procedures: Out of a total of 58 ECC, 36% (n = 21) accepted to participate in this study. Environmental data was collected for each ECC in two seasons (i.e. summer and winter) starting from November 2011 till August 2013. The Portuguese version of BOLD (Burden of Obstructive Lung Disease) was administered to the older people from September 2012 to April 2013, along the winter season environmental sampling campaign correspondent to each ECC. All participants (n = 143) gave their informed consent and should had ≥ 65 years old and live in the ECC for more than 2 weeks. Results and Conclusions: Cough (23%) and sputum (12%) were the major respiratory symptoms, and allergic rhinitis (18%) the main self-reported illness. Heart troubles were reported by 37% of the residents. Overall PM2.5 median concentration was above reference levels both in winter and summer season. Also, peak values of PM10, total volatile organic compounds, CO2, bacteria and fungi exceeded the reference levels, compromising indoor air comfort and worsening the already existent respiratory chronic diseases. The winter predicted mean vote (PMV) index was below references, between the ‘slightly cool’ (1) and ‘cool’ (2) points in the thermal sensation scale, which may potentiate respiratory tract infections. Predicted percentage dissatisfied and PMV indices also showed significant differences by room and by season (p < 0.01). Older people exposed to PM10 above and temperature below the reference levels presented a higher odds of allergic rhinitis (OR = 2.9, 95% CI: 1.1 – 7.2) and (OR = 0.8, 95%CI: 0.0 – 1.0) respectively.
765 Does Pharmaceutical Exposure Mediate Risks Associated with Exposure to Heavy Metals? An Epidemiological Investigation of NHANES

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 Pharmaceutical & personal care products (PPCPs), while intended to improve health, may have unintended health effects and are emerging contaminants of concern. One potential example which has received limited attention is the effect of PPCPs on the risks associated with exposure to common environmental contaminants such as toxic heavy metals. Here, the objective was to understand if pharmaceutical use can be associated with biomarkers of exposure to toxic heavy metals (total blood mercury [Hg], total blood lead [Pb] and total blood cadmium [Cd]). We analyzed prescription drug use and metals biomarker data from the U.S. National Health and Nutrition Examination Survey (NHANES) 2009-2010 (n=7724 for Hg; 7994 for Pb, Cd), accounting for age, sex, income, and race/ethnicity, as well as fish consumption for Hg. Overall prescription drug use was a significant predictor for Hg and Pb, but not Cd. More specific information on prescription drugs revealed drug categories that were significant predictors of Hg (e.g., cardiovascular agents), Pb (e.g., metabolic agents), and Cd (e.g., cardiovascular agents). There were differences between males and females in a number of drug categories, including lower blood Pb and Cd among only females taking hormones/hormone modifiers. These results suggest that exposure to PPCPs commonly utilized by society may mediate exposures to other contaminants, such as toxic heavy metals. Future work is needed to resolve the underlying mechanisms and determine the hazards associated with such real-world exposures to chemical mixtures.

766 Important Covariates of the Cord Blood Transcriptome

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Development of –OMICS technologies over the past decade have enabled the implementation of molecular biology in the field of epidemiology. An important challenge in (molecular) epidemiology is how to deal with influencing factors (e.g., gender, age, ethnicity, …). Gender specific transcriptional responses have been reported in many studies. However, it is reasonable to assume that the same set of confounders and/or covariates in exposure-effect relationships does not apply to each transcript among the transcriptome. Based on stepwise multiple regression, we propose an approach that may show potential in different –OMICS fields. As a proof of concept, we aimed to identify covariates and/or confounders in the associations between environmental exposure and the cord blood transcriptome at the gene specific level. In 195 samples collected immediately after birth, we identified the set of most important determinants for each gene among the transcriptome. Gestational age was the most prominent covariate of the neonatal transcriptome, as it was associated to over 60% of the genes. Other important covariates in the study population were gender of the newborn, maternal blood lipid content and season of birth associated with expression of about 20 to 30 percent of the genes. Interestingly, exposure to cadmium and paracitize matter influenced a similar proportion of genes. Genes associated with the most important determinants among the transcriptome were mainly involved in hematological system development and function, and in inflammatory and immune response. Ongoing analysis will reveal whether networks of related genes can be identified from the gene expression data. The studies of the Flemish Center of Expertise on Environment and Health were commissioned, financed and steered by the Ministry of the Flemish Community. The research received funding from the European Community’s Seventh Framework Programme (FP7/2007–2013) under grant agreement OBELEX 227391.

767 Examining the Association between Urinary Concentrations of 2, 5-Dichlorophenol and Blood Pressure in US Adults

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Urinary concentrations of 2,5-dichlorophenol (2,5-DCP) is a reliable biomarker for measuring exposure to para-dichlorobenzene (p-DCB) in indoor environments. Recent studies suggest that urinary levels of 2,5-DCP is associated with obesity, earlier age of menarche, and possibly other related metabolic effect. In this study, we examined the association between urinary concentrations of 2,5-DCP and blood pressure in U.S. adults who participated in the 2007-2010 National Health and Nutrition Examination Survey. After excluding pregnant women and participants with missing covariates, a total of 3005 adults participants, aged 20-79 years, were included in the analyses. Participants were categorized as having normal blood pressure (<120/80 mmHg) and having a systolic blood pressure ≥120 mmHg or a diastolic blood pressure ≥80 mmHg (including prehypertension and hypertension). Of the 3005 participants, 49.9% were having prehypertension or hypertension. Median urinary concentrations of 2,5-DCP was 7.0 μg/L (interquartile range: 2.1-29.2). Geometric means of urinary concentrations of 2,5-DCP differ by gender, race/ethnicity, poverty status, education, and physical activity, which indicates biological mechanisms and social or behavioral factors related to the exposure. Multivariate logistic regression analyses found that the third quartile of urinary 2,5-DCP had an increased odds of prehypertension or hypertension, as compared to the lowest quartile. The odds ratio for the association was 1.47 (95% CI: 1.12, 1.92) after adjusting for urinary creatinine, 1.39 (95% CI: 1.03, 1.87) after adjusting for urinary creatinine, age, gender, race/ethnicity, poverty status, and educa-

768 Evaluation of US EPA’s NonCancer Risk Assessment of Libby Amphibole Asbestos

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US EPA’s Integrated Risk Information System (IRIS) evaluates information on human health effects that may result from exposure to environmental contaminants. In 2011, US EPA released a draft IRIS assessment for Libby amphibole asbestos. This is the first risk assessment for a specific type of asbestos; once it is finalized, it will likely have implications for other types of asbestos. US EPA included a Reference Concentration (RfC) calculation based on pleural plaques (which are considered by many to be benign biomarkers of exposure) rather than an endpoint associated with established adverse effects, such as asbestosis or diffuse pleural thickening. US EPA estimated the point of departure by modeling the dose-response relationship between Libby amphibole asbestos exposure and pleural plaques in Vermiculite workers in Marysville, Ohio, and calculated the RfC by applying several uncertainty factors (UFs) to this estimate. We critically evaluated the methods US EPA used to derive the RfC. Using a weight-of-evidence (WoE) approach, we demonstrated that pleural plaques do not cause clinically significant lung function decrements and, therefore, should not be used as a basis for the RfC. We further evaluated the dose-response modeling approaches and UFs selected by US EPA, finding several instances in which other choices – which had large impacts on the RfC – would have been better supported by the science. These alternative choices should be considered further before the IRIS assessment is finalized.

769 Steatohepatitis Associated with Adipocytokine Abnormalities in the Anniston Community Health Survey

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Background: Polychlorinated biphenyl (PCB) exposures have been associated with steatohepatitis in prior epidemiological studies. A highly-exposed residential adult cohort (Anniston Community Health Survey, BCHS) was assembled to study polychlorinated biphenyl (PCB) effects on adverse health outcomes. The present study investigates the effects of PCB exposure on mechanistic serum steatohepatitis biomarkers within BCHS (n=744). Methods: Cytokeratin-18 whole (CK18 M65) and caspase-cleaved fragment (CK18 M30) were measured by ELISA, and adipocytokines were measured by Luminex. Ortho-substituted PCBs were measured by GC/MS. Linear regression models were used to examine relationships between ΣPCBs and adipocytokines after stratifying the cohort based on liver disease status as determined by CK18: no liver disease (NLD, M30<200 U/L, M65<300 U/L), toxicant-associated steatohepatitis (TASH, M30>200 U/L, M65>300 U/L) and other liver disease (OLD, M30>200 U/L). Log-transformed ΣPCBs (whole weight) and biomarker variables were adjusted for total lipids, age, race, gender, BMI, diabetes status, and alcohol. Results: The overall prevalence of liver disease was 60% (TASH 49% and OLD 11%). The prevalence of TASH was significantly higher in men than women (50% vs. 46%) and in non-Hispanic whites than African Americans (57% vs. 39%). Insulin, PAI-1, IL-1B, and IL-6 were significantly higher in subjects with TASH vs. NLD. Insulin and leptin were inversely associated with PCB levels. Conclusions: The prevalence of biomarker-induced liver disease
in ACHS was exceptionally high and gender/ethnic differences were noted. Most affected subjects had a CK18 ratio consistent with hepato cellular necrosis, rather than apoptosis, which is consistent with chemical liver injury (TASH) - possibly implicating PCB exposures. Likewise, TASH was associated with insulin resistance and pro-inflammatory cytokine elevation.

**Aflatoxin B1 Exposure Modulates HIV to AIDS Pathogenic Progression**


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Aflatoxin B1 (AFB) is a common contaminant in food in the developing world and it is a human carcinogen. Animal studies have demonstrated AFB-induced immune toxicities on T-cell-mediated cellular immunity, and limited human studies showed association of AFB exposure with HIV infections, which are endemic in sub-Saharan countries. However, convincing evidence of this association needs more investigations in prospective cohort studies. In this study we measured AFB-lys adduct levels from an existing HIV prospective cohort study conducted in Uganda and examined the correlation between AFB exposure and HIV pathogenesis. Based on median of AFB adduct level in serum (3.91 pg/mg albl), HIV infected participants were divided into two groups: high AFB exposure group (n=79) and low AFB exposure group (n=78). Kaplan meier estimators were obtained using the length of incubation periods (HIV infection to AIDS development). The estimators were significantly different in two groups (p=0.0349). Ten years after HIV infection, 8% of the low AFB exposure group progressed to AIDS, compared to 18% in the high AFB exposure group. The Cox PH model showed that hazard to progress to AIDS increased in the high AFB exposure group after adjusted for baseline CD+ T cell counts, viral replication, and demographic risk factors. In order to understand possible mechanism, cytokine/chemokine was further analyzed in the subset of these groups. AFB exposure was positively correlated to increased expression of cytokines in inflammatory pathway, including MIP-1x (p=0.017), GM-CSF (p=0.07), IL-15 (p=0.03), IL-8 (p=0.031), and cytokine related to angiogenesis VEGF (p=0.059), respectively. These results suggest chronic inflammation by AFB exposure might be the partial cause of fast pathogenic progression from HIV to AIDS in high AFB exposure group.

**Polychlorinated Biphenyls, Diabetic Status, and Inflammatory Cytokines**

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Associations between serum concentrations of ortho-substituted polychlorinated biphenyls (PCBs) and concentrations of inflammatory cytokines were examined, as stratified by diabetic status, in adult participants of the Anniston Community Health Survey. Thirty-five ortho-substituted PCB congeners were measured using high-resolution gas chromatography/isotope-dilution high-resolution mass spectrometry. Inflammatory biomarkers IL-1β, IL-6, IL-8, TNFα, and plasminogen activator inhibitor (PAI)-1 were measured by multi-analyte chemiluminescent de- tector (Luminox) and commercially available ELISA kits. Linear regression models were used to analyze associations between ΣPCBs and inflammatory biomarkers. Of the 741 participants, 50.7% were classified with normoglycemia, 21.5% were used to analyze associations between ΣPCBs and measured significant but suggest that the direction of association between ΣPCBs and measured biomarkers may differ by diabetes status, with potential inverse associations in the prediabetic group and positive associations in other groups. Preliminary results from the follow-up study of this cohort show a 25% higher median PAI-1 level (p=0.001) 8 years after baseline. PCB-mediated prothrombotic PAI-1 upregulation may be an important driver of cardiovascular risk in diabetics.

**Circulating Oxidized LDL and Conventional Biomarkers of Cardiovascular Health in a Navajo Cohort Exposed to Uranium-Mining Metal Contaminants**

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Numerous abandoned mines (AUMs) within the Navajo Nation contribute uranium, arsenic and other metals to the soil and groundwater. Environmental exposure to metal contaminants may promote or exacerbate cardiovascular disease (CVD) through the oxidation of LDL cholesterol. In recent decades, the prevalences of CVD and type 2 diabetes have increased among the Navajo community. Residents currently living in close proximity to AUMs have an increased risk for hypertension. To assess the potential impact of these contaminants on the cardiovascular health of exposed individuals, we examined conventional (CRP, IL-6) and novel (oxLDL) plasma biomarkers in 20 communities of the Navajo Nation. Samples and data were obtained in partnership with the DNHE Project. OxLDL has not been evaluated in this population, so we first assessed oxLDL levels as related to demographic and clinical data. Biomarker data were then linked to geospatial and well water data on contamination sites using linear regression and Bayesian models. Diabetes, hypertension, and obesity are prevalent in this cohort as determined by self-report and clinical measures. OxLDL correlates with CRP and HBAlc. Estimated annual intake of arsenic is a significant predictor of oxLDL. To examine a possible mechanism for this finding, and because copper, another metal, is known to directly oxidize LDL, proof-of-concept assays were performed to investigate if arsenic and uranium could also directly oxidize LDL. We found that arsenic, but not uranium, oxidizes the apo-B protein but not the lipid component of LDL as measured by ELISA and TBARS, respectively. In summary, oxLDL seems to trend with arsenic intake. Arsenic may directly oxidize circulating LDL, a potential contributing mechanism to explain chronic CVD health outcomes in arsenic-exposed populations.

**The Occurrence of Cyanobacterial Toxins in Alberta Recreational Waters**

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Occurrences of cyanobacteria (blue-green algae) blooms and the presence of microcystin toxins (MCs) in fresh water have been reported worldwide. MCs are highly potent hepatotoxins with a diverse structure and health consequences. As part of the Alberta Cyanobacteria Monitoring Program, a total of 2706 water samples were collected from recreational beach areas across Alberta in late spring and summer months from 2010 to 2014. All water samples were screened for MCs using the protein phosphatase inhibition assay (PPIA). Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) was used to analyze 960 water samples for differentiating MC congeners and for quantification. The PPIA results showed that 1% (2010), 3% (2011), 2% (2012), 2% (2013), and 2% (2014) of samples had MC concentrations exceeding 20 μg/L (the Canadian Recreational Water Guideline), mostly in Central and Northern Alberta lakes from July to early September each year. Over 80% of the samples had MC concentrations below 1.5 μg/L (the Canadian Drinking Water Quality Guideline). The maximum concentrations were 35, 78, 100, 64, and 340 μg/L in the years 2010 to 2014, respectively. Multiple MC congeners co-occurred in some water samples. MC-LR was found to be the most prevalent (60% in 2011, 79% in 2012, and 57% in 2013 of samples contained MCs). MC-RR was also detected in 3% of the samples in 2011, and 17% of the samples in 2012. Our results show the geographic distribution and the temporal trend of the MCs occurrence in Alberta recreational water during spring and summer months. The results of the monitoring program assist in the development of policies and procedures for safe use of beaches (e.g., advisories) and better public health management.
The risk of lung cancer among chromate production workers has been used in several quantitative hexavalent chromium [Cr(VI)] risk assessments, and one of the most studied cohorts is from the Painesville Ohio facility. The last follow-up of this cohort was completed in 1997, including 482 workers and 14,048 total person-years at risk. Short-term workers (<1 year of employment) were excluded, limiting information in the low exposure range. We conducted an updated study of 714 Painesville workers employed from 1940-1972, including 198 short-term workers and 47 with unknown vital status at the end of previous follow-up, to calculate standardized mortality ratios (SMRs) and improve upon the data available for cancer risk assessment. The average length of follow-up (through 2011) was 34.4 years, including 24,535 total person-years at risk. Vital status was confirmed for 690 workers (658 deceased, 32 alive); 24 were lost to follow-up. A comprehensive job exposure matrix was used to estimate Cr(VI) exposures for each worker for cumulative exposure and highest monthly exposure, which is the highest exposure estimated. Significant excess in mortality were observed for the entire cohort from cancers of trachea/lung/bronchus (n=77; SMR 186, 95% CI 145 to 228) and other circulatory system diseases (n=60; SMR 153, 95% CI 114 to 191). In addition, there was a significant decreasing trend in lung cancer deaths according to year of hire (p=0.04) and an increasing trend by duration of employment (p=0.01). Lung cancer death was significantly elevated in the cohort with increasing Cr(VI) exposure for both highest monthly exposure (p=0.03) and cumulative exposure (p=0.01). Although short-term workers had lower Cr(VI) exposures compared to the entire cohort, they had higher all-cause mortality, which was significant, indicating poorer health status. These data provide new data for dose-response modeling and the cancer risk assessment for airborne Cr(VI).
Cardio-Oncology Concerns Encourage Novel Approaches to Pharmaceutical Risk Assessment


Recent successes in prolonging the life of cancer patients with optimized use of traditional approaches and the addition of novel classes of drugs (e.g., tyrosine kinase inhibitors) has raised the visibility and interest in managing the cardiovascular sequelae of a number of these therapeutic regimens. These concerns have energized the development of the unique and emerging field of cardio-oncology. A number of recent workshops have explored significant gaps and opportunities for improving both clinical and nonclinical approaches. This symposium intends to explore the knowns, unknowns, and opportunities for improvement in nonclinical approaches to cardiovascular risk assessment of anticancer drugs. The ultimate goal of these discussions is to identify opportunities to further enhance translation of nonclinical approaches and enhance relevance to patient outcomes.

Clinical Challenges for Managing Cardiovascular Risk in Cancer Patients in an Era of Prolonged Life Expectancy

D. Sawyer, Vanderbilt School of Medicine, Nashville, TN. Sponsor: S. Pettit.

Cancer patients—both young and old—are often exposed to complicated and toxic therapeutic regimens. Historically effective cancer drugs (e.g., adriamycin) and novel classes of antineoplastics (e.g., antibody based targeted therapies, tyrosine kinase inhibitors) are effectively and significantly extending the life of patients with a number of common malignancies. An unfortunate detractor to that success is the specter of unintended cardiovascular toxicity. This presentation will explore how these effects factor into the risk stratification of cancer patients anticipating and undergoing treatment, the potential for delayed and chronic sequelae to treatment, and the challenge of recognizing and managing drug-related cardiac toxicity in these patients, particularly during the development of novel anti-cancer therapeutics. The utility of an expanding array of imaging and biomarker tools in monitoring cardiac function and stress during both drug development as well as in clinical oncology practice will be discussed. In addition, the contribution of co-morbidities such as hypertension, obesity, and diabetes to risk of these adverse effects, as well as the strategy to prevent cardiovascular effects of cancer therapy will be discussed.

The Intersection of Pharmacology and Toxicology in an Age of Targeted Cancer Therapies

B. R. Berridge, GlaxoSmithKline, Research Triangle Park, NC. Sponsor: S. Pettit.

Cardiotoxicity has been a recognized liability for the historically effective cytotoxic anthracyclines for many years but our understanding of the mechanisms of that toxicity is still evolving. The emergence of more targeted and pharmacologically effective cancer drugs like tyrosine kinase inhibitors, HDAC inhibitors and proteasome inhibitors are improving the profile of cancer patients but have often maintained liabilities for cardiovascular toxicity increasing our interest in their mechanisms. Investigations are revealing unexpected associations with pharmacologic targets as well as complicated interactions between co-therapies. This presentation will explore the molecular pharmacology of historic and novel cancer drugs and apply developing evidence to understand potential interactions between pharmacology and mechanisms of cardiotoxicity.
A "CAR-T" cells can recognize selected extracellular targets and kill target-expressing cells. Surprisingly, preclinical studies for these biologic immunostimulants have been underutilized. Together, these talks should paint a detailed landscape of biologic cancer immunotherapeutics, and challenges facing toxicologists.

**S 786** “CAR-T” Cells—A Crash Course in Immunostimulant Safety Concerns


Recent clinical oncology trials have evaluated the therapeutic re-administration of autologous T cells genetically modified *ex vivo* to express "new" T cell receptors (TCRs) or chimeric antigen receptors (CARs, essentially scFv fragments of therapeutic mAbs). These neo-TCR-T cells or CAR-T cells recognize antigen-MHC or extracellular protein targets, respectively, on hematologic or solid tumor cancer cells. This *ex vivo* gene/cell therapy field has been championed by academic and clinical research centers, with the late entrance of pharmaceutical industry partners. The preclinical evaluation of potential safety concerns for these immunostimulants has been challenging—and underutilized. While showing great promise in treating certain cancers, numerous “unexpected” or “unpredicted” serious adverse events, including death, have occurred. This presentation will focus on considerations for the design of translational preclinical programs to address the safety concerns for these novel immunostimulants.

**S 787** Integrated Nonclinical Safety Evaluation of a PD-L1 Antagonist with Impacts on Dosing

R. Prell. Genentech, South San Francisco, CA.

Programmed cell death 1 (PD-1) is a receptor expressed on T cells following activation. Binding to its ligand, programmed cell death 1 ligand 1 (PD-L1), down-regulates the quality and magnitude of T-cell responses. Many neoplastic cells express PD-L1 and evade destruction by the immune system. MPDL3280A, an effectectorless (FcγR-binding deficient) human IgG1 mAb that blocks PD-1/PD-L1 interactions, is in development as a potential therapy for solid tumors. This presentation will focus on the nonclinical safety studies conducted in mice and cynomolgus monkeys for MPDL3280A and integrate it into the context of known PD1/PDL1 pathway liabilities involved in autoimmune and infectious diseases. Taken together, this information, which led to a more conservative first in human starting dose than the standard NOAEL approach, will also be described.

**S 788** Balancing Safety and Efficacy of Novel Immune System Agonists to CEA and OX40 Targets

R. Dixit. MedImmuno/ASTA-Zeneca, Gaithersburg, MD.

Many immune system agonistic antibodies, including a CD28 super-agonist antibody (TGN1412) and 4-1BB agonist antibody have had severe life-threatening adverse immune-mediated toxicities in humans. Super-agonist immune-monoclonal biologics pose significant safety challenges that may limit their clinical use even in advanced cancer settings. The presentation will describe smart approaches of using translational immunopharmacology based *in vivo* and *in vitro* models to characterize and predict the safety of immune system agonistic biologics. Specifically, safety/toxicology data from TGN1412, CEA-BiTE and OX40 agonistic biologics will be discussed.

**S 789** Overcoming the Safety Challenges of a CD137-Agonist Immuno-Oncology Therapeutic

H. G. Haggerty. Bristol-Myers Squibb, New Brunswick, NJ.

The development of novel therapies that harness the body’s immune system is transforming the way cancer is treated. Despite the encouraging results that have been observed, monitoring the immune system can lead to severe adverse immune-mediated reactions, such as the grade 4 hepatitis caused by an agonistic antibody against the costimulatory receptor, CD137 (41BB). CD137 agonist treatment activates T cells, NK and/or NKT cells resulting in enhanced cytokine production, T cell survival and proliferation, promoting tumor-specific immune responses. This presentation will discuss the nonclinical and clinical safety assessment of anti-CD137 agonistic antibody and the investigative work conducted to enhance our understanding of the mechanism of CD137-mediated liver toxicity, including that observed in mice treated with anti-murine CD137 mAb.

**S 790** Thinking beyond General Toxicology Studies for Immunotherapeutics

W. Helms. US FDA/CDER, Silver Spring, MD. Sponsor: L. Black.

Traditionally regulators have relied heavily on general toxicology studies to anticipate the potential clinical toxicities of a drug but newer generation cancer immunotherapeutics (CITs) have challenged this approach. A much clearer understanding of the pharmacologic mechanism of action of the drug has been important for accurately predicting clinical toxicity, thus, as has been the case for many other biological products, the nonclinical safety evaluation for CITs can require assigning higher significance to the pharmacology studies submitted to support the development of the drug. This presentation will provide a regulatory perspective on the considerations and challenges associated with developing novel immunotherapy products for cancer indications. Case studies of recent challenges with immunotherapies submitted to the FDA will be presented with an emphasis on the anti-PD-1 example.

**S 791** Nrf2 Signaling Pathways in Model Systems: A Master Regulator of Neurotoxicity and a Potential Therapeutic Target

R. M. Nass. Pharmacology and Toxicology, Indiana University School of Medicine, Indianapolis, IN.

The nuclear respiratory factor 2 (Nrf2), a bZip transcription factor, plays a critical role in maintaining cellular redox homeostasis in normal physiology, and in initiating environment- or pathophysiology-associated stress response. The high conservation of Nrf2’s structure, target genes, and downstream signal transduction pathways across animal phyla, in concert with the emerging evidence that the transcription factor is a regulator of protein degradation, neurotoxicity, and cell death, suggests that exploring Nrf2-associated molecular pathways in invertebrate and vertebrate genetic models will have significant relevance to human toxicology. In this symposium, we describe novel insights and strengths, as well as limitations, of increasingly complex genetic models including the nematode, fly, fish, and rodents to identify the molecular pathways involved in Nrf2-associated neuronal protection, as well as the utility of Nrf2-mediated therapeutic targets. Dr. Richard Nass will describe his studies utilizing the nematode *C. elegans* to explore the genetic and molecular basis of Nrf2- and sirtuin-associated DA neuronal vulnerability. Dr. Leo Pallanck will discuss his studies using the genetic fruit fly model *D. melanogaster* to identify Nrf2-induced compounds to inhibit PD-associated neurodegeneration. Dr. Evan Gallagher will describe his research utilizing the genetic zebrafish model *D. rerio* to elucidate the role of Nrf2 in maintaining sensory behaviors following cadmium exposure. Dr. Jeff Johnson will discuss his studies on how astrocytic Nrf2 activation inhibits PD-associated genetic- and chemical-induced neuropathology in rodents. Finally, Dr. Donna Zhang will discuss her studies involving the regulation of Nrf2 by E3 ubiquitin ligases in rodents and humans, and the opportunities this regulation provides for identifying novel therapeutic targets and leads.

**S 792** The Identification and Characterization of an SKN-1/Nrf2 Pathway Involved in Toxicant-Associated *C. elegans* Models of Parkinson’s Disease

B. Arbuckle1, N. YanDuy2, W. Li1, J. Trinidad1 and R. M. Nass1.

1Pharmacology and Toxicology, Indiana University School of Medicine, Indianapolis, IN and 2Chemistry, Indiana University, Bloomington, IN.

Idiopathic Parkinson’s disease (PD) is an oxidative stress-related disorder that results in abnormal dopamine (DA) signaling and cell death. Although the origin of the pathogenesis in PD remains unclear, correlative evidence suggests both genetic and environmental contributions. Recently we have shown that the PD-associated transcription factor SKN-1/Nrf2 is expressed in the DA neurons of the genetic model *Caenorhabditis elegans* (*C. elegans* and inhibits PD-associated DA neurodegeneration. The high conservation of the *C. elegans* genome, biosynthetic, and metabolic pathways with mammalian systems, as well as an array of robust genetic tools provides significant opportunities to dissect the molecular basis of human neurotoxicities. In this study we asked what are the genes and molecular pathways involved in DA neuron vulnerability to PD-associated toxicants. We demonstrate that SKN-1 functionally interacts with a sirtuin, a protein whose activation has been shown to regulate cell cycle and longevity, also regulates DA neuron vulner-
ability to 6-OHDA, rotenone, and heavy metals. We show that a sirtuin mutant renders the DA neurons up to 10-fold more resistant to the neurotoxants relative to WT, and that overexpression results in a 2-fold increase in DA neurodegeneration. The generation of the first C. elegans acetylome as well as reverse genetics and biochemical assays indicates protein acetylation and interactions between SKN-1, sirtuins, and the ubiquitin proteasome system (UPS) modulates neuronal vulnerability. This study also identifies novel genes and molecular pathways involved in DA neuron vulnerability, and describes the utility of C. elegans to identify novel Nrf2- and sirtuin-associated therapeutic targets. Support: NIEHS ES014459, ES003299, IUCRC, and FNDR Fund to RN, IUCRC to JT, and PEAR to WL.

793 Identifying Neuroprotective Factors from Coffee and Tobacco
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Parkinson’s disease is a common neurodegenerative movement disorder caused by the death of dopaminergic neurons in the substantia nigra. Epidemiological studies have revealed a significantly reduced risk of Parkinson’s disease among coffee and tobacco users. To test whether this reduced risk reflects a neuroprotective role of coffee and tobacco, we previously tested the neuroprotective potential of coffee and tobacco extracts using fruit fly models of Parkinson’s disease. Our work demonstrated that coffee and tobacco extracts are neuroprotective, and that this neuroprotection requires the transcription factor NRF2. One of the neuroprotective components of coffee is cafestol, a known NRF2 activator. However, the neuroprotective compound(s) in tobacco were unknown, so we have subjected a tobacco extract to reverse phase HPLC fractionation to identify these compounds. This work has revealed a single peak of NRF2 activity in our fractionated tobacco extract. We have also found that exposing two different fly models of Parkinson’s disease (parkin mutants and alpha-synuclein expressing flies) to food containing 1% of our peak NRF2-inducing fraction partially suppressed the degeneration of dopaminergic neurons that occurs in these models. We also found that exposing parkin mutants to our peak NRF2-inducing fraction partially attenuated their shortened lifespan. Finally, using a vertebrate cell culture system we found that our peak NRF2-inducing fraction was capable of inducing the NRF2-responsive genes, NQO1 and Gclc in an NRF2-dependent manner. We are currently subjecting our peak fraction to additional purification so we can initiate studies to determine the chemical structure of the NRF2-activating compound from tobacco. We anticipate that these compounds can potentially serve as therapeutics for Parkinson’s disease.

794 Role of Nrf2 in Regulating Cellular Antioxidant Responses of Fish
E. P. Gallagher, L. Wang, R. Ramburg and M. Mills. Environmental and Occupational Health Sciences, University of Washington, Seattle, WA.

The Nrf2-Keap1 pathway functions as a master regulator of antioxidant defense, but is less characterized in fish than in higher vertebrates. Obstacles to characterizing fish Nrf2 responses include the lack of molecular tools, as well as the duplication of Nrf2-Keap1 complex and loss of effector function, respectively. We have performed a comprehensive transcriptome analysis of fish liver to identify Nrf2 targets and to predict the full complement of Nrf2-responsive genes across teleost-specific whole genome duplication. Unlike most other teleosts, zebrasfish (Danio rerio) are an excellent model system to understand the role of nrf2 in regulating cellular defenses against oxidative stress due to the availability of transgenics, imaging capabilities, and molecular tools. We have shown that cadmium (Cd), an nrf2 activator and inhibitor of olfactory function, elicits a dose-dependent induction of nrf2-regulated genes associated with adaptation to oxidative stress (e.g., heme oxygenase 1, glutathione S-transferase pi, and glutamate-cysteine ligase catalytic subunit) in zebrafish larvae, and in olfactory tissues of adult zebrafish. Cd-mediated increases in gene expression were attenuated by knockdown of nrf2a, which also further disrupted olfactory behavior and coincided with the loss of olfactory sensory neurons. Transcriptome analysis of liver tissues from Cd-exposed adult zebrafish suggests that liver nrf2a is involved in protection against Cd-induced cellular oxidative stress. Our studies are also utilizing a recessive loss-of-function zebrafish mutant of Nrf2 (nrf2a<sup>−/−</sup>) to further define the role of Nrf2 in maintaining zebrafish sensory behaviors under chemical exposures. We are also cloning the promoters of fish genes containing consensus electrophile responsive elements (EpREs) and analyzing their responsiveness in transfected cells exposed to environmental chemicals to better understand the responsiveness of potential Nrf2-regulated genes in other teleosts. In light of these data gaps and the presence of paralogues in fish nrf2, care should be exercised in extrapolating fish Nrf2 induction responses across aquatic species. Supported by NIEHS P42-004696 and NSF 339637.

795 A Role for Astrocytic Nrf2 Activation in Neuroprotection
I. A. Johnson. Pharmacology and Toxicology, University of Wisconsin-Madison, Madison, WI.

Increased oxidative stress has been associated exposure to many environmental chemicals. In addition, oxidative stress has been implicated as a major component in neurological damage or disease. The list includes but is not limited to traumatic brain injury, stroke, pesticide exposure, metal exposure, Alzheimer’s disease, Parkinson’s disease, Huntington’s disease, and amyotrophic lateral sclerosis. Transcriptional activation of protective genes is mediated by a cis-acting element called the antioxidant responsive element (ARE) that binds the transcription factor Nrf2 (NF-E2-related factor 2). Activation of this pathway protects cells from oxidative stress-induced cell death. We hypothesize that Nrf2-ARE activation in astrocytes confers resistance to a variety of neurotoxic insults. In primary neuronal cultures, Nrf2 activation in astrocytes is a primary mechanism conferring resistance to oxidative stress. Similar cultures generated from ARE-hPAP (human placental alkaline phosphatase) reporter mice demonstrated selective activation of the Nrf2-ARE pathway in astrocytes suggesting that Nrf2 activation in the astrocyte somehow confers resistance to naïve neurons. To determine if this applied in vivo, mice overexpressing Nrf2 in astrocytes GFAP-Nrf2 were generated. Genetic models of Parkinson’s and Alzheimer’s disease are currently being examined for changes in oxidative stress, mitochondrial dysfunction, and altered proteostasis. This presentation will discuss how astrocytic Nrf2 activation modulates the ability of neurotoxic insults to disrupt these critical cellular pathways. (Supported by ES08089 and AG03514).

796 The Molecular Mechanisms of Nrf2 Regulation beyond Keap1: Developing Therapeutics Targeting the “Correct” E3 Ubiquitin Ligase for Nrf2 Activation

The cellular defense response regulated by Nrf2 is crucial in maintaining cellular homeostasis. Dysregulation of the Nrf2 pathway has been found in many human diseases including neurodegenerative diseases. Through detailed mechanistic investigations, Keap1 has been revealed as the primary regulator of Nrf2 by functioning as a substrate adaptor of the Keap1-Cul3-Rbx1 E3 ubiquitin ligase. Many efforts have been geared to identify small synthetic molecules or natural products that inactivate Keap1-mediated ubiquitylation of Nrf2, thus activating the Nrf2 pathway. From these efforts, many Keap1-targeting Nrf2 activators have been identified. These Nrf2 activators have proven to be useful in protecting against both cancer and neurodegenerative diseases in rodent models and in human clinical trials. The pros and cons of these Nrf2 activators will be discussed. Subsequent to Keap1, other E3 ubiquitin ligases of Nrf2 have been identified. First, β-TrCP-Skp1-Cul1-Rbx1 was identified as an E3 ubiquitin ligase for Nrf2. More recently we identified Hdrl1, an endoplasmic reticulum membrane integral protein, as another E3 ubiquitin ligase for Nrf2. Activation of the XBP1-Hdrl1 arm of the unfolded protein response transcriptionally up-regulated Hdrl1, resulting in enhanced Nrf2 ubiquitylation and degradation and, ultimately, attenuation of the Nrf2 signaling pathway. In this talk, I will discuss how Nrf2 is regulated by different E3 ubiquitin ligases under physiological and pathological conditions and the therapeutic importance of targeting the correct E3 to prevent Nrf2 loss during the progression of neurodegenerative diseases.

797 Evaluating and Quantifying Stress for Inclusion in Cumulative Risk Assessment

The environmental justice movement has long recognized disproportionate exposure to chemical and nonchemical stressors in vulnerable populations. While chemical stressors are clearly defined, the term nonchemical stressor covers a broad landscape from physical (e.g., radiation, heat) to psychosocial (e.g., fear of violence). Here, we define nonchemical stressors as factors that stimulate a physiological stress response, with a particular focus on stressors that are relevant to people living in vulnerable communities. Both nonchemical and chemical stressors can contribute to multiple diseases (e.g., cardiovascular disease, asthma) that have higher incidence in vulnerable communities. However, there are many challenges to moving forward with quantitative risk assessments that accurately account for chemical and nonchemical stressors. Progress toward this goal requires focused research attention on developing and validating approaches for measuring the physiological effects of nonchemical stressors and interactions between chemical and nonchemical stressors. Additionally, advancement will require developing and evaluating case studies
that adapt available approaches from epidemiology, toxicology, and risk assessment to estimate cumulative risk from chemical and nonchemical stressors. This workshop will bring together experts to discuss the latest science aimed at evaluating chemical and nonchemical stressors and incorporating them into cumulative risk assessment. Discussion will encompass a broad range of diseases (cardiovascular disease, neurodevelopmental delay), chemicals (air pollutants, metals), and stress types (maternal stress, chronic stress). Throughout the workshop, speakers will discuss promising approaches, knowledge gaps, and suggested future research. The concerted, multidisciplinary effort embarked in this workshop will help to shed light on the real impact of exposure to chemical and nonchemical stressors on health and disease in our most vulnerable communities. (This abstract does not reflect US EPA policy.)

W 800  Quantifying “Stress” in Epidemiological Studies
D. B. Miller. CDC-NIOSH, Morgantown, WV.

Using animal data in assessing the cumulative risk caused by exposures to chemical and non-chemical stressors requires extrapolation from animal to human. Incorporating epidemiological data in the evaluation obviates this need but requires methods able to quantify the levels of stress and their association with disease. Prolonged exposure to non-chemical stressors of both a psychological and physical nature (i.e., chronic stress) as well as to various pollutants can play an etiological role in disease including cardiovascular disease (CVD). Dr. Miller will discuss the measurement of stress and the most suitable metrics for quantifying stress in epidemiology studies while emphasizing the caveats and limitations in their use. Data from the Buffalo Cardio-Metabolic Occupational Police Stress (BCOPS) study provides information on the body systems (e.g., hypothalamic-pituitary-adrenal (HPA) axis and autonomic nervous system (ANS)) involved in responding to stressors and their possible relationship to CVD. Police officers are a susceptible and marginalized population who, relative to the general population, suffer greater CVD morbidity and mortality that may be linked to inherent police work stressors. These include long work hours, shift work, trauma, high demand, and pollutant exposure. The longitudinal and multi-factorial design of the BCOPS study will allow us to determine whether stress measures/biomarkers are associated with the chronic progression of subclinical CVD, metabolic derangements and psychiatric disorders. In cross-sectional data a deranged HPA response, as measured by the salivary cortisol response to challenge, is associated with impaired brachial artery reactivity and metabolic syndrome – predictive indicators of CVD. Self-evaluation of police stressors revealed gender specific associations with poor HPA response and low heart rate variability. Our findings suggest a complex association between these various endpoints that often differ by gender but are potentially interpretable and predictive using allostatic models of causation.

W 801  A Framework for Examining Social Stress and Susceptibility to Air Pollution in Respiratory Health

There is growing interest in disentangling the health effects of spatially clustered social and physical environmental exposures and in exploring potential synergies among them, with particular attention directed to the combined effects of psychosocial stress and air pollution. Both exposures may be elevated in lower-income urban communities, and it has been hypothesized that stress, which can influence immune function and susceptibility, may potentiate the effects of air pollution in respiratory disease onset and exacerbation. In this presentation, I will review the existing epidemiologic and toxicologic evidence on synergistic effects of stress and pollution, and describe the physiologic effects of stress and key issues related to measuring and evaluating stress as it relates to physical environmental exposures and susceptibility. Finally, I will identify some of the major methodologic challenges ahead, as we work to validate current methods to clustered social and physical exposures and accurately describing the interplay among these exposures. As this research proceeds, recommendations include careful attention to: the relative temporalities of stress and pollution exposures, non-linearities in their independent and combined effects, physiologic pathways not elucidated by epidemiologic methods, and the relative spatial distributions of social and physical exposures at multiple geographic scales.

W 802  Quantifying Chronic Stress Exposure for Cumulative Risk Assessment: Lessons Learned from a Case Study of Allostatic Load

Although multiple methods of quantifying environmental chemical exposures have been validated for use in human health risk assessment, quantifying chronic stress exposure is more challenging. Stress is a consequence of perceiving an “exposure” (e.g., violence, poverty) as more than one can handle (i.e., “stressful”). Humans respond to stressful exposures via measurable physiological changes mediated by the hypothalamic-pituitary-adrenal axis. Chronic stress can result in physiologic dysregulation of the stress response, which can be quantitatively estimated via allostatic load (AL). AL has been defined as the number of physiological measures across multiple systems, responding outside of the normal range. While chronic stress has been quantitatively estimated as AL, chronic stress is rarely considered in environmental human health risk assessment. In such assessments, the hazard index (HI) approach is used to estimate risk from multiple stressors. These stressors have
Infant Formula Innovations for Improved Infant Health

R. Clemens, Horii/USC School of Pharmacy, University of Southern California, Los Angeles, CA.

Since implementation of the Infant Formula Act (1980) and its amendments (1986), and the FDA’s critical overview of infant nutritional requirements (1996), there have been few innovations. Those few innovations include technologies and ingredients to reduce cow milk and soy protein allergenicity, addition of docosahexaenoic acid for improved cognitive development and visual acuity, introduction of some strains of probiotics to maintain gut health, and inclusion of an array of oligosaccharides which may also maintain gut health. As the scientific and medical communities increase their understanding of human milk biology, progressive safety and clinical research may contribute to infant formula innovations. Several case reports that illustrate these fundamental research approaches required for FDA review and prior to product commerce within the United States will be discussed.

The Neonatal Pig As a Research Model for Infant Formula Safety Assessment

B. A. Thorsrud, Developmental & Reproductive Toxicology, MPI Research, Mattawan, MI.

Preclinical testing of novel ingredients planned to be used in infant formula requires evaluation of their safety during the lactation period. The most common animal research models for preclinical toxicity testing are the rat and mouse. However, there are inherent limitations to using these species for infant formula testing. In addition, their dietary differences and the inability to metabolize high-fat diet in a manner similar to humans does not make it an attractive or appropriate model for testing new substances in infant formula. The neonatal pig seems to be a good test model as it covers the period from birth to weaning facilitating the animal developmental time frame experimentally feasible (weeks versus months). Furthermore, the neonatal piglets have a similar physiology and development to humans with respect to the gastrointestinal tract and the immune system hence consumption and metabolism of some strains of probiotics to maintain gut health, and inclusion of an array of oligosaccharides which may also maintain gut health. As the scientific and medical communities increase their understanding of human milk biology, progressive safety and clinical research may contribute to infant formula innovations. Several case reports that illustrate these fundamental research approaches required for FDA review and prior to product commerce within the United States will be discussed.
Graphene, a one-atom-thick monolayer of carbon, is an engineered nanomaterial (ENM) with physical and chemical properties that may offer application advantages over other carbonaceous ENMs, such as carbon nanotubes (CNT). As use of graphene nanomaterials (GNMs) in a variety of industries and manufacturing increases, the potential for respiratory exposure, particularly in the workplace, also rises. Unlike CNT, toxicity of GNMs has not been well defined. In addition, GNMs can vary in dimension, surface chemistry, number of layers, and other physico-chemical parameters, which in turn may affect toxicological potency of the material. The goal of this workshop is to present the most recent toxicological research findings in the field of GNMs and gain an understanding of the hazard and risk for exposure. The workshop will cover the physico-chemical characteristics and applications of a variety of GNMs, potential exposure in occupational settings, toxicity related to size and composition following various methods of pulmonary exposure in animal models, and comparative toxicity to well-defined carbonaceous ENMs. The outcome for the session is to establish whether GNM exposure poses a potential health hazard by providing an understanding of GNMs and conveying the most recent material science expertise and toxicological research related to respiratory exposure to various forms of GNMs.

**W 808 Pulmonary Toxicity of Graphene Nanomaterials: An Emerging Concern in Manufacturing and Applications?**

J. R. Roberts and A. Erdely. HELD/PPRB, NIOSH, Morgantown, WV.

Manufacturing of graphenes (Gr) may increase the risk of respiratory exposure to workers. The goal of this study was to assess toxicity of three non-oxidized Gr of different sizes [20 μm lateral x 7-10 nm thick (Gr20), 5 μm lateral x 7-10 nm thick (Gr5), and <2 μm lateral x 1-2 nm thick (Gr1)] following respiratory exposure. Carbon black (CB, 15 nm diameter), was used as a particle control. Particles were characterized for surface area (SA), structure, zeta potential, surface reactivity, and agglomeration in vehicle (dispersion medium; DM). Gr samples were found to be similarly composed of two graphite structures, were not surface-reactive, and consisted of 64-72, 75-84, and 28-30 layers for Gr20, Gr5, and Gr1, respectively. Gr1 had the greatest SA followed by CB, then Gr20 and Gr5. Agglomeration in DM ranged from ~5-300 and 0.5-60 μm, for Gr20 and Gr5, respectively, and from ~0.2-5 μm for both Gr1 and CB, with no differences in zeta potential. In vivo, male C57Bl/6J mice received 4 or 40 μg of Gr1, Gr5, or Gr20, or 40 μg of CB, or DM by pharyngeal aspiration. At 4 h (0 d), 1d, 7d, 1m, and 2m post-exposure, pulmonary, lung, and systemic inflammation and oxidative stress, distribution, clearance, and histopathology were evaluated. Gr deposition in airways vs alveoli, and lung clearance, were size-dependent. No toxicity was observed in any of the low doses. Gr20 and Gr5 increased indices of lung inflammation and injury in lavage fluid and tissue gene expression to a greater degree and duration than Gr1 and CB. Gr5 and Gr20 also showed no to minimal lung epithelial hypertrophy and hyperplasia with resolution over time. In addition, the aorta and liver inflammatory and acute phase genes were transiently elevated in Gr5 and Gr20. When compared to similar doses of carbon nanotubes in the literature, non-oxidized Gr were less potent inducers of toxicity.

**W 809 Physical and Chemical Properties of a Variety of Graphene Nanomaterials—Engineering Materials for Specific Applications**

A. Kyridis and I. Chaudhuri. Cabot Corporation, Billerica, MA.

Graphene-based materials are a new family of carbon products that hold significant promise because of their unique combination of electrical, mechanical, and optical properties. These materials may lead to ground-breaking new applications in electronics and displays. They may also find uses in many of the legacy applications of carbon additives by enhancing functionality such as reinforcement of elastomers, electrical and thermal conductivity in polymers, among many others. The performance characteristics of graphene-based materials in these applications will be driven largely by their morphology and surface chemistry as well as the quality of their dispersion in the host matrix. There are over a dozen different routes for the synthesis of graphene-based materials relating to the diversity of the potential applications of graphenes in order to meet all performance requirements. These routes produce materials with very different physicochemical characteristics that are quantified through certain key analytics such as their surface area, thickness, lateral size, and surface chemistry. Some of these characteristics can vary over several orders of magnitude for many of these materials. This presentation will provide an overview of the major synthetic routes and the relevant physicochemical properties of the intermediates and materials produced by these routes. Properties relevant to specific commercial applications will be explored. Understanding how these characteristics relate to the toxicology of these materials via different exposure routes will be an important factor in engineering safer graphene products.

**W 810 Occupational Exposures along the Graphene Product Value Chain: Production, Formulation, and Use**

C. M. Sayes. RTI International, Durham, NC.

Graphene is a two-dimensional crystalline allotrope of carbon with atoms in a densely-packed regular hexagonal lattice. It displays high strength and durability for current density, high surface area/weight ratio, and high light transparency. These characteristics make graphene appealing to industry sectors such as electronics, energy, transportation, and building materials. Because of this unique electronic and chemical properties. Currently, little is known about the potential toxicity associated following an inhalation exposure. A 5-day repeated inhalation toxicity study for graphene was conducted using a nose-only inhalation system for rats. A total of three groups (20 rats per group) were compared: (1) control (ambient air), (2) low-dose (0.08 ± 0.14 mg/m³ graphene) and (3) high-dose (3.86 ± 0.94 mg/m³ graphene). Rats were exposed to graphene for 6 h/day for 5 days, followed by recovery for 3, 7 and 28 days. Bioaccumulation and macroparticle ingestion of graphene were evaluated in the rat lungs. Exposure of graphene did not change body weight or organ weight of rats after the 5-day exposure and during the recovery period. Graphene was readily ingested by alveolar macrophages in the exposed groups. There was, however, no statistically significant difference in the level of lactate dehydrogenase, protein and albumin between the exposed groups and the control group, indicating very little lung injury following exposure. These results suggest that a 5-day repeated exposure of graphene produce a minimal toxic effect at the concentrations and time points selected in this study.
Comparative Inhalation Toxicities of Graphene and Other Carbonaceous Nanomaterials

R. Landiesle, BASF Product Safety - Experimental Toxicology and Ecology, Ludwigshafen, Germany.

Graphene, graphite nanoplatelets, carbon nanotubes and carbon black are carbon-based nano-materials with broad technological applications. Carbon nanotubes and carbon black possess different inhalation toxicities, whereas less is known about graphene and graphite nanoplatelets. In order to compare the inhalation toxicity of the mentioned carbon-based nanomaterials, male Wistar rats were exposed head-to-tail to atmospheres of the respective materials for 6 hours per day on 5 consecutive days. Target concentrations were 0.1, 0.5, or 2.5 mg/m³ for multi-wall carbon nanotubes and 0.5, 2.5, or 10 mg/m³ for graphene, graphite nanoplatelets and low-surface carbon black. Toxicity was determined after end of exposure and after three-week recovery using broncho-alveolar lavage fluid and microscopic examination of the entire respiratory tract. No adverse effects were observed after inhalation exposure to 10 mg/m³ graphite nanoplatelets or relatively low specific surface area carbon black. Increases of lavage markers indicative for inflammatory processes started at exposure concentration of 0.5 mg/m³ for multi-wall carbon black. Increases of lavage markers indicative for inflammatory and low-surface carbon black. Toxicity was determined after end of exposure and after three-week recovery using broncho-alveolar lavage fluid and microscopic examination of the entire respiratory tract. No adverse effects were observed after inhalation exposure to 10 mg/m³ graphite nanoplatelets or relatively low specific surface area carbon black. Increases of lavage markers indicative for inflammatory processes started at exposure concentration of 0.5 mg/m³ for multi-wall carbon black.

Challenges in the Life Cycle of a Toxicologist

T. E. Levine¹ and W. I. Brock². ¹Retired, Arlington, VA and ²Brock Scientific Consulting, Montgomery Village, MD.

Toxicologists face different challenges at different stages of their career life cycle. This session will explore some of these challenges, and offer potential solutions to those challenges. Industry, academia, and government employ 47 percent, 21 percent, and 14 percent of toxicologists, respectively. For students and postdoctoral trainees applying for jobs in these sectors, the initial challenge in getting that first position is presenting oneself on paper and in person. The goal of the first presentation will be to demystify the US federal hiring process with specific emphasis on how to describe oneself on paper as a toxicologist in order to be considered for a government position. The second speaker will address early-career toxicology positions in industry, and how the roles and responsibilities of the entry-level toxicologist contribute to the developing career. For the mid-career toxicologist, the challenge is often how to keep progressing, whether to pursue a technical or managerial track, and whether to consider transitioning to peripheral disciplines. The mid-career toxicologist speaker will provide guidance on how to develop a broad skill-set to enhance career opportunities. The fourth speaker will address how work/life satisfaction can be attained in the context of careers in science, which are very often a way of life and full more than a job. The tools presented will assist attendees in identifying strategies that can have the biggest impact on their work/life satisfaction and in developing their own personal action plan, whatever their career stage. The final challenge for many toxicologists is how to transition to semi- or full retirement; many toxicologists continue to work either full or part-time as consultants. Some choose to pursue interests long deferred due to the demands of full-time work. The last presentation will explore challenges encountered by the toxicologist as “retirement” and the twilight of a career approach. At the end of the session, a panel discussion will convene to address specific issues that arise in the career of the attendees, and discuss strategies for advancing the toxicology career.

The Nuts and Bolts of Getting Hired As a Government Toxicologist

T. E. Levine, Retired, Arlington, VA.

Getting your first professional job is often daunting. Finding the best way to describe yourself on paper so your resume is seriously considered and presenting yourself successfully at an interview are challenges common to all jobseekers. However, the Federal government’s system for hiring toxicologists can be particularly challenging to those coming out of graduate school or post-doctoral fellowships, or seeking a mid-career change. Most Federal hiring uses a front-end process in which people with no scientific expertise make decisions about who is qualified to be interviewed based on highly prescribed standards. These standards pertain to school coursework and leave no room for experiential modification. Sometimes toxicology jobs are advertised under other titles in order to circumvent these strict standards. Without a connection to someone on the inside, who can help navigate the hiring process, qualified applicants may miss job opportunities because they do not understand how to negotiate the government application process. This presentation will attempt to demystify the government hiring process by explaining what the standards require, how to present an application so that a non-scientist can easily recognize your qualifications and how to identify toxicology jobs in the Federal government. This session should be of interest in particular to those who might want to work in the Federal government as well as people administering training programs seeking a better understanding of how to structure their programs and advise students.

Taking the Leap: Myths and Realities of Starting Your Career As an Industry Toxicologist

J. S. Molfino, FORUM Pharmaceuticals, Inc., Boston, MA.

Industry careers in toxicology represent the largest percentage of jobs within the field, but effectively encompass a diverse background of career options. Entry level positions for an industry toxicologist can therefore vary significantly amongst traditional industry employers, such as Contract Research Organizations (CROs), the chemical or cosmetic industries, and biotech or pharmaceutical companies. Roles and responsibilities for entry level toxicology positions within these employment areas will be discussed and contrasted. In addition to the breadth of industry employment opportunities, these careers can be highly competitive given the traditionally lucrative benefits and flexible work environments. Availability of industrial toxicology jobs are also subject to vacillations of the global economy and regulatory environments, which can result in expansive hiring to meet demands at any costs or rapid contractions of the workforce. For a toxicologist looking to start a career in industry, standing out amongst candidates with existing industry experience can be challenging, particularly during periods of surplus talent. Strategies for leveraging non-industry experience will be explored, in the context of what skills and expertise are important to industry employers. This session is directed primarily toward graduate students, post doctoral fellows, and early career toxicologists interested in industry positions, with a focus on understanding entry level toxicology career options and the skills necessary to obtain these positions.

Mid-Career Challenges and Opportunities for the Toxicologist


This presentation will provide perspective of being a toxicologist in small, mid-size and large companies spanning US Environmental Protection Agency and the US Food and Drug Administration regulated arenas. The speaker will discuss the unique challenges and opportunities presented by the multiple “hats” that are required, and the value of being active in the scientific community. This presentation will also discuss how to prepare early in the career for later stages and how/whether to consider transitioning to peripheral disciplines.

Improving Your Work/Life Satisfaction

D. J. Dean, Association for Women in Science, Hedgesville, WV. Sponsor: T. Levine.

Given the work environments and expectations for individuals in science, and the fact that a career in science is very often a way of life and far more than a job, work-life satisfaction can be elusive. This presentation will present personal work-life strategies that can enable toxicologists to effectively navigate competing and conflicting demands on their time and energies. Session attendees will come away with the ability to define for themselves what work-life satisfaction is and is not. A context for an action plan that can be implemented immediately or in the future will be presented. Seven key aspects of achieving and maintaining work-life satisfaction will form the framework for the presentation. This framework will provide ways that individuals can (1) think about their current personal or professional choices that may be out of alignment with their priorities and values, (2) examine the short and/or long term benefits of their choices; (3) ponder why certain choices are important to them and the ‘costs’ of choices; and (4) find the personal work-life satisfaction strategies that work for them within the systems in which they work.
819 Challenges for the Late-Career Toxicologist

T. B. Knudsen

174 SOT 2015 Annual Meeting

Agency, Research Triangle Park, NC, 3Lockheed Martin, Research Triangle Park, NC.

M. C. Leung1, 2,  J. Phuong2,  N. C. Baker3,  N. S. Sipes1, 2,  G. Klinefelter4,
ter that drives toxicologists is when to stop working. This can be due to various reasons such as:
1. Decompression period: This is the time after retirement when toxicologists transition to a new phase of life.
2. Reentering the workforce: Many toxicologists may choose to return to work in a different capacity.
3. Volunteering: Some toxicologists may choose to volunteer in various roles.

820 Comparing OASIS Estrogen/Androgen Receptor Binding QSAR Predictions to Results from ToxCast II Estrogen/Androgen Receptor Binding Assays


One goal of the US-EPA ToxCast program is to use a combination of in silico and high-throughput (HT) assay signatures to screen untested chemicals as a means of prioritizing in vivo testing. In ToxCast II, >1800 compounds were tested in HT enzyme screening assays. We compared the three respective NovaScreen HT assays for estrogen - ER (human, bovine and mouse) and androgen - AR (human, chimpanzee and rat) receptor binding to the predictions of OASIS, a three-dimensional quantitative structure-activity relationship (3D-QSAR) model developed to assess the binding affinity to the mammalian nuclear receptors. Analysis of the ER - QSAR model predictions indicated the in domain chemicals in the three assay platforms' results have low sensitivity (< 56%) but high specificity (95%). Analysis of the AR - QSAR model predictions indicated the in domain chemicals had very high sensitivity (92-100%) and acceptable specificity (70-81%). When HT results were restricted to compounds within the respective domain of the ER and AR - QSAR model and showing consistent agreement of ER and AR binding at AC50 (< 1 μM for all three binding assays, the QSAR models accurately predicted binding for the compounds 100% of the time. These results suggest the OASIS ER/AR QSAR models can be used to screen potential ER/AR binding but highlight the need to better understand concordance between HT assay platforms and sensitivity of the models.

821 Systems Toxicology of Male Reproductive Development: Profiling 774 Chemicals for Molecular Targets and Adverse Outcomes

M. C. Leung1, 2,  J. Phuong2,  N. C. Baker3,  N. S. Sipes1, 2,  G. Klinefelter4,

M. T. Martin1, K. W. McLaurin1, 4,  W. Setzer1,  S. P. Darney1,  R. Judson4 and T. B. Knudsen1, 3Oak Ridge Institute for Science and Education, Oak Ridge, TN.

National Center for Computational Toxicology, U.S. Environmental Protection Agency, Research Triangle Park, NC, 3Lockheed Martin, Research Triangle Park, NC and 4National Health and Environmental Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, NC.

Adverse trends in male reproductive health have been reported for increased rates of testicular germ cell tumor, low semen quality, cryptorchidism, and hypospadias. An association with prenatal environmental exposure has been inferred from human and animal studies underlying male reproductive developmental defects. The present study established the links between environmental chemicals, molecular targets, and adverse outcomes using U.S. EPA animal study (ToxRedDB) and high-throughput screening (HTS; ToxCast) databases. This systems-based approach revealed a phenotypic hierarchy of testicular atrophy, sperm effects, and malformations across 63 of 774 (8%) chemicals in ToxRedDB, resembling what might be expected in testicular dysgenesis syndrome (TDS) in humans; 48 of these 63 chemicals had ToxCast data, interacting with 126 of 286 (44%) molecular targets in HTS. Although estrogenic and anti-androgenic activities have been extensively studied in TDS, the present study showed these receptor targets to be only a subset of the potential landscape of molecular targets. Clusters of chemical classes (e.g. phthalates, conozoles, carboxamides, and phenol compounds) and in vitro bioactivity (e.g. nuclear receptors, vascular remodeling proteins, and cytochrome-P450 reductases) suggested multiple novel pathways in male reproductive developmental defects. This points to the need for computational models that capture multiple adverse outcomes and simulate spatial dynamics to advance a predictive and mechanistic understanding of TDS from in vitro profiling. [This abstract does not necessarily reflect US EPA policy].
The prediction of developmental and reproductive toxicity (DART) in preclinical species using early screening assays such as the zebrafish ZETA assay is considered an important contribution to implement predictive toxicology in drug discovery. The optimization of assay conditions and evaluation of exclusion criteria is a key point. The assay protocol was established and validated with 37 reference pharmaceutical compounds. As 1% (v/v) DMSO was shown to be tolerated, all compounds were dissolved in DMSO to maximize compound concentrations. Egg incubation was performed under controlled conditions at 28.5°C and 12h dark-light cycle for 5 days. To get a better understanding of the time course of the effect, the morphological evaluation was performed at 2 days post fertilization (dpf) and 4 dpf. The evaluated parameters were the relative abundance of morphological alterations (embryotoxicity) and of lethality. Calculation of the 50% embryotoxic and lethal concentrations (EC50 and LC50) using non-linear regression and the ratio of LC50/EC50 to generate a teratogenic index (TI) were used to determine the outcome of the assay. In order to increase the accuracy of TI determination at least 1 replicate experiment with optimized concentrations were performed. We could demonstrate that the inclusion of an early time point and optimization of the concentration spacing improved the interpretation of the results. Based on the results of the reference compounds in ZETA in comparison with in vivo results the threshold TI for positive outcome was defined (>1.2) and correction criteria were set to improve predictivity (e.g. exclusion of effects > 1mM). The assay protocol resulted in overall excellent concordance with preclinical in vivo developmental toxicity of approximately 80%. Inclusion of bioanalysis will be the next step for further optimization. These validation results showed that the ZETA assay can be used effectively and early in development for de-risking developmental toxicity of drug candidates.

Peer-reviewed experimental studies on endosulfan were studied to discern how it initiates toxicity pathways to create adverse outcomes in developing and mature organs. The Mode of Action (MOA)/species concordance/human relevance framework, created by staff of US EPA and international organizations (ILSI, IPCS, and Health Canada), provides a weight-of-evidence approach to risk assessment and Health Canada), provides a weight-of-evidence approach to risk assessment and Health Canada), provides a weight-of-evidence approach to risk assessment and Health Canada), provides a weight-of-evidence approach to risk assessment and Health Canada), provides a weight-of-evidence approach to risk assessment and Health Canada), provides a weight-of-evidence approach to risk assessment and Health Canada), provides a weight-of-evidence approach to risk assessment and Health Canada), provides a weight-of-evidence approach to risk assessment and Health Canada), provides a weight-of-evidence approach to risk assessment and Health Canada), provides a weight-of-evidence approach to risk assessment. The framework’s use for carcinogens now includes noncarcinogens in which the degree of toxicity involves species-specific mechanisms or age-specific effects on developing organs. Five MOAs causing male reproductive toxicity in rodents were discerned during gestation, adolescence, and maturity, plus one for neurotoxicity. Endocrine disruption (MOA 1D) occurs in the developing brain during gestation. Oxidative stress (MOA 2D) occurs in the developing male rat reproductive system during gestation and continues throughout the first wave of spermatogenesis which begins at birth and ends after puberty. It can occur in humans during gestation, and after a silent period from early infancy until the first wave of spermatogenesis initiates puberty. Inhibition of gap junction intercellular communication (GJIC), MOA 3D, is most critical during the first wave of spermatogenesis in rodents and humans. Neurotoxicity (MOA 1A) dominates in adults. Oxidative stress (MOA 2A) occurs in adults to a lesser degree, depending on diet, because antioxidant enzymes are established, and inhibition of GJIC (MOA 3A) is not critical in adults, so toxicity is less and is reversible due to feedback inhibition. The key events of the MOAs observed in animal and human short-term in vivo and in vitro data increase confidence that endosulfan causes toxicity in humans. A child-specific reference dose (chRD) from exposure to >6.5 x 10^-5 mg/kg/d was calculated from an in vitro experiment in rats (Sinha et al., 2001).
and dietary components; these exposures also alter the microbiota species composition and microbiome. Breakthroughs in analytical methods and tools have accelerated the understanding of the roles of the microbiome in human health and disease. Metagenomics represents a powerful approach to define the microbiota species composition in a given sample through detection of their genes and gene products, and the role of altered microbiomes in disease. Vast microbiota diversity exists within and between humans, each population producing a large array of their own metabolites and products, which contributes to regulating the overall host biology within this symbiotic relationship. Metabolic profiling strategies, including new mass spectrometric and bioinformatics techniques, are being developed to analyze the microbial metabolism to generate chemical maps that describe the molecular connections and communications between host cells and microbiome. Understanding microbiome-metabolome-host interactions will drive the identification of novel drug targets and development of new therapeutic interventions for many diseases. Rapid advances linking microbiome, the microbiota, and metabolome and their role in health and disease represent an important frontier from a toxicological perspective. The goal of this session is to feature eminent scientists who have made important contributions and advances to current knowledge of the microbiome. Integrated areas that will be explored include metagenomic characterization of microbiomes in human and environmental ecosystems, changes in the microbiome from birth to death and important applications to forensics, the molecular characterization of the microbiome and the challenges of linking large amounts of genome sequencing and mass spectrometric data, and the metabolic crosstalk between the host and the symbiotic microbiome and its influence on disease and therapeutic, personalized medicine interventions.

**829 Metagenomic Approaches for Understanding the Microbial Foundations of Complex Ecosystems**


Advances in DNA sequencing technology have created new approaches to characterizing the microbial composition of diverse ecosystems. The same strategies used for shotgun sequencing of individual genomes are now being applied to sequencing of the collective DNA of diverse environments as common as soil, the open ocean, and the human gastrointestinal tract and as specialized as deep earth subsurface, natural springs at pH 10, and hospital air. Based on its universal conservation, extensive reference sets and low cost analysis of regions of the gene encoding 16S ribosomal (r)RNA is generally a first option for defining the microbial composition of an environmental sample. 16S rRNA profiling has a number of limitations, however, including imprecise mapping of sequence reads to organisms, challenges in defining appropriate statistical schemes for dataset comparison, and perhaps most important – it provides no information about genome content or function.

Metagenomic sequencing, which involves shotgun sequencing of the entire isolated DNA from an environment, produces tremendous amounts of novel information that also presents analytical challenges. Limitations in assembly of short-read sequence metagenomes datasets means that most genes are not present in full-length form and cannot be linked into genomes for all but the most abundant organisms. The extent of strain-level diversity is also just beginning to be appreciated, changing our view of what it means for an organism to be “present” in a sample. Applications of metagenomic analysis to human disease and to the interaction of geochemical parameters with microbial diversity will be presented.

**830 Dynamics of the Human Microbiota**

R. Knight1, 2. 1Department of Pediatrics, University of California San Diego, San Diego, CA and 2Department of Computer Science and Engineering, University of California San Diego, San Diego, CA. Sponsor: P. Goering.

Advances in technology allow us to understand the human microbiota not as a static entity but as a complex, changing ecosystem that records substantial data about our lifestyles, our environmental exposures, and the people we live with. Key advances include understanding changes in the human microbiota associated with early life events such as delivery mode and antibiotics, subsequent development of the microbiome throughout life, and even the development of the microbiome after death and its potential utility for forensics. Of particular value for toxicological studies is the ability to transfer the microbiome to germ-free mice, allowing individualized tests of function and subsequent therapies to be tested in personalized rodent models. Relating human to rodent microbiome timescales, especially for dietary studies, and relating multi-omics data in a time-series context, remain outstanding challenges. Rapid progress being made in these areas will allow far more powerful studies of the effects of drugs in mammalian hosts and our ability to stratify responders from non-responders for a wide range of therapies.

**831 A Community-Based Molecular GPS from Microbes to People–Implications for Forensics, Health Monitoring, and Therapeutics**

P. C. Dorrstein. Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California San Diego, San Diego, CA. Sponsor: P. Goering.

The chemical make-up of biology is incredibly complex. A single host is made up of molecules that come from host cells, microbes, the food they eat, environmental exposure and the same molecules in a variety of metabolized states. As mass spectrometers are becoming faster and more sensitive, we can detect a lot of molecular information. There are now mass spectrometers that can analyze 10,000 samples a day. However, there is no infrastructure to analyze this amount of molecular information or to correlate this information to other Big Data generating approaches such as sequencing. On average, however, only 1-5% of all the molecular information that is collected by mass spectrometry can be annotated. There is simply too much information for one person or lab to analyze with the existing tools that are available. In this lecture, we will explore the strategies for organizing and visualizing the massive amount of information obtained in mass spectrometry based microbiome projects. The key is that this information needs to be digested in intuitive and informative ways. More importantly, we will show how crowd sourcing of the molecular analysis by 1000s of users globally is changing the way this type of data is analyzed. Using these tools, we will highlight the molecular and microbial make-up of human lungs as well as human skin and its impact on xenobiotics, antibiotics and global microbial-based specialized metabolism.

**832 Microbiome-Host Metabolic Axes—New Dimensions in Personalized and Public Healthcare?**

J. K. Nicholson. Department of Surgery and Cancer, Faculty of Medicine, Imperial College London, London, United Kingdom.


**833 Alternative Models to Study Classical Toxicants: A Mechanistic View**

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Alternatives to in vitro toxicity testing are increasingly necessary due to regulatory mandates (e.g., REACH legislation), cost and time constraints, as well as ethical considerations. Models are being used in government, industry, and academia to develop a system for chemical screening and prioritization framework, and to study the mechanistic action of compounds traditionally studied in vitro. Graduate and postdoctoral researchers will describe their studies of classical toxicants using cell-based and alternative animal models that demonstrate how in vitro systems can be developed to elucidate mechanistic action of classical toxicants. The first presenter will describe the evaluation of ToxCast compounds to characterize multidimensional developmental and neurotoxicological effects using zebrafish. The second speaker will describe his efforts studying the developmental effects of RyR-active PCBs using embryonic zebrafish as a novel in vivo model. The third presenter will discuss the use of C. elegans in a genetic screen assessing MeHg-associated dopaminergic neuron degeneration, highlighting involvement of SKN-1/Nrf2 and MRP-7. The fourth speaker will present the development of an in vitro omics approach to identify pathways of developmental neurotoxicity, and discovery of gene expression and metabolic changes relating to pesticides, pharmaceuticals, and metals. The fifth presenter will discuss a novel in vitro model for ozone adaptation, analyzing expression of proinflammatory and oxidative stress genes and discovery of the role of histone acetylation in the epigenetic control of ozone adaptation. The
There are tens of thousands of man-made chemicals in the environment, with limited human health hazard information. Relevant biological platforms and new computational tools are needed to prioritize safety testing of these chemicals. The ToxCast program, was developed by the EPA to predict the potential toxicity of chemicals. The high throughput screen assays used in the ToxCast program lack biological complexity and represent an artificial biological environment for testing. However, the versatility of the zebrafish makes it an ideal model to bridge in vitro and mammalian data. We evaluated all 1,060 unique US EPA ToxCast Phase 1 and 2 compounds using the embryonic zebrafish in a high-throughput assay designed to characterize multidimensional in vivo effects at 5 concentrations for developmental and neurotoxicity. We found that 487 induced significant adverse biological response, such as neurotoxicity (e.g.: caffeine, chlorpyrifos, dieldrin, endosulfan, fipronil and nicotine) or affected notochord/lower axial bend formation (e.g: thiram, zinc, retinoic acid and dazomet). The utilization of 18 simultaneously measured endpoints means that the entire system serves as a robust biological sensor for chemical hazard identification. The sensitivity of the developmental embryonic zebrafish assay is able to detect 78% of neurotoxicants identified in the literature. The experimental design enabled us to describe global patterns of variation across tested compounds, evaluate the concordance of the available in vitro and in vivo Phase 1 data with this study, highlight specific mechanisms/value-added/novel biology related to notochord development, and demonstrate that the developmental zebrafish detects adverse responses that would be missed by less comprehensive testing strategies.

### A Zebrafish Model of PCB Developmental Neurotoxicity

G. W. Miller, K. M. Walter, E. B. Frisch and P. Lein, University of California Davis, Davis, CA.

Despite being banned since the late 1970s, polychlorinated biphenyls (PCBs) remain persistent environmental toxicants that pose significant risk to the developing nervous system. We have demonstrated that some non-dioxin-like PCBs alter neuronal connectivity by promoting dendritic arborization in cultured hippocampal neurons via ryosine receptor (RyR)-dependent mechanisms. Structure activity relationships (SAR) of RyR sensitization by PCBs have been established using mammalian cell culture. However, whether this SAR translates to in vivo development and neurotoxicity of PCBs has yet to be tested. Low throughput and high costs make it challenging to perform extensive in vivo SAR studies in rodent models. To address this gap, we are evaluating the embryonic zebrafish as an in vivo model of PCB developmental neurotoxicity. As an initial assessment of the zebrafish model, we tested PCB congeners that exhibit differing RyR activity in mammalian cells: those with negligible activity (PCB 66 and 77), moderate RyR activity (PCB 52 and 153), and high RyR activity (PCB 95). Dechorionated embryos were statically exposed to varying concentrations (0.1-10 μM) of these congeners from 6 to 120 h post fertilization (hpf). Embryos were observed at 24 and 120 hpf by stereomicroscope for mortality and gross malformations, and were subjected to a light/dark stimulus test for locomotive behavior at 120 hpf. Significant concentration-related increases were seen for adverse outcomes in embryos treated with either moderately or highly RyR active congeners, with severity significantly greater in highly RyR active congeners. No differences were observed between treatment groups at 24 hpf, and low activity congeners were not significantly different from vehicle (DMSO) or untreated controls after 120 hpf. These data correlate with the relative potencies of these PCB congeners on RyR activity, suggesting the feasibility of using embryonic zebrafish as a novel in vivo model to study the developmental effects of RyR-active PCBs.

### Identification and Characterization of Molecular Modulators of MeHg-Induced Toxicity in the Genetic Model C. elegans

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Methylmercury (MeHg) exposure from occupational, environmental, and food sources is a significant threat to public health. MeHg poisonings can result in developmental and neurological deficits, and may contribute to the progression to develop the dopamine (DA) neurodegenerative disorder Parkinson’s disease (PD). Although MeHg poisoning has been studied for decades the molecular bases for the toxicities are largely ill-defined. We explore the utility of C. elegans to recapitulate mammalian models of MeHg toxicity, and to ask what genes and molecular pathways are involved in MeHg-induced whole worm and DA neuron pathology. We examine whole animal, embryo, and DA neuron vulnerability to MeHg, utilizing a multi-assay approach to identify and characterize genes involved in MeHg-associated toxicity. Chronic exposure of MeHg conferred embryonic defects, developmental delays, decreases in brood size and animal viability, and DA neuron degeneration. Toxicant exposure also resulted in a robust induction of glutathione S-transferases, at both the mRNA and protein level, that were largely dependent on the PD-associated phase II antioxidant transcription factor SKN-1/Nrf2. A whole genome reverse genetic screen of over 17,000 genes identified 92 genes that affect whole animal viability. A novel multidrug resistant protein, MRP-7, was also identified, and shown to modulate MeHg-associated induction of stress response proteins associated with the endoplasmatic reticulum, golgi apparatus, and mitochondria; and inhibits toxin-associated DA neurodegeneration. This study describes the development of a C. elegans model for MeHg toxicity that will likely prove useful in identifying novel molecular pathways and therapeutic targets that inhibit MeHg-induced cellular pathologies in humans.

### 3D Models and ‘Omic Approaches to Study Pathways of Developmental Neurotoxicity

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In line with the National Research Council report, “Tox-21” proposed paradigm shift in toxicology, we have developed novel 3D models and gene expression to identify pathways of developmental neurotoxicity (DNT). Considering the importance of cell-to-cell interactions in the brain, our laboratory has previously made use of a 3D rat primary neuronal organotypic model granted by the FDA. However, due to interspecies differences causing large problems in drug development our group has established a humanized model of the 3D rat model using human iPSCs. The 3D rat model was exposed from day 7 up to 21 to suspected (developmental) neurotoxins including pesticides (Mebane), drugs (Valproic Acid) and metals (Lead Chloride; PbCl2). Treatment with the different compounds significantly modified the expression of selected genes, related to the different stages of neuronal and/or glial cell development. The mass spectrometry analysis showed differences in metabolic levels between control and treated in concentration dependent manner. In addition, treatment with diverse compounds induced different metabolic profiles. The metabolite inosine has shown to increase glial survival and promote axonal growth. Exposure to PbCl2, and Maneb significantly decreased this metabolite and could disturb the neural development. Indeed, gene expression analysis indicates decreased neuronal/axonal loss in patients. Gene expression studies implied the same with decrease in NF-200 and MBP. Several metabolites involved in glutamate and amino sugar metabolism were lower after exposure to PbCl2, and Maneb, indicating perturbation in these pathways. Moreover, PbCl2, and Maneb exposure lowered several metabolites in the pathway of the neuronal specific metabolite N-acetyl aspartate, an indication of neuronal/axonal loss in patients. Gene expression studies implied the same with decrease in NF-200 and MBP. This study demonstrates that gene expression and metabolomic analysis can be sensitive endpoints for DNT assessment. Moreover, the metabolomics approach has the potential to prioritize and/or group compounds with similar mechanisms based on the metabolic profile.

### Modern Techniques and an Old Mystery: Exploring Mechanisms of Ozone Adaptation

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Exposure to air pollutants, including ozone, is associated with increased morbidity and mortality; hence it is important to understand how these effects are mediated. Controlled human exposure studies have demonstrated that acute ozone exposure leads to reduced lung function and airway inflammation. However, repeated expo-
sure to oxygenates these effects, indicating that sub-chronic exposure results in an adaptive response. Despite being first reported 50 years ago, the mechanisms underlying ozone adaptation remain obscure. We developed an in vitro model using human primary bronchial epithelial cells (pHBECs) grown at an air-liquid interface, to assess ozone adaptation on a cellular level. We monitored the expression of pro-inflammatory and oxidative stress genes, including IL-8, IL-6, COX-2, and HO-1, in response to repeated ozone exposure. We observed that daily two-hour ozone exposures over five days elicited gene expression patterns in pHBECs that are consistent with adaption responses observed in human exposure studies. IL-8 was induced approximately eight-fold on the first day of exposure, but steadily declined with subsequent exposures resulting in a 70% reduction from peak induction by the fifth exposure day. Expression patterns for other target genes followed a similar pattern. Interactions between environmental pollutants and the epigenome can dictate cellular responses in future exposures. Thus, we investigated the role of epigenetic changes in the initiation and maintenance of ozone tolerance. Using chromatin immunoprecipitation we examined the promoter region of the aforementioned genes and found that histone acetylation, an activating modification, varied in a manner that was consistent with changes in gene expression. We have shown that ozone adaptation may be recapitulated in vitro, providing a mechanistic foundation for pollutant adaptation that can be used as a model for the effects of subchronic toxicant exposure, to predict adverse health effects, and identify populations that may be particularly susceptible.

**839 Functional Genomics Approach in Yeast Identifies Mechanisms of Trichloroethylene Toxicity**

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Trichloroethylene (TCE) is an environmental contaminant and human carcinogen that remains an environmental health hazard decades after its introduction. Studies have identified the metabolite dichlorovinyl cysteine (DCVC) as the penultimate mediator of TCE renal toxicity. While evidence from rodent and epidemiological studies suggest a mutagenic mode of action mediating TCE kidney toxicity, there remains a need for mechanistic evidence to support the association between TCE exposure, mutagenesis and cancer. Advances in genomic technologies make a functional genomics approach in Saccharomyces cerevisiae and avian DT40 cells appealing in vitro platforms for elucidating toxicology mechanisms. Initial yeast profiling studies revealed genotoxicity as an important contributor in DCVC toxicity. The phenotypic profile generated by DCVC showed high similarity to those of known DNA interstrand crosslinking agents, implicating DNA damage and mutagenic DNA repair as a potential mechanism of renal toxicity. Specifically, mutagenic translesion synthesis (TLS), nucleotide excision repair (NER) and homologous recombination pathways were required for tolerance to DCVC. Additionally, we found that DCVC damage elicits repair predominately by the low fidelity polymerase Rev3 (Polɛ). A combination of functional studies in the avian DT40 system and human cell lines revealed translesion synthesis repair was required whereas interstrand crosslink repair was not, suggesting an alternate mechanism of DNA damage and recombination independent repair. From our results, we propose that DCVC causes non-distorting DNA lesions that are repaired by translesion synthesis and template switching. These findings are significant as we provide mechanistic evidence suggesting a mutagenic mode of action mediated by the nephrotoxicant metabolite DCVC. Furthermore, we show that a functional genomics approach in yeast is a viable method for examining toxicity mechanisms and more specifically its utility in characterizing genotoxicity mechanisms.

**840 Immune Responses to Different Classes of Inhaled Particulates: Unique vs. Shared Responses and Mechanisms**

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Inhaled particulates, including silica, asbestos, particulate matter, and nanoparticles, induce pulmonary inflammation, lung fibrosis and, often, systemic disease. As these materials each range in size, shape, durability, composition, and surface properties, they are often evaluated for immunotoxic effects individually rather than collectively. Often they are considered “distinct” classes of toxicants, and information that could be used to advance the overall knowledge about immunotoxicity of inhaled particulates is not frequently exchanged or evaluated in the context of a single toxicant class, viz., particulates. Taking a more unifying approach, some common findings about immunotoxicities (and associated mechanisms) have begun to emerge. For example, with each class of material, an initial pulmonary response is induced that is mediated via the innate immune system and, in turn, drives an early inflammatory response. Similarly, an adaptive immune response is then triggered that appears to be responsible for the onset of systemic diseases/pathologies.

Nevertheless, each of these four particulate classes is capable of inducing some unique pathologies; consequently, there are likely some innate/adaptive responses (and associated mechanisms) induced by each type of particulate that somehow also specifically differ. The purpose of this symposium is to bring together experts to discuss, utilizing new cutting-edge information obtained about the immunotoxicity of these materials, the uniqueness of the innate and adaptive immune responses to each of the different particulates. A final presentation will then review areas of commonality and uniqueness. Based on these presentations, the symposium will seek to build a consensus about particle immunotoxicology that will help accelerate research for all particles and improve cross fertilization of research.

**841 Diverse Immune Effects of Silica and Mineral Silicates/Asbestos**

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Typical monkeys are caused by exposure to silica particles and asbestos fibers. Although both silicates induce lung fibrosis, the complications of exposed patients are different. Silicosis patients often suffered from autoimmune diseases such as RA, SLE, SSc and ANCA-related vasculitis. However, the most important issue of asbestos-exposed patients is the occurrence of malignant tumors such as mesothelioma. To understand this difference, we have analyzed the effects of silica and asbestos (chrysotile) on regulatory T cells (Treg), since excess autoimmunity and reduced tumor immunity are considered opposing activities of Treg function. The analyses using peripheral blood mononuclear cells from healthy donors and silicosis patients indicated that silica particles induce early loss of Treg cells via through CD95/Fas overexpression by chronic activation. However, cell line models of Treg cells showed enhanced Treg function as well as increased proliferative features by continuous and long-term exposure to chrysotile. The mechanisms, such as the differences of size and configuration as well as responses of innate immunity, causing these differences will discussed.

**842 Unique Aspects of the Immunotoxicity of Silicates**

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Recent research has uncovered the vital role of the innate immune system in determining the immunotoxicity of silica. Studies have identified the importance of silica particle size and surface modifications in innate immune cell activation, and the essential role of scavenger receptors in modulating the severity of the innate pulmonary immune response. The silica-induced inflammatory response also requires MyD88 and signaling of toll-like receptors (TLR) to drive proinflammatory cytokine expression. Also essential is activation of the inflammasome and caspase 1 to enable cleavage of some of the expressed cytokines, particularly IL-1β. Indeed inflammasome activation appears to be a common property of inhaled particulates such as silica, asbestos, particulate matter and nanoparticles and has recently been argued to contribute to the severity of systemic autoimmune disease. Many of the genes required for silicosis, including IL-12, IFN-γ, and IL-17, are important in the development of autoimmunity. Thus understanding the mechanisms required for innate sensing of silica and other inhaled particulates will contribute significantly to our understanding of how the innate immune response directs the adaptive immune response leading to disease including autoimmunity. Vital to the integration of these findings with human health outcomes will be the development of new models, including animal models, that provide a better understanding of how genetically heterogeneous human populations respond to toxicants such as silica and the other inhaled particulates discussed at this symposium.

**843 Influence of Source and Chemistry on Immune Effects by Ambient Particulate Matter**

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Epidemiological studies continue to show that exposure to air pollution leads to increased incidence and severity of allergic and infectious lung disease. There is also increasing evidence that hospital admissions for asthma increase during wildfire episodes. Early studies explained these effects through mechanisms associated with the smoke of host defenses such as macrophage phagocytosis, however it is becoming clear that innate immune responses influence both the quality and the strength of specific immune response profiles. Particulate matter air pollution is a heterogeneous mix of primary and secondary particles arising from a plethora of point and mobile sources. Studies from our laboratory have demonstrated wide differences in the chemistry of commonly studied combustion samples such as diesel
and woodsmoke in association with a range of immunotoxic effects. In addition, we have also recently demonstrated that particulate size fractions obtained from a peat wildfire episode have differential toxicity between the pulmonary and cardiac components. This presentation will illustrate how the physico-chemical attributes of air pollutants dictate their effects in the lungs and influence the development of specific immune response profiles associated with infectious and allergic lung diseases. (This abstract does not reflect EPA policy).

S 844 Molecular Mechanisms of Particulate-Induced Pulmonary Inflammation: Intrinsic Adjutant and Allergy

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Recently, the number of patients suffering from allergic diseases has increased, especially in developed countries, and recent literature has shown that particulate pollutants may be involved in the exacerbation of allergic responses. Of special interest, nanometer-sized particulates could enter into the respiratory tract and settle deep in the lung, causing pulmonary chronic inflammation such as asthma. These particulates are known to induce type 2 immune responses, which are characterized by the elevation of antigen-specific serum IgE in vivo. However, the mechanistic basis by which particulates elicit type 2 responses is unclear. Here, we will show that particulates engulfed by alveolar macrophages induce cell death and release dead cell derived factors (damage-associated molecular patterns: DAMPs), in turn, DAMP’s function as an intrinsic adjuvant for the induction of lung inflammation and IgE production.

S 845 Commonalities and Differences of the Distinct Particulates That Characterize Their Unique Properties in Both Stimulating Immunity and, Subsequently, Disease

A. Holian, CEHS, University of Montana, Missoula, MT.

A summary of the commonalities among the adaptive/innate immune responses (and relevant mechanisms) induced by the different particulates will be reviewed. The overarching question being addressed is whether the immune effects responding to each particulate type sufficient to explain the observed diseases? If not, what are the indirect effects of immunity? This will be followed by a series of questions developed by the speakers for the audience to discuss with the goal of defining the gaps of knowledge that need to be addressed in order to better understand the immunotoxicology of all the particles.

S 846 Local and Systemic Toxicity from Cobalt and Chromium-Containing Hip Prostheses

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Over 500,000 patients in the US have received a metal-on-metal hip prosthesis. Movement of loosened components in a failing prosthesis and friction between bearing surfaces can result in increased local and systemic metal concentrations, principally of cobalt and chromium. Metallic debris affects bone health through direct effects on bone cells and through indirect inflammatory signalling. These effects vary with the metal, its physical and chemical properties, and valency. Cobalt and chromium localize at nuclear and perinuclear sites in osteoblasts, suggesting uptake through cell membrane transporters, and is modulated by P2 receptor blockade. Metallic debris induces a range of cellular responses by direct cytotoxicity mediated through activation of redox reactions or the substitution of other bivalent cations in biological pathways, and through cytokine induction through inflammasome and other signalling pathways. Pro-inflammatory cytokine responses to particulate metal is greater in individuals with a susceptibility to bone loss versus those that do not demonstrate this response. This variability in response is associated with genetic variability within the population.

S 847 Hip Prostheses: What Toxicologists Need to Know

A. Vale, School of Biosciences and College of Medical and Dental Sciences, University of Birmingham, Birmingham, United Kingdom. Sponsor: A. Vale.

There are two main approaches to hip replacement. Most commonly, the head of the femur (thigh bone) and acetabulum (socket in the pelvis) are replaced with new, artificial parts (total hip replacement). Alternatively, resurfacing the original socket and the ball of the femur may be undertaken. In the traditional total hip replacement, the non-moving parts are most commonly made from titanium alloys with minor additions of aluminium and vanadium; cobalt/chromium with traces of molybdenum and nickel; or stainless steel (iron containing small amounts of chromium and other metals). The bearing surfaces may be made of cobalt/chromium, stainless steel, ceramic, or polyethylene, and components may be used in various combinations. Metal-on-plastic (a metal ball with a plastic socket) is the most widely used combination. Ceramic-on-plastic (a ceramic ball with a plastic socket) or ceramic-on-ceramic (where both parts are ceramic) are often used in younger, more active patients. Over 500,000 patients in the United States have received a metal-on-metal (a metal ball with a metal socket) prosthesis. Movement of loosened components in a failing prosthesis and friction between bearing surfaces can result in increased local and systemic metal concentrations, principally of cobalt and chromium. The generation of large numbers of nanoparticles of 25–50 nm diameter is of toxicological importance due to the ability of these particles to enter cells and cause tissue damage. In vivo studies have shown that cobalt/chromium wear particles are cytotoxic and adversely affect bone cell function, and may contribute to prosthesis failure. Whole blood and serum concentrations of cobalt and chromium usually rise only modestly after insertion of a metal-on-metal prosthesis, reaching a maximum of 1.3–2.5 μg/L during the first year after replacement. However, in those with failing prostheses, concentrations can be much higher, with systemic metal concentrations up to several mg/L.

S 848 Adverse Local Tissue Responses to Metal

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Wear and corrosion at prosthetic surfaces result in the release of metallic, ceramic, and plastic nano- and micro-particles and metallic ionic complexes into the peri-prosthetic environment. Local tissue responses to this debris include a combination of direct cytotoxic, innate, and adaptive immune responses. Histologically they may be broadly characterised as a macrophage-dominated foreign body giant cell reactions presenting as a granulomatous mass that replaces bone; with direct cytotoxicity presenting as tissue necrosis; or as a lymphocyte-dominated vasculitis-associated lesion characterised on imaging as a cystic or solid ‘pseudotumour’. The clinical manifestations of these responses include bone loss leading to prosthesis loosening or fracture, masses that cause pain or space occupying effects, and tissue necrosis. Metallic debris affects bone health through direct effects on bone cells and through indirect inflammatory signalling. These effects vary with the metal, its chemical properties, physical form and valency. Cobalt and chromium localize at nuclear and perinuclear sites in osteoblasts, suggesting uptake through cell membrane transporters and is modulated by P2 receptor blockade. Metal species localize to the basolateral membrane in osteoclasts, suggesting cell entry by endocytosis and trafficking through a functional secretory domain. Metallic debris induces a range of cellular responses by direct cytotoxicity mediated through activation of redox reactions or the substitution of other bivalent cations in biological pathways, and through cytokine induction through inflammasome and other signalling pathways. Pro-inflammatory cytokine responses to particulate metal is greater in individuals with a susceptibility to bone loss versus those that do not demonstrate this response. This variability in response is associated with genetic variability within the population.

S 849 Mechanisms of Cardiovascular, Neurological, and Thyroid Effects of Cobalt Toxicity

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Cobalt is an essential component of (vitamin B 12). Normally, concentrations of cobalt in blood are < 1 μg/L. However, patients with metal-on-metal joint prostheses, particularly of the hip joint, may have considerably higher concentrations. Data on the effects of cobalt exposure comes from animal and epidemiological studies, exposed-worker evaluations, and clinical experience. The latter is from an episode where excessive concentrations were added to beer, cobalt used to treat anemia, and patients with hip prostheses. These sources of human experience have raised concerns about effects cobalt on the cardiovascular, visual and auditory systems, and the thyroid gland. The cardiovascular effects of cobalt derive from an episode where cobalt was added to beer to improve the quality of the ‘head’, causing heavy
drinkers, if malnourished, to develop cardiomyopathy. Animal models have shown that under conditions of protein deficiency, causing depletion of cobalt-chelating amino acids, cobalt inactivates lipoate, a co-factor for pyruvate dehydrogenase, thus inhibiting the Krebs cycle. The possible sensory effects of cobalt are based primarily on anecdotal reports. Animal studies, in which cobalt doses maintained blood concentrations in the range of some patients with hip prostheses, reported depletion of retinal ganglion cells, axonal swelling and myelin thinning of the optic nerve, and a reduction of hair cells of the Organ of Corti. Cobalt chloride, given for the treatment of anemia, was associated with goiter and hyperthyroidism. Subsequent studies documented small changes in T3 concentrations in occupationally exposed cohorts. This effect appears to be due to the inhibition of tyrosine iodinase and the subsequent decrease in thyroid iodine uptake and incorporation of iodine into thyroid hormones.

Eighteen patients with systemic toxicity in association with a metal-containing hip have been reported. Eight of the 18 patients had received a metal-on-metal (MoM) prosthesis; the remaining 10 had undergone revision of a fractured ceramic bearing component to a metal-containing bearing. The reported systemic features fell into three main categories: neuro-ocular toxicity (14 patients: peripheral neuropathy (six cases), sensori-neural hearing loss (seven), cognitive decline (five), ocular toxicity (six)), cardiotoxicity (11 patients) and thyroid toxicity (nine patients). All these features except cognitive decline have been described previously in association with cobalt poisoning. Where blood metal concentrations were reported (n=17), the median cobalt concentration was 398 [range 13.6–398.6] μg/L, compared to a median chromium concentration (n=14) of 48 [range 4.1–221] μg/L. Thus, cobalt rather than chromium appears to be the greater systemic hazard in these patients. Those patients who had received a MoM prosthesis (n=8) had a median peak blood cobalt concentration of 34.5 [range 13.6–398.6] μg/L; those with a metal-containing revision of a failed ceramic prosthesis (n=9) had a median blood cobalt concentration of 506 [range 353–6521] μg/L. The most common treatment was removal of the Co/Cr prosthesis, undertaken in all but one patient. This was usually associated with a fall in circulating cobalt concentration and a reduction in some or all symptoms. Assessing the toxicological likelihood that systemic toxicity is associated with placement of a cobalt-containing hip prosthesis requires consideration of several criteria including: symptoms consistent with the known cardiac, neurological, or thyroidal effects of cobalt; increased blood cobalt concentrations; a fall in blood cobalt concentration after treatment, accompanied by signs of clinical resolution. Ten of the 18 reported cases are likely to be related to cobalt exposure when judged by these criteria.

The Adverse Outcome Pathway (AOP) framework guides the formal characterization of the series of key events starting with chemical perturbation of a molecular initiating event (MIE) and resulting in an adverse outcome relevant for regulatory decision-making. AOPs should be chemical agnostic to allow general use in interpreting high-throughput assays developed based on the MIE, but practical application of AOPs in risk assessment requires comparison between the concentration expected to result in an adverse outcome based on the extent of MIE stimulation and the biologically effective target tissue dose for a chemical. This requirement in turn speaks to the critical need to consider absorption, distribution, metabolism, and excretion (ADME) of a chemical, which may render an otherwise toxic chemical inaccessible to molecular targets in AOPs. Considerations of ADME not only link biological responses to chemical exposure, but are essential when extrapolating in vivo assays to in vitro conditions and across species and lifestages. With the maturation of the AOP framework, this workshop seeks to open a dialogue within the Society on how to apply this same rigor to developing a framework that incorporates ADME events connecting environmental chemical exposure and AOP initiation. To provide the broadest array of perspectives on this problem, scientists from around the world have been invited to discuss toxicity pathways, adverse outcome pathways, pharmacokinetic modeling, and chemoinformatic tools. Following the presentations, there will be a discussion period to facilitate open discussion among workshop attendees on the state of the science in connecting ADME to AOP research. Participants should leave with a better appreciation of how ADME and AOPs together can improve toxicity predictions based on in vitro measurements. The overarching goal of this workshop is to enhance the use of the AOP framework in chemical-specific risk assessment by better integrating knowledge and data between ADME and AOPs.
on embryonic behavior are thought to be useful to predict lethality for neurotoxic compounds. However, for some compounds, behavioral effects in embryos do not relate directly to lethality in adult fish, probably due to high metabolic rates modifying target available concentrations in adult fish. A straightforward approach to include strings in the ADME Module through a name lookup is the analysis of internal concentrations and biotransformation in fish embryo. We have shown that internal dosimetry characterization is possible for whole embryo extracts. However, if in vivo data are lacking, and for a refined tissue-based, comparative assessment of internal concentrations between species or life stages, the application of mathematical models is essential. Therefore, we have initiated the establishment of compartment-based and, even more ambitious, the development of physiologically-based toxicokinetic (PBTK) models for adult fish and fish embryos. The PBTK models are based on physiological, measured parameters, avoiding any estimations based on fitted parameters. A particular problem, however, for the validation of a fish embryo PBTK model is the determination of local, tissue specific concentrations. Combining the PBTK model with AOP-linked assessment of predictive endpoints in fish embryos would increase perspective of regulatory applications.

**855 Translation of *In Vitro* Concentration-Response Relationships of Key Events to Human *In Vivo***

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The vision for the next generation risk assessment is to use "high-throughput" *in vitro* systems to identify the series of key events in an adverse outcome pathway (AOP). Inevitably, this requires the translation of *in vitro* to *in vivo* concentration response relationships, which is a significant challenge. The physiologically-based toxicokinetic (PBTK) models offer an effective platform for conducting quantitative *in vitro to in vivo* extrapolation (QVIVE) at both an individual and population level. Inter-individual differences in anatomy, physiology and biochemistry at both organ and cellular levels, coupled with Bayesian inference, can be used to reconstruct probability distributions of exposure or dose. In this approach, the description of variability in the human population is converted to a problem of describing variability via probability distributions in individuals organ or aggregated compartments. When many compartments are defined discretely, logical constraints are required to prevent violation of tissue mass balance and regional blood flow. This in turn requires re-parameterization. Further, there is an inconsistency in the literature as to whether the distribution assigned to a given organ or aggregated compartment mass or blood flow should be normal or lognormal. This invariably has resulted in nonsystematic measures to address these issues which have led to the prediction of much more diverse, and in some cases implausible physiologies, than actually exist within the human population. The addition of cellular systems biology pathway models describing perturbations at the cellular level that must be related to an exposure or dose regime at a different level of biological organization represents a significant additional tier of complexity and parameters for the PBTK models. We have established a prior distribution for the physiology of the adult working population that provides a framework and realistic bounds on human physiology. The combination with demographic information such as sex, ethnicity, age and height, this prior distribution can be used to substantially reduce uncertainty in the reconstruction of exposure from human biomonitoring data compared with current approaches.

**856 Cheminformatic Tools in Support of Pharmacokinetics and ADME Profiling**


The Adverse Outcome Pathway framework can effectively link high-throughput screening (HTS) toxicity predictions corresponding to a molecular initiating event (MIE) with adverse outcomes of regulatory concern. In an analogous manner, dosimetry modeling, such as physiologically based pharmacokinetic (PBPK) modeling, can effectively link external exposures to target tissue doses required to trigger the MIE. However, current HTS approaches can screen thousands of compounds per year in contrast with a few hundred existing PBPK models. In this study, a comprehensively curated PBPK-related corpus was developed to provide model predictions based on chemical structure similarity. First, publications that contain PBPK models in either title or abstract were retrieved from the literature. Chemical Named-Entity Recognition (NER) tools were then used to annotate chemical names, which were checked for redundancies and mapped to chemical SMILES strings and CAS numbers through a name lookup in several molecular repositories. In addition, the 3-dimensional molecular geometries, as well as both 2D and 3D molecular descriptors, were computed for this entire chemical dataset using the Molecular Operating Environment (MOE 2013.08.02, Chemical Computing Group Inc, Montreal Canada). From 1977 through October 30 2013, a total of 340 unique chemicals were identified for which PBPK models have been developed, corresponding to a total of 798 published models in a PBPK literature corpus containing a total of 1707 articles. This compiled molecular/bibliographic database provides a chemical structure-centric basis for identifying relevant PBPK modeling literature for new chemical entities that need to be modeled. Within suitable distance metrics, models for nearest neighbor chemicals can closely mimic the pharmacokinetics of a novel chemical structure when a chemical lacks previous PBPK models. This tool, when coupled with HTS toxicity and external exposure predictions, can provide a risk-based rather than hazard-based prioritization of chemicals for regulatory purposes.

**857 An “ADME Module” in the Adverse Outcome Pathway Knowledgebase**


The Adverse Outcome Pathway (AOP) framework has generated intense interest for its utility to organize knowledge on the toxicity mechanisms, starting from a molecular initiating event (MIE) to an adverse outcome across various levels of biological organization. While the AOP framework is designed to be chemical agnostic, it is widely recognized that considering chemicals’ absorption, distribution, metabolism, and excretion (ADME) behaviors is critical in applying the AOP framework in chemical-specific risk assessment. Currently, information being generated as part of the Organisation for Economic Co-operation and Development (OECD) AOP Development Programme is being consolidated into an AOP Knowledgebase (http://aopwiki.org). To enhance the use of this Knowledgebase in risk assessment, an ADME Module has been developed to contain the ADME informative chemical events (MIEs) and other key events in an AOP for specific chemicals. The conceptual structure of this module characterizes the potential of a chemical to reach the target MIE based on either its structure-based features or relative rates of ADME. The key features of this module include (1) a framework for connecting biology-based AOP to biochemical-based ADME and chemical/ human activity-based exposure pathways; (2) links to qualitative tools (e.g., structure-based cheminformatic model) that screen for chemicals that could potentially reach the target MIE; (3) links to quantitative tools (e.g., dose-response model) that evaluate feedback from biological perturbations to target tissue doses. Feedback from workshop participants will be incorporated into the refinement of the ADME module to ensure that it covers the diverse needs of scientists and regulators looking to make better use of AOPs. Disclaimer: This abstract does not necessarily reflect U.S. Environmental Protection Agency policy.

**858 Regulatory Neurodevelopmental Testing: New Guiding Principles for Harmonization of Data Collection and Analysis**

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There is increasing concern worldwide about the potential for chemicals to affect neurodevelopment in children. In 2012, OECD published the extended one-generation neurodevelopmental toxicity guideline 443, which will generate new auditory startle, motor activity, and morphometric data. Since these endpoints will have increased regulatory significance, their conduct and interpretation requires increased attention. New evaluations of older DNT studies conducted according to US EPA guidelines (including learning and memory) in global regions are also playing a larger role in children’s health risk assessment. Yet, data from the same study has resulted in different risk assessments in different countries, leading to potential trade barriers. In addition, data are sometimes incompletely reported or analyzed, adding to inconsistencies in evaluations. These issues also apply to juvenile and pre-natal studies conducted for pharmaceuticals. Although various regulatory bodies have different risk management frameworks, this workshop will provide an opportunity to develop more harmonized scientific approaches for evaluating DNT data. One major reason for varying regulatory decisions based on the same data is expectations for variability of DNT data. Speakers from industry, academia, and government with regulatory neurotoxicology expertise will discuss inherent and controllable variability, suggest guiding principles for assessment of DNT data, and selection of benchmark response levels that take into account different variability. Speakers will address shortcomings in study conduct, data reporting, and analysis that are encountered by regulatory authorities, and will propose approaches to harmonize evaluation of data using different DNT endpoints as case studies. The workshop will end with discussion led by two discussants from industry and government. This session is especially timely as laboratories in different world areas are developing new capabilities to conduct the OECD 443 guideline and regulatory bodies place new emphasis on evaluation of DNT data for children’s health risk assessments.
Neurobehavioral testing is a foundational component of studies designed to assess developmental toxicity and developmental neurotoxicity with pharmaceuticals and chemicals, including pesticides. Computer-automated tests of motor activity and the acoustic startle response are common methods of such studies. Variability of neurobehavioral endpoints is often relatively high, especially at early stages of development, which undermines reviewer confidence in study results and has impacted the interpretation and application of additional safety factors for infants and children. Thus, it is important to control environmental factors that contribute to variability and to understand what is normal or excessive for different endpoints. This presentation will set the stage for the workshop, by using new data comparisons of neurobehavioral endpoints from different types of guideline studies to illustrate how variability in control animals is increased, due to study design requirements, relative to what is achievable under optimal conditions. This presentation will illustrate how to examine variability in the context of particular stages of development, including how to differentiate between excessive variability and highly-consistent results that are inherently more variable.

Motor activity (MA) testing is a requirement of both EPA and OECD DNT guidelines as well as the new OECD extended one-generation reproductive toxicity test guideline. Thus, MA will continue to play an important role in children’s health risk assessment. The test guidelines are relatively non-specific about test procedures and data reporting, and high variability in MA data directly impacts interpretation of both study conduct reliability as well as decisions on whether there are effects on the developing nervous system. Similarly, shortcomings in the reporting of MA data from testing laboratories also contribute to uncertainty about test results. This presentation will discuss the type of data that needs to be reported so that regulators can assess the quality of activity data including “normal” activity patterns for the strain, species and equipment. This presentation will provide guidance on expected MA variability under required testing conditions based on recent retrospective analysis of control data from DNT studies submitted to Health Canada. The impact of software and equipment settings on activity of control animals at different developmental stages will be presented. The use of different covariates that can address MA variability will be compared.

DNT studies conducted for regulatory purposes incorporate multiple endpoints that vary in the specific parameters measured as well as the metrics used to evaluate results. Regulatory guidelines provide flexibility in study design, placing responsibility on the individual investigators to precisely define the hypothesis being tested and design appropriate analyses. Available equipment can record multiple parameters, not all of which convey biologically meaningful information. Thus it is useful to focus the study design on detection and analysis of those effects whose biological significance is meaningful in the risk assessment context. Although general guidance has been provided, practical recommendations on implementation of such approaches have been limited, and differences in guideline specifications suggest different biological hypothesis that could be tested within the general guidance provided. For example, the new OECD extended 1-generation study includes analysis of auditory startle habituation at PND 24 in 1 male or 1 female/litter. OECD DNT guidelines specify some test of sensory and motor function at two ages in 1 male and 1 female/litter (i.e. a test of habituation is not required). Analysis of auditory startle data could include treatment, sex, trial block, and day and potential factors in a hierarchical model; analyses of these data could include multiple main effects as well as a many levels of interactions, for multiple measured parameters. Using auditory startle as an example, types of parameters typically measured, ways in which measured parameters may be summarized or combined, and the utility of various types of information in evaluating biological effects of treatment will be explored.

Discussion will focus on identifying parameters that have biological relevance for use in risk assessment, and how that and other practical considerations (e.g. type of device) might impact evaluation and graphical and tabular presentation of data. This is an abstract of a proposed presentation and does not reflect the policy of the US Environmental Protection Agency.
The US Environmental Protection Agency (US EPA) Endocrine Disruptor Screening Program (EDSP) has been evolving since its inception in 1998. The two-tiered program was initiated in response to a mandate by Congress to investigate the potential for pesticides and drinking water contaminants to have adverse effects on endocrine signaling. The collection of 11 assays included in EDSP Tier 1 were compiled to provide a standardized battery by which compounds could be screened for the potential to interact with estrogen, androgen, or thyroid (EAT) hormone signaling pathways. Compounds showing potential EAT activity in the Tier 1 battery are anticipated to undergo further, more comprehensive testing in EDSP Tier 2, which will provide dose-response data on adverse endpoints for use in quantitative risk assessment. Since 2009, when the US EPA issued the first set of testing orders for Tier 1 screening, 52 compounds have been screened through this battery of assays. Many challenges were encountered during this initial testing, including meeting assay performance criteria, interpreting data, and allocating time and resources for such a large-scale screening battery. One of the most significant and challenging questions that transpired following this first attempt at EDSP Tier 1 testing was, “Where do we go from here?” Addressing this question will be paramount in effectively implementing this program. Issues related to the dynamic process of optimizing the screening portion of the EDSP include challenges associated with Tier 1 screening, proposed approaches for streamlining the screening program, the future of the program as EDSP21, endocrine screening in non-mammalian species, and lingering questions on the aptness of the Tier 1 battery. Discussions focused on these topics are timely, with impending Tier 1 test orders on the horizon for List 2 compounds and upcoming decisions regarding the future for Tier 1 positive compounds as relates to moving into Tier 2 testing.

The workshop will end with 35 minutes of discussion led by two Discussion Leaders from industry and government experienced in evaluating DNT endpoints within the context of the entire toxicity database for risk assessment purposes. They will develop a straw proposal list of general guiding principles for reporting and evaluation of DNT endpoints based on input from speakers and their respective colleagues. Workshop attendees will be invited to contribute to this open forum discussion.
The current Tier 1 screening regimen for detecting potential endocrine disrupting chemicals (EDC) focuses largely upon three important endocrine signaling pathways: estrogen, androgen, and thyroid (EAT). However, the wealth of endocrine signaling pathways that utilize other nuclear receptors to mediate hormone action raises the likelihood that screening for EAT activities alone, is inadequate for the protection of humans and wildlife populations. For example, tributyltin avoids detection as an EDC using the Tier 1 testing regimen. Yet tributyltin binds and activates both the retinoid X receptor (RXR) and the peroxisome proliferator activated receptor (PPAR) resulting in excess adipocyte differentiation, lipid accumulation, and weight gain. Considering that RXR is a partner to many nuclear receptors, activation of the receptor by tributyltin may result in occult, pleiotropic responses. Not all species contain the same repertoire of nuclear receptors. Prostosome invertebrates typically do not possess ligand-activated estrogen, androgen, and thyroid receptors, but do express numerous receptors that are not found in vertebrates. Thus, the present Tier 1 screening battery is not protective of these species against endocrine disrupting chemicals. For example, the hormone mabey farnesoesate is responsible for sex determination in some crustaceans (e.g., Daphnia). This hormone acts through activation of the mabey farnesoeate receptor (MFR) which is not present in any of the species used in the Tier 1 battery. The insecticide pyriproxyfen would not screen positive in the current Tier 1 battery, yet this compound activates the MFR and alters sex ratios in daphnids at concentrations orders-of-magnitude below those that are acutely toxic to the species. It may be impractical to screen for all receptor-mediated endocrine signaling pathways in a Tier 1 screening battery involving whole animals. Whereas, assays involving cell based approaches (e.g., reporter gene assays) or that target several endocrine pathways simultaneously (e.g. microarrays) would be a more sagacious approach to initial screening.

Species Extrapolations for EDSP—Are Species Adequately Evaluated in the EDSP

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A question that comes up when assessing the potential hazards posed by endocrine active compounds is whether cross species surrogates are appropriate. Various ligand binding and receptor reporter activation assays have been used to assess the activity of various environmental estrogens and these have shown a high fidelity of binding and receptor reporter activation assays have been used to assess the active compounds is whether cross species surrogates are appropriate. 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appropriate risk-avoidance and healthy behavior—hence, these aids reduce decision errors. Risk information presented visually is judged as easier to understand and recall. Visual presentations often result in an “Ah-ha” moment and require less time to comprehend than traditional presentation formats of the same information. As demonstrated with specific examples, a series of figures is used to display relationships between toxicologic/epidemiologic endpoints and potential for human risk. These figures are meant to convey in an easy-to-understand way the relationships between toxicologic/epidemiologic observations, data uncertainty and variability, commonly used risk metrics (e.g., RfDs, ADIs, RSDs) and estimates of potential exposure. In addition, the approach can include information on guidance values developed by other countries/authorities, which gives the audience an understanding of how the same underlying database can yield different guidance values. Technological advances allow a range of information to be strategically linked to relatively simple figures. The final figure in the progression shows how guidance values can be portrayed within the context of biomonitoring information, derived from NHANES. This approach is likely to have significant value to risk managers and members of the public who are not as well acquainted with the risk assessment process, its uncertainty and variability, and its role in the risk management process.

Appropriately designed visual aids can improve comprehension of risks associated with different medical treatments, screenings, and lifestyles. Often, individual and societal risk-based decisions stem from anecdotal narratives. Visual aids also foster appropriate risk-avoidance and healthy behavior—hence, these aids reduce decision errors. Risk information presented visually is judged as easier to understand and recall. Visual presentations often result in an “Ah-ha” moment and require less time to comprehend than traditional presentation formats of the same information. As demonstrated with specific examples, a series of figures is used to display relationships between toxicologic/epidemiologic endpoints and potential for human risk. These figures are meant to convey in an easy-to-understand way the relationships between toxicologic/epidemiologic observations, data uncertainty and variability, commonly used risk metrics (e.g., RfDs, ADIs, RSDs) and estimates of potential exposure. In addition, the approach can include information on guidance values developed by other countries/authorities, which gives the audience an understanding of how the same underlying database can yield different guidance values. Technological advances allow a range of information to be strategically linked to relatively simple figures. The final figure in the progression shows how guidance values can be portrayed within the context of biomonitoring information, derived from NHANES. This approach is likely to have significant value to risk managers and members of the public who are not as well acquainted with the risk assessment process, its uncertainty and variability, and its role in the risk management process.

As chemical hazard and dose-response assessments increase in their complexity and robustness, the support documents can easily grow to several hundred pages in length. These assessments often include components (e.g., PBPK modeling, dose-response modeling, regression analyses of epidemiology data) that remain a “black box” to even seasoned risk assessors. For this reason, it becomes a challenge to present this information in a clear and concise manner to those who are applying the assessment to make a decision (i.e., risk managers). A successful dose-response summary will be able to distill the assessment down to key steps and decision points in a simple yet clear manner. To accomplish this goal, we have developed example summary tables that illustrate how to visually present the key information in a manner that facilitates communication. These tables and electronic versions can be readily extended to allow users to interact with the table by selecting alternative options to allow stakeholders, including risk assessors and risk managers, to see the impact that decision point changes have on the overall derived value. This presentation will provide case studies to demonstrate this tool and explain with specific examples how the elements and the tool can be used to: (1) help communicate the impact that decision point changes have on the overall derived value. This presentation will focus on advancements in the characterization and presentation of uncertainty in human health assessments developed within the IRIS Program.
Characterization of a Resazurin/Resorufin Assay for Reactive Acyl Glucuronides with Dicumarol and Reactive Aldehyde Trapping Agents

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We have used a resazurin conversion to resorufin assay in HepG2 cells and hepatocytes to characterize reactive acyl glucuronides (RAGs). In characterizing the resazurin to resorufin response to presumptive RAG exposed aldehydes, it is important to be able to distinguish between 1) increased fluorescence due to RAGs interacting with resazurin vs 2) parent molecules of RAGs, or the RAGs themselves, inhibiting the glucuronidation of resorufin. Glucuronidation of resorufin removes fluorescence and its inhibition would produce essentially the same signal as the increases in resorufin postulated to form from RAG aldehydes interacting with resazurin. Dicumarol (135 uM), a presumptive diaphorase/NQO1 inhibitor, increased the fluorescence signal of resazurin (presumably by inhibiting removal of resorufin via diaphorase) and also blocked most of the resazurin response to RAGs (but non-toxic disulfiram concentrations did not). This may reflect block of the same resorufin removal pathway(s) by dicumarol and RAGs. While we do see identical increases in fluorescence with dicumarol and many high doses of RAGs, we did observe an additional increase in fluorescence with ketoprofen (675 uM) which we attribute to inhibition of glucuronidation of formed resorufin; ketoprofen is a well-known glucuronidation inhibitor (UGT7b7 best characterized). Most importantly, the aldehyde trapping agents hydralazine, methoxamine, and pyridoxamine gave inhibition profiles identical to those reported in the literature (Burcham et al, 2002; Aldini et al, 2006), with 8 mM hydralazine blocking all of the response. Further studies are underway to characterize direct interaction of resazurin with RAGs, which has so far been limited by the sensitivity of the approaches used.

880 Evaluation of an In Vitro Cytotoxicity Assay in FBS-Free Medium to Select Safer Compounds

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In vitro cytotoxicity assays using cultured cells have helped prioritize compounds for subchronic toxicity studies. However, some compounds selected for toxicity studies that did not demonstrate cytotoxicity, and were also not promiscuous in a panel of Cerep binding assays, caused toxicity at low free plasma concentrations. Our recent results demonstrated that fetal bovine serum (FBS)-free cytotoxicity assays may be useful in detecting cytotoxicity of compounds with low free fraction which might be masked by the presence of FBS, and ranking these compounds prior to acute toxicology studies. Here, we have studied 130 Pfizer proprietary compounds from various programs and marketed drugs across therapeutic areas with low free fraction (0.0005-0.09) in rodent plasma in an ADP-depletion assay using normal rat kidney epithelial cells (NRK52E) at normal (10%) or reduced (0% or 5%) concentrations of FBS after 24 hours of exposure. We have observed that 76 acidic compounds decreased LC50s (3-fold) in FBS-free medium compared to 10% FBS whereas basic and neutral compounds with similarly low free fraction did not decrease LC50s at 0% FBS. Analysis of physicochemical properties revealed that 13 of 52 compounds with low or medium permeability determined by Madin-Darby canine kidney cell line (MDCK-II) permeability (LE) permeability had reduced LC50s in FBS-free medium, whereas none of 29 high permeable compounds decreased LC50s at 0% FBS. Considering that increased intracellular concentrations of compounds result in increased cytotoxicity, FBS-free medium may contribute to higher intracellular concentrations of compounds with lower permeability. Taken together, these results suggest that reduced-FBS cytotoxicity assays are useful to differentiate inherent cytotoxicity of compounds with low free fraction and low permeability in acidic chemistry space.

881 Nrf2 Stress Response, a Suitable Biomarker for Oxidative Stress and Reactive Metabolites Formation

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Drug-Induced-Liver-Injury (DILI) remains the cause of approximately one-third of unexpected toxicities leading to attrition during drug development and post-marketing. DILI can be caused by the parent drug, but in many cases, formed reactive metabolites (RM) are responsible for liver damage. Cells are protected from the formation of RM by the activation of the transcription factor NF-E2-related factor-2 (Nrf2), which is known to protect against oxidative stress by inducing a wide variety of genes that aid in the detoxification and elimination of drugs. The objective of the present study is to investigate if the Nrf2 stress response is a suitable biomarker for oxidative stress and RM formation using 2 different approaches and 10 reference compounds. The reference compounds used are ticlopidine, clozapine, diclofenac, acetaminophen, tienilic acid, BHA as the positive compounds and caffeine, levofloxacin, fluoresmide and aspirin as the negative compounds. In a first approach, an index is calculated based on the expression pattern of 7 genes under the regulation of Nrf2. To calculate the index, the normalized sum of all AACT values for each gene above one were counted and divided by 7, the number of genes under investigations. Depending on the human donor used, 5 or 6 of the positive compounds were correctly classified. In the second approach, the Antioxidant Response Element Reporter Cell Line, AREc32, is evaluated for measuring Nrf2 activation using the same set of reference compounds as used above. The AREc32 cell line is a stable transfected MCF7 cell line that provides a rapid quantification of Nrf2 mediated activation of ARE. It is concluded from this study that the AREc32 assay is suitable to detect the Nrf2-inducing compounds from our compound list, 5 of the 6 positive compounds were correctly classified. Taken together, the present study indicates that Nrf2 regulation, using both approaches in parallel, is a relevant biomarker for the detection of cellular consequences associated with the presence of reactive metabolites.

882 An In Vitro Assay Panel to Predict Drug-Induced Mitochondrial Toxicity

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Mitochondrial toxicity (MT) is responsible for curtailed use and post-market withdrawal of many pharmaceuticals and there is no one assay available that reliably predicts MT in early drug screening activities. We have developed an in vitro assay panel of in vitro assays that encompass three major adverse events resulting from MT: (1) energy metabolism disruption (2) increased oxidative stress and (3) altered apopotosis. BxPC-3 (human adenocarcinoma) and SNU-475 (human hepatocarcinoma) cells were cultured with 100uM of the known antiviral MT inducers didanosine (ddl), Stavudine (d4T) and Zalcitabine (ddC) for two weeks. Pravastatin (P) and Insorne (In) with no reported MT were also tested. Cells were harvested on Days 7 and 14 following drug treatment and lysates generated were tested in assays that detect oxidative phosphorylation (OXPHOS) complex 1 – 5, ATP content (Abcam), total glutathione, ROS/RNS content (CellBio labs) and caspase 3 activity (Achrom). In comparison to no drug treated control, a 70-90% decrease in OXPHOS complex 1 level was observed for d4T, ddt and ddc treated BxPC-3 cells and a 40-60% decrease for ddt and ddc treated SNU-475 cells. OXPHOS complex 3 and 4 levels also decreased by 20-60% in d4T, ddt and ddc treated BxPC-3 cells and in ddc treated SNU-475 cells. Increases in caspase 3 activity and glutathione content was observed for both cell lines treated with all three antivirals. No changes in OXPHOS complex 2, 5 and ATP content were observed following drug treatment in both cell lines. For both cell lines ddc induced the most MT and BxPC-3 was the most susceptible cell line to all three drugs. Decreases (10-40%) in OXPHOS complex levels were also observed in BxPC-3 cells following two week treatment with both P and In with no impact on cell growth. This in vitro assay panel successfully mapped MT profile of five drugs in two cell lines and the approach can be utilized to predict MT of drug candidates. Work supported by NIAID Contract N01-AI-70043.

883 A Novel Cell-Based Assay System for Risk Assessment of Drug-Induced Liver Injury Considering Immune- and Inflammation-Related Gene Expression

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Drug-induced liver injury (DILI) is one of the leading causes of failure in drug development and post-marketing drug withdrawal. Although some in vitro methods for risk assessment of DILI that utilizes hepatic cell death or cellular stress as markers have been developed, their predictive performance remains unsatisfactory. We previously developed in vitro mice models for DILI and found that several immune- and inflammation-related genes were commonly upregulated in the liver of mice administered DILI-positive drugs. In this study, we sought to develop a cell-based assay system for risk assessment of DILI considering drug metabolism and immune- and inflammation-related gene expression. For this purpose, human liver carcinoma HepaRG or HepG2 cells were treated with 79 withdrawn or pharmacutical drugs with different DILI risks and the cultured media were collected. Human promyelocytic leukemia HL-60 cells were subsequently treated with the collected drug-conditioned media and mRNA expression levels of S100A9, IL-1β, MCP-1, IL-8, and TNFα were investigated by real-time PCR. An area under the receiver operating characteristic curve (AUC-ROC) was calculated to evaluate
predictive performance of the mRNA levels as markers to discriminate between drugs with no- or less-DILI concern and drugs with most-DILI concern. The expression of IL-8 in HL-60 cells treated with drug-containing conditioned medium from HepaRG cells (HL-60/HepaRG) exhibited the highest AUC-ROC value of 0.74, followed by the expression of IL-1β in HL-60/HepaRG (AUC-ROC = 0.71). Notably, AUC-ROC values of any genes were consistently higher in HL-60/HepaRG than those in HL-60/HepG2, suggesting that HL-60/HepaRG has a more potent ability to detect metabolic activation of drugs with DILI concern. Collectively, we developed a novel cell-based assay system for risk assessment of DILI and suggested that this assay has a potential utility in screening for DILI in preclinical drug development.

Mitochondrial Deregulation: A Potential Explanation for Drug-Induced Organ Toxicity

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To enable selection of the safest compounds at earlier and less expensive stages of drug development pharmaceutical industry seeks for predictive in vitro screening approaches. One of these approaches is to target a common cellular toxicity mechanism underlying different organ toxicities. Mitochondrial toxicity is considered to be one of the main underlying mechanisms of drug induced hepatoc- and cardiototoxicity. Using HepG2 cells we show the predictivity of an in vitro system to correctly identify mitotoxic (n=18) and non-mitotoxic (n=6) drugs. We defined mitotoxic drugs as drugs that induced liver injury (DILI) in human and in addition are reported to have a mitotoxic effect in vitro. Following compounds met that criteria: amiodarone, bromfenac, busprofen, HCL, clozapine, diclofenac, etanopage, flutamide, furazolidone, ibufenac, ibuprofen, indomethicin, rosiglitazone, tamoxifen, tolcapone, troglitazone and valproic acid. Non-mitoxot drugs are defined as drugs with no reported DILI in human nor mitochondrial toxicity in vitro. Following drugs were selected as non-mitotoxicate-betaine HCL, diphenhydramine, enalapril maleate salt, lamotidine, isoprotorenol HCL and primidone. Mitochondrial function as a measure of cellular metabolism through the measure of the oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) was assessed by the use of the Seahorse Bioscience XFe analyzer. The OCR is a measure for the respiration rate via oxidative phosphorylation in the mitochondria and ECAR is a measure for glycolysis. Overall, a good predictivity was obtained with a specificity of 6/6 and a sensitivity of 13/18. In addition, we highlight important variations in predictivity values depending on the medium composition and use of mitochondrial stressors.

Evaluation of SEAware™ Computational Approach to Predict Toxicological Liabilities of Small Molecules

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Small molecules pharmaceuticals can act on diverse protein targets, some of which may be unrelated by conventional molecular metrics. Discovering the unintended ‘off-targets’ by empirical methods can be daunting and is not always successful. SeaChange Pharmaceuticals, Inc. has developed SEAware™ software, a chemoinformatic and statistical tool that may help in uncovering unintended ‘off-targets’ that are associated with adverse drug reactions (Keiser et al., Nat Biotechnol, 2007; Keiser et al., Nature, 2009; Lounkine et al., Nature, 2012). Here we describe the evaluation of this computational approach in predicting off-target hits for small molecule pharmaceuticals. We selected 18 compounds from 4 different Genentech programs as the evaluation set. Off-target hits for these diverse set of compounds were determined using receptor binding and functional assays, and they were also correlated with specific in vitro findings. These off-target interactions and their associated effects spanned a diverse array, and included: 1. sedation and decreased intestinal transit in mice due to agonistic activity on mu-opioid receptors, 2. muscle fasciculations in mice due to interactions with acetyl cholinesterase and muscarinic receptors, 3. CNS-driven behavioral changes in mice due to inhibition of the dopamine transporter (DAT), and 4. hypotension in dogs due to inhibition of adrenergic α1A receptors. Negative control compounds for each program were also included in this assessment. Of the 4 programs under evaluation, the mu-opioid receptors off-target for the first group of compounds was successfully identified. However, the remaining off-targets were not uncovered by this computational method. The limited predictivity of the SeaChange approach for these compounds is likely due to differences in the chemical space between the public CHEMBL database which is used by SEAware™ and our internal chemical space, and the predictivity can potentially increase if the approach is customized and trained to a more pharmaceutically-relevant chemical space.

Increased Mitochondrial ROS Formation by Acetaminophen Is Associated with Disrupted Expression of Mitochondrial Electron Transport Chain-Related Genes in Human Hepatic Cells


Acetaminophen (APAP) overdose results in hepatotoxicity. Although this APAP intoxication is generally associated with the formation of reactive metabolites, oxidative stress and mitochondrial dysfunction, the underlying molecular mechanisms are still incompletely understood. To examine the mechanistic effects of APAP-induced hepatotoxicity in depth, HepG2 cells were exposed to a low and high dose of APAP (0.5 and 10mM) and analyzed at four time points (12, 24, 48 and 72h) for genome-wide gene expression. Mitochondria were isolated and electron spin resonance (ESR) analysis was performed to ascertain the mitochondrial radical formation as a toxic effect marker. Finally, the yield of ATP was measured to confirm the impact of toxic dose of APAP on cellular energy production. Our results indicate that, in particular, 10mAPAP exposure significantly influences expression levels of mitochondrial protein-encoding genes in association with a clear-cut increase in mitochondrial reactive oxygen species (ROS) formation. We also reveal that 10mAPAP affects the expression of many genes encoding the subunits of electron transport chain (ETC) complexes, which may alter normal mitochondrial functions by disrupting the assembly, stability structural integrity, electron transport of ETC complexes, and ultimately, lead to a measureable depletion of ATP, and cell death. Additionally, the expression of mitochondria-specific antioxidant enzyme, SOD2, is reduced. The decreased expression of the mitochondrial antioxidant may damage the ROS scavenging ability, cause imbalance of the mitochondrial ROS homeostasis, and, eventually, boost the formation of mitochondrial ROS. Overall, this study shows that in-depth analysis of transcriptomics data may provide mechanistic explanations for the observed APAP-induced increase of mitochondrial ROS formation and, related to this, the APAP-induced oxidative stress.

Are Variable Changes in Acylcarnitine Profiles Diagnostic of Effects on Mitochondrial Functions?

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Mitochondrial impairment can lead to numerous adverse sequelae including cytotoxicity, organ injury or failure. Acylcarnitines are key intermediates in mitochondrial function and metabolomic profiling has confirmed that changes in plasma acylcarnitine profiles are common findings in toxicity studies. Acylcarnitines facilitate entry of long-chain fatty acids into the mitochondria via the carnitine shuttle where they serve as substrates for the electron transport chain-related genes in human hepatic cells. The expression of IL-8 in HL-60 cells treated with drug-containing conditioned medium from HepaRG cells (HL-60/HepaRG) exhibited the highest AUC-ROC value of 0.74, followed by the expression of IL-1β in HL-60/HepaRG (AUC-ROC = 0.71). Notably, AUC-ROC values of any genes were consistently higher in HL-60/HepaRG than those in HL-60/HepG2, suggesting that HL-60/HepaRG has a more potential ability to detect metabolic activation of drugs with DILI concern. Collectively, we developed a novel cell-based assay system for risk assessment of DILI and suggested that this assay has a potential utility in screening for DILI in preclinical drug development.
Hepatotoxicity is a major cause of attrition during pharmaceutical development. While newer models have offered improvements in predicting incidence of common (high frequency) hepatotoxic events, the ability to detect idiosyncratic (low frequency) drug-induced liver injury (DILI) has remained elusive. While rodent models involving external or internal manipulation have enabled mechanistic study of certain drugs, there remains a need for an animal model that can detect liver liabilities where the mode of action is unknown. A critical issue is that conventional models lack genetic diversity, which in several instances has been shown to play a role in adverse drug reactions. The Diversity Outbred (DO) mice comprise a genetically diverse population with variability that surpasses that of the human population, but in which the minor allele frequency is greater in the DO (12.5% on average). We hypothesized that the DO could provide a model for low frequency DILI in patient populations. In this study, female DO mice (N=50/group) were administered orally one of three drugs associated with rare liver toxicity that are still used clinically (diclofenac, zileuton, isoniazid) or 0.5% methylcellulose vehicle. ALT was not elevated by 0.5% methylcellulose (P>0.05). Fold Mice were dosed (i.g.) daily up to 14 days and blood samples were taken before still used clinically (diclofenac, zileuton, isoniazid) or 0.5% methylcelulose vehicle. Mice were dosed (i.g.) daily up to 14 days and blood samples were taken before dosing and at necropsy. As a group, diclofenac and zileuton both caused significant elevations in alanine aminotransferase (ALT) from the pre-dose (baseline) values at necropsy (P<0.05). ALT was not elevated by 0.5% methylcellulose (P>0.05). Fold elevations in ALT ranged from 0.2-8.3 fold for diclofenac and from 0.2-13.6 fold for zileuton, and group means±SEM for diclofenac and zileuton post-dosing were 82.8±7.3 U/L and 123.8±10.0 U/L, respectively, compared to 32.39±5.4 U/L in the vehicle group. While preliminary, the data provide an important first step to qualifying the DO mouse population as a tool for improved prediction of rare safety liabilities that may call for personalized prescribing strategies.

Non-immunologically triggered hypersensitivity is well known for radiocontrast agents, antibiotics, anesthetics, and some peptides/proteins and has a clear impact on their efficacy/safety testing. As more parenterally administered, e.g. therapeutic antibodies, are being developed, these histaminergic reactions gain importance. The effect of infusion rate on probability and severity of histamine release in several species has previously been observed. Preliminary results from a dose range finder study with intravenously dosed Aprotinin (Trasylolef), a known inducer of hypersensitivity, suggested that a) the rat is more prone to the induction of histamine release than mouse and that b) infusion over 6 hours clearly reduced histamine release in mouse and rat. To substantiate this finding, 5 rats and 5 mice each, surgically implanted with an indwelling femoral vein catheter, were bolus injected or infused over 6 hrs with 240,000 or 300,000 U/kg Aprotinin. Rats showed clear histaminergic symptoms which were more pronounced (number of rats affected, number of symptoms/rat) after bolus injection. In mice, clinical signs were observed only after bolus dosing in 1 mouse per dose. Predose histamine levels in mice were lower but still partly in the range of predose rat histamine levels. In both species histamine levels after infusion were in the respective predose range while after bolus injection they were clearly increased. The increase after bolus injection was markedly more pronounced in rats (20 to 90fold) and related to dose. In mice the internal stores were apparently exhausted at both dose levels. These results confirm that histamine release and thereby induced clinical symptoms can be modified by the infusion rate in both rats and mice. In the mouse the intronic stores appear to be lower than in rat. Furthermore, the feasibility of infusing freely moving mice via indwelling vein catheter was demonstrated, thereby avoiding local tail (vein) damage and dosing variability as seen with the traditional tail vein injection.

Antibody-drug conjugates (ADCs) have advantages over traditional chemotherapies in that they allow for target-dependent delivery, exhibit increased half-life, and reduced body burden of cytotoxic agents. These agents have different dispositional and consequently toxicities from that of their respective drug payloads alone. Uptake of ADCs and their potential toxicities are likely driven by antigen-dependent and independent processes, the labile nature of the antibody-drug linker, and pharmacology of the payload. Therefore, complex in vitro models are needed to assess potential organ toxicity and mechanisms thereof. In this regard, we evaluated nine ADCs, chosen based on preclinical in vitro safety findings, in InSphero liver microtissues (LiMTs) from rat and human in relation to their effects on primary human hepatocytes. Cytotoxicity was determined by IC50 values based on total ATP content for the ADCs and their respective payloads alone on primary human hepatocytes (2-day single-dose treatment) and LiMTs (6-day repeat-dose treatment) in parallel. As expected, ADCs exhibited less cytotoxicity in both platforms per molar equivalent than the free-payload alone. Leftward shifts in their IC50 values were observed without exception in the LiMTs, with differences as much as 10-fold between platforms. In general, human LiMTs were more sensitive to cytotoxicity from ADCs than rat LiMTs, supporting potential involvement species- and/or
antigen-specific mechanisms. Furthermore, studies were conducted to assess target gene expression and the effects of the non-parenteral cells in accounting for differences observed between primary hepatocytes, rat LiMTs, and human LiMTs. In conclusion, spherical LiMTs are a stable co-culture system capable of treatment durations not attainable by traditional primary hepatocyte cultures. Additionally, this platform appears to be a promising tool for assessing mechanisms of ADC-mediated toxicity to support investigative toxicology studies in safety assessment.

893 Background Data on Functional Observational Battery in the Common Marmoset

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To compare neurobehavioral function in common marmosets (Callithrix jacchus) with macaque monkeys, the functional observational battery (FOB) for use of the central nervous system (CNS) drugs was assessed. One advantage of using the common marmoset is that smaller amounts of test compounds can be used compared to macaque monkeys because the marmoset is as small as a rat. From a drug evaluation standpoint, this species is also attractive as a candidate animal to consider as an alternative to rodents in drug efficacy tests for biologics. Three to four animals were assigned and administered Vehicle, Cocaine (Coc: 1 or 2 mg/kg, i.v.), Chlorpromazine (Cpz: 1 or 3 mg/kg, i.v.), Diazepam (Dzp: 1 or 3 mg/kg, i.v.) or Pentobarbbitone (Pbt: 3 or 10 mg/kg, i.v.). All animals were evaluated using a FOB, once before administration and 6 times after administration beginning at 5 minutes up to 6 hours. (Dzp group is 5 times expect 5 minutes.) The Coc group showed continual movement, hyperirritability and hyperthermia. The Cpz group showed ataxia, hypomotility, hypoactivity, myosis, attenuation of auditory and pain response, and systemic suppression, muscle relaxation. The Dzp group showed ataxia, hypomotility, hypothermia, myosis, attenuation of auditory and pain response, systemic suppression and muscle relaxation. The Pbt group showed ataxia, hypoactivity, hypoactivity, myosis, and pain response. The results showed that the FOB for the common marmoset is an effective tool for evaluating CNS drug and biologics.

894 The Influence of Body Weight Changes on Organ Weight Variations in GLP Safety Assessment Studies in Rats


Best practice guidelines recommend that the weights of liver, heart, kidneys, adrenals gland, brain, thyroid glands, pituitary glands, and testes (from mature males) should be collected for rats in all multidose GLP studies that last from 7 to 365 days. The body weight (BW) is one of the most frequently studied parameters and formulation. In the study that was used to optimize infusion rate, clinical signs of toxicity related to cell administration rate were observed following administration of a bolus IV injection of 1.5x10^6 UTC suspended in PBS by over 30 to 40 seconds. Dosing of PBS and 6x10^4 UTC was well tolerated, adverse clinical signs were observed following IV injections at the 1.5x10^6 UTC dose with the subsequent death of one rat. Histopathology revealed pulmonary arterial thrombosis and focal minimal myocardial findings. By slowing the injection rate to administer UTC over 1 to 2 minutes, clinical and pathological signs of toxicity were mitigated and an infusion rate of 1 x 10^6 cells/minute was used for subsequent studies in rats with human UTC. To support the clinical use, UTC were administered in a pharmacologically active dose in rats via IV infusion. Control rats were administered either saline or TICF. The components of TICF were cryoprotectant formulation (CF), DMEM-Lite and 10% v/v dimethylsulfoxide (DMSO). Transient clinical signs of swollen snouts and swollen limbs/paws were observed in rats dosed with all CF-containing formulations including both the CF vehicle group and all UTC groups. A series of studies were conducted to evaluate these findings and identified a CF formulation component, dextran, as the cause of swelling in rats. Infusions of UTC were well tolerated using a new formulation that did not contain dextran. Taken together, these results identified two components that must be considered to ensure safety use: 1) as suspensions of particles infusion rates for cell based therapies must be controlled for systemic administration; and 2) the complex formulations used to both cryopreserve and administer cells may contain pharmacologically active components that must be identified.

895 Mitigation of Infusion Reactions for Cell-Based Therapies

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The development of an umbilical tissue-derived cell (UTC) product as a therapy for ischemic stroke was supported by a series of toxicity studies to define infusion parameters and formulation. In the study that was used to optimize infusion rate, clinical signs of toxicity related to cell administration rate were observed following administration of a bolus IV injection of 1.5x10^6 UTC suspended in PBS by over 30 to 40 seconds. Dosing of PBS and 6x10^4 UTC was well tolerated, adverse clinical signs were observed following IV injections at the 1.5x10^6 UTC dose with the subsequent death of one rat. Histopathology revealed pulmonary arterial thrombosis and focal minimal myocardial findings. By slowing the injection rate to administer UTC over 1 to 2 minutes, clinical and pathological signs of toxicity were mitigated and an infusion rate of 1 x 10^6 cells/minute was used for subsequent studies in rats with human UTC. To support the clinical use, UTC were administered in a pharmacologically active dose in rats via IV infusion. Control rats were administered either saline or TICF. The components of TICF were cryoprotectant formulation (CF), DMEM-Lite and 10% v/v dimethylsulfoxide (DMSO). Transient clinical signs of swollen snouts and swollen limbs/paws were observed in rats dosed with all CF-containing formulations including both the CF vehicle group and all UTC groups. A series of studies were conducted to evaluate these findings and identified a CF formulation component, dextran, as the cause of swelling in rats. Infusions of UTC were well tolerated using a new formulation that did not contain dextran. Taken together, these results identified two components that must be considered to ensure safe clinical use: 1) as suspensions of particles infusion rates for cell based therapies must be controlled for systemic administration; and 2) the complex formulations used to both cryopreserve and administer cells may contain pharmacologically active components that must be identified.

896 Microscopic Changes in Various Organs Due to Restricted Feeding in Wistar Rats


Safety evaluation of pharmaceuticals is an integral part of the drug development. Some of the chemicals when administered at high concentration reduce the feed consumption. Decreased feed consumption, secondary to the high dose interfere the interpretation of overall safety of the chemical in question. There are reports available on the effect of various parameters generally considered important for safety evaluation but none of them were actually addressed the effects of decreased feed consumption at microscopic levels in different tissues except one or few organs like bone marrow. To address this problem we undertook the study to evaluate the effects of feed restriction on microscopic changes in different organs of Wistar rats. Groups of rats (10/sex/group) were fed ad libitum (control), given 50% (moderately restricted), 75% (moderately restricted), 50% (markedly restricted) and 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. Various organs were evaluated for microscopic changes. Microscopic changes were observed in many organs e.g. liver, pancreas, thymus, spleen, bone marrow, adrenals, thyroid etc 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 leads to various types of microscopic changes in many of the organs and should be considered during the safety evaluation of xenobiotics.

897 Use of Instant Glucose Measurements for Detection of Acute Hypoglycaemia in Rats

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In rats, identification of animals suffering from hypoglycaemia is a challenge as clinical signs often appear at very low glucose levels and are not easily distinguishable from clinical signs of other conditions. Instant glucose measurement in small blood samples (one drop) provides clear benefits, both ethically and practically. In the present study, we evaluated the One Touch glucometer for measurement of blood glucose levels in rats, with special attention on low glucose concentrations. The focus was on (1) the direct comparison of glucose measurements in plasma (at least 0.25 ml of blood), and in whole blood (one to a few drops of blood) and (2) comparison the sites of blood sampling (tail vein and sublingual vein). To create a
broad range of blood glucose concentrations, blood samples were collected before and after treatment with Actrapid® (human insulin). Samples were collected from the tail vein (whole blood) and from sublingual vein (whole blood and plasma).

There was a good linear correlation (R2=0.87) between whole blood glucose values obtained from the samples collected sublingually and corresponding glucose levels in plasma. The lowest level of glucose measured by glucometer used in the study corresponds to 2.5 mmol/L plasma glucose, which is a clearly hypoglycemic state (before treatment plasma glucose was 5.8±2.8 mmol/L). Correlation between values obtained from the tail vein and plasma was not reliable (R2=0.51). Worse still, the glucose values measured in the tail samples tended to overestimate the glucose levels, thus creating the risk of missing the animals at risk. Therefore, this type of sampling, although much easier to perform, was regarded as not appropriate for use in rapid diagnostics of hypoglycemia. In conclusion, a few drops of blood collected sublingually can be used for quick evaluation of glucose status of rats. The method reduces sampling volume and permits quick and correct handling of the animals at risk of hypoglycemia, thus increasing the ethical standards and the general quality of the studies.

Use of Histopathology, MRI Images, and Plasma Biomarkers to Identify and Monitor Drug-Induced Slow Myofiber Selective Myopathy

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Drugs affecting lipid metabolism may induce myopathies both preclinically in animals and clinically in patients. One of the myopathies is slow myofiber selective and is difficult to be detected by CK values. This study aimed to develop sensitive methods for detecting and monitoring slow myofiber toxicity. A PPARα agonist (AZD8677)1) was used in this study to induce slow myofibre selective myopathy. Groups of 9 male Wistar rats were orally dosed for 4 weeks with AZD8677 and vehicle, respectively. At Days -1, 7, 14, 22 and 28, blood samples were collected to measure skeletal muscle toxicity biomarkers, MRI images of the right hindlimbs were recorded and time series of T2 relaxation time were measured. At day 29 the rats were necropsied and multiple muscles from the hind limbs were sampled for histopathological and immunohistochemical analyses. The histopathology at day 29 showed myofiber degeneration/regeneration and interstitial fibrosis. The severity of the lesions was directly correlated to the proportion of slow myofibers in each muscle, with the soleus being the most affected muscle. Corresponding to histopathology, the MRI images showed hyper intensity in the region of the soleus. This change appeared as early as day 7 and was maintained at a similar degree at day 14, 22 and 28. The skeletal muscle biomarkers sTnI, FABP3 and MYL3 in plasma showed the same pattern with increases at day 7 that were maintained throughout the remaining of the study. Additional biomarkers were measured at day 28, including ALT, AST and ALP (increased levels) and CK (not increased). This study demonstrated alternative approaches for detecting drug induced slow fiber myopathy in rats. Three sensitive and specific methods have potential utility for monitoring drug induced slow myofiber selective myopathy in patients.

Developing a Heat Map for CNS Profiling Based on the Irwin Test


The Irwin test, first described by Irwin in 1968, consists of a series of behavioural, neurological and autonomic observations in rodents; and is part of the safety pharmacology core battery recommended by the ICH S7A before human testing. UCB BioPharma has been using the Irwin test in mice for over 30 years and amassed a considerable amount of data on over 400 reference compounds relevant to different internal projects. To maximize the value of such dataset, the aim of the project was to classify and represent the data into a meaningful heat map of effects across different classes of reference compounds. After data transformation and careful analysis, it appeared that 10 out of the 55 parameters measured were only influenced by less than 10% of compounds and 15/53 parameters (mainly related to motor activity where correlations were observed between these parameters) were affected by more than 90% of the same compounds. The heat map of the mean results per parameter also revealed which observations are the most influenced by different pharmacological, chemical or therapeutic classes of compounds. For example GABA A Negative Allosteric Modulators and GABA antagonists (anxiogenic and pro-epileptic agents) are characterized by a lack of effects on “impaired gate” and “righting reflex” compared to GABA A Positive Allosteric Modulators (mainly sedative and anxiolytic drugs). From our extensive dataset, we could conclude that only 70% of the parameters measured during an Irwin test, mainly related to locomotor activity, are affected by a drug and that this analysis highlighted that reference compounds producing an increase in monoamines are by far the class of compounds showing the most effects in the Irwin test. Further analyses are ongoing to increase the accuracy and strategic application of the Irwin test as an internal decision making criteria for target organ liability evaluation.

Effects of Different Vehicles Used in Preclinical Studies on the Neurobehavioral Profile and General Activity of Sprague-Dawley Rats


In the early phases of preclinical drug development, new chemical entities are seldom entirely optimized and may be difficult to solubilize. Consequently, the use of various excipients is required to formulate the test compounds for in vivo administration. The objective of the current study was to evaluate the effects of 19 vehicles in rats by using the Irwin test and actometers (Attrack, Panlab®). The excipients tested are suspending agents, surfactants, anti-foam, NaCl, solvents and cyclodextrins. For the Irwin test, 53 parameters were recorded on each Sprague Dawley rat before vehicle administration (n=6/group), and 15, 30, 60 and 120 min post-dosing (intraperitoneal). Data were analysed using a home-made algorithm extracting the main effects on central activity/reactivity, neuromotor tone, neurovegetative reflexes, and autonomic system. 48 hours later, the same groups were placed into an actometer for 60 min just after a new administration of the same vehicles; distance travelled and rearing counts were recorded. Five vehicles out of the 19 tested (including Soluphor® P 10%, Methocel® E3 1%) showed similar neurobehavioral profile and general activity as NaCl 0.9%; inducing slight hypotonicity that could be related to the fact that the animals were handled several times. In contrast, sedation was seen in both tests with 2 excipients, Solutol® HS15 ≥10% showed hypothermia, sedation, bradypnoea, decreased distance travelled and rearing. With Dimethylacetamide ≥10% more than 20 Irwin parameters were modified and confirmed by the significant decrease in the parameters collected from the actometer. Sodium Lauryl Sulfate at 0.2% should not be used from an ethical point of view for clear discomfort of the rats (writhing and dyspnoea). The other vehicles tested showed excitatory signs or hypotonicity that could biased the characteristics of the pharmacological in vivo model used. These data confirm the importance of a careful vehicle choice in particular if used in neurobehavorial tests.
The Differential Effect of Nembutal and Ketamine/Xylazine Anesthetic on Doxifludide-Induced QT Interval Prolongation

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Previous work from our group has suggested that anesthetics with additional inherent IKs blockade such as sodium pentobarbital may sensitize the animal to agents that prolong the electrocardiographic QT interval. In the present work, we used doxifludide to assess the vulnerability of both the Nembutal and ketamine/xylazine anesthetized guinea pig. Male guinea pigs (400-550g) were anesthetized with Nembutal (60 mg/kg IP, maintenance dose continuous IV infusion 6 mg/kg/h) or ketamine/xylazine (87/5 mg/kg IP, maintenance dose 44 mg/kg IP) and surgically instrumented with a Millar catheter to measure arterial pressure and heart rate. PR interval, QRS duration, QT/QTc interval and arrhythmogenesis were determined from continuous surface electrocardiograms. Animals anesthetized with Nembutal received vehicle (10% hydroxypropyl beta cyclodextrin in saline) or doxifludide (0, 0.0125, 0.025, 0.05, 0.1, 0.2, and 0.4 mg/kg), and animals anesthetized with ketamine/xylazine received vehicle (10% hydroxypropyl beta cyclodextrin in saline) or doxifludide (0, 0.0025, 0.005, 0.01, 0.02, 0.04, and 0.08 mg/kg) at 0.5 mL/kg over a period of 5 minutes followed by 10 minute-recovery period. Administration of doxifludide to either Nembutal or ketamine/xylazine anesthetized guinea pigs significantly increased QTcB interval at all doses tested compared to time-matched vehicle control (< 0.05). QTcB interval maximally increased 22% at a doxifludide dose of 0.08 mg/kg in the Nembutal group. In ketamine/xylazine anesthetized guinea pigs, a dose of doxifludide five times higher (0.4mg/kg) increased QTcB by only 12% maximally. No changes in mean arterial pressure, HR, QRS interval or PR interval were observed in Nembutal or ketamine/xylazine groups treated with doxifludide compared with each respective vehicle control. Our results demonstrate that sodium pentobarbital anesthetized guinea pigs are more sensitive to QTc interval prolongation; this should be the anesthetic of choice when screening agents for the potential to prolong QTc interval.

RNA-Seq and Microarray Gene Expression Vie for Toxicogenomics Superiority

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Transcriptomics technologies are the key tools for toxicogenomics. To determine if emerging transcriptomics technologies provide additional information to advance the understanding of complex toxicological processes, they must be compared carefully to existing, well-established technologies. To this end, data on gene expression alterations were obtained using the new Illumina RNA-Seq approach and compared with standard Affymetrix microarrays to obtain an understanding of which might provide more biological elucidation. Specifically, we use a comprehensive study design to generate gene expression data using both transcriptomics technologies from the same liver samples of rats exposed to varying degrees of perturbation (e.g., by 27 chemicals representing multiple modes of action (MOAs). The cross-platform concordance in terms of differentially expressed genes (DEGs) or enriched pathways is linearly correlated with treatment effect size (R2=0.8). Furthermore, the concordance was also affected by transcript abundance and biological complexity of the MOA. RNA-seq outperforms microarray (90% versus 76%) in DEG verification as assessed by qPCR, with the gain mainly due to its improved accuracy for low-abundance transcripts. Nonetheless, classifiers to predict MOAs performed similarly when developed using data from either platform. Therefore, the chemical studied and MOA, transcript abundance and genomic application are important factors in toxicogenomics research.

Integrating Differential Gene Expression with Hepatic, Cardiovascular, and Respiratory (NCR) Assessments Using Oral Administration of Amphetamine and Acepromazine to Conscious Beagle Dogs

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Cigarette smoking is the leading cause of emphysema and chronic obstructive pulmonary disease (COPD) worldwide. The receptor for advanced glycation end products (RAGE) is a cell surface membrane protein that recognizes AGE ligands produced by cigarette smoke and is an activator of both inflammatory and immune responses. Mice lacking RAGE protein expression show reduced oxidant-induced lung injury compared to mice that express RAGE. We hypothesized that RAGE null mice exposed to cigarette smoke will show reduced lung inflammation and oxidative stress compared to wild-type mice. RAGE null and C57BL/6 wild-type mice (n=3-4 group) were exposed to whole body cigarette smoke for one week and lung alveolar macrophages were removed and total RNA isolated for RNA sequencing. Illumina read files were aligned to the mouse Mm10 reference genome and differentially expressed genes were determined using DESeq negative binomial statistics. 354 genes were differentially expressed (Fold ≥ 1.5, FDR < 0.05) in macrophages by genotype and/or cigarette smoke exposure. Using Ingenuity pathway analysis software we identified multiple upstream transcription factors, including Nfe2l2 (Nrf2), Nfk1a and Nphp1 (ER stress transcription factor), whose increased activity (z-score ≥ 2) would account for the RNA expression changes observed in wild-type mice exposed to cigarette smoke. However, in RAGE null mice, none of these transcription factors reached a statistically significant z-score. Increased mRNA expression of stress response genes Cyp2a5 and Hsp1 was confirmed by qPCR analysis in wild-type mice. Increased expression of ER stress proteins Park (Eif2ak3), Ire1α (Ern2) and Pdi (Pdia3) was observed in wild-type mice but not RAGE null mice exposed to cigarette smoke. These results suggest that RAGE null mice exposed to cigarette smoke display a diminished oxidative and ER stress response and that inhibition of RAGE activity in vivo might be an effective therapeutic target for patients with a high risk of developing emphysema and COPD.
energy metabolism, immune response, and fibrosis. Targeted LC-MS/MS analysis detected 126, 72 and 130 metabolites in the hepatic extract, serum and day 26 urinary metabolites, respectively (vehicle, 3, 10 and 30 μg/kg only). Cytoscape was used to integrate differential expression and metabolite data onto 15 disrupted KEGG pathways that converged to common metabolites such as NADPH, and acetyl-CoA. For example, xanthine dehydrogenase (Xdh) expression increased 2-fold with concomitant decreases in serum xanthine and hypoxanthine levels indicating increased purine metabolism that may represent an important sink for NADPH in addition to cytochrome P450’s. The increased expression of the rate-limiting enzyme hexose-6-phosphate dehydrogenase (H6PD) in the pentose pathway (PPP) and altered hepatic levels of PPP metabolites (2-2-fold increase in ribulose-5-phosphate levels, 2-5-fold decrease in 6-phosphogluconic acid, erythrose-4-phosphate, fructose-6-phosphate, ribose-5-phosphate and glucose-6-phosphate) are consistent with increased pentose phosphate pathway (PPP) flux required to support induced several NADPH-dependent reactions. Collectively, integration of transcriptomic and metabolomic data suggests TCDD elicits systemic metabolic dysregulation associated with the dose-dependent progression of steatosis to steatohepatitis with fibrosis. Funded by SRP P42ES04911.

907 Mathematical Modelling of the Mevalonate Pathway As a Tool in the Development of a Quantitative Adverse Outcome Pathway for Cholesterol Biosynthesis Inhibitors

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Cholesterol, a vital membrane component and risk factor for cardiovascular (CV) disease, acts as a steroid, a vital component of the fungal cell wall, are synthesized by the mevalonate pathway. Inhibitors of the mevalonate pathway are used in CV therapy, and as anti-fungal agents (CYP51 inhibitors). The pathway is responsible for the biosynthesis of both cholesterol and isoprenoids and is under multivalent negative feedback control to permit the independent synthesis of both sterol and non-sterol components. The extent to which altering this pathway is associated with the carcinogenic and developmental effects of CYP51 inhibitors is unclear and complicated by the complex control. Therefore we have formulated and solved a deterministic nonlinear ordinary differential equation model of the mevalonate pathway (in a hepatocyte that describes transcription, by sterol regulatory element binding protein (SREBP), and translation of mRNA to enzymes 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR) and Squalene Synthase (Sqs), HMGR and Sqs reacting with substrates within the pathway to synthesise intermediary products and cholesterol, negative feedback regulation of SREBP by cholesterol, the regulation of Sqs degradation via cholesterol, and the regulation of HMGR degradation via Geranyl-PP, farnesyl-PP, lanosterol & cholesterol. Parameterised data from the literature, we show the pathway is very sensitive to changes in the transcription and translation of HMGR, the established rate limiting steps in this pathway: Sqs acts as a second rate limiting step; and the dose-response of cholesterol effects on SREBP permits the model to exhibit monotonic, damped or pure oscillations. Local feedback control the degradation of HMGR and Sqs, however the system is most sensitive to changes in HMGR with farnesyl-PP concentration tightly controlled. [1] Brown and Goldstein J Lipid Res (1980) 21:505.

908 Integrative Data Mining of High-Throughput In Vitro Screens, In Vitro Data, and Disease Information to Identify Adverse Outcome Pathway (AOP) Signatures

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The adverse outcome pathway (AOP) framework provides a systematic way to describe linkages between molecular and cellular processes, which can be measured via high-throughput screening (HTS), and organism or population level effects. Our goal is to generate computationally-predicted AOPs (cpAOPs) via data mining in order to accelerate AOP assembly and provide a more comprehensive coverage of biological space. We used frequent itemset mining (FIM) to find associations between the gene targets of ToxCast HTS assays and disease phenotypes from the Comparative Toxicogenomics Database (CTD). The method was also applied to map gene expression data in CTD to disease information also from CTD. This approach allows for indirect linkages between genes that have undergone HTS and disease through genes with expression changes in CTD. ToxCast phase II chemicals common to both datasets were used as aggregating variables for both analyses. After FIM analysis, 10,877 gene to disease associations met our cut-off (lift >1) for the ToxCast data and 82,055 for CTD. Considering genes and diseases as nodes and the FIM associations as edges, a cpAOP network was defined. Further analysis of this network using more stringent filters (increased support, confidence and specificity) provided a graphical representation of the links and highlighted indirect associations. One indirect association is illustrated by our finding of a link between AhR and Glaucoma in both Toxicast and CTD gene data via FIM. Though AhR isn’t found in CTD gene-disease pairs for glaucoma, it is a regulator of CYP1B1 which is not screened in ToxCast but was linked to glaucoma both by FIM analysis for CTD and a CTD query. This example highlights the value in integrating multiple data sources when defining cpAOPs for ToxCast assays. 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. EPA.

909 Formaldehyde-Associated Changes in Gene and Cytokine Expression Profiles within Nonhuman Primate Nasal and Circulating Blood


Formaldehyde (FA) is a ubiquitous environmental contaminant that has recently been shown to disrupt genomic and epigenomic profiles related to inflammation- and metabolism-associated signaling in the rodent model, a finding of high interest to policy makers and toxicologists during the ongoing formaldehyde assessment. This study set out to further analyze genmic signaling and cytokine expression levels associated with FA exposure using nonhuman primates exposed to either filtered air or 6 ppm FA for two days of 6-hour, whole-body inhalation exposure. Genome-wide transcript analysis showed 63 genes altered by FA in the nose and 53 genes altered in circulating white blood cells (WBCs), with 9 genes overlapping. Both gene sets showed alterations in signaling related to prostanooid metabolism. Prostanoids are compounds involved in inflammation responses, and are related to FA-associated health effects, including asthma and nasopharyngeal cancer. A nonhuman primate-specific cytokine panel assay identified eight cytokines in the nose and two cytokines in the circulating plasma with decreased expression associated with FA. No cytokines showed increased expression. The cytokine with the most significantly decreased levels in response to FA exposure was monocyte chemotactic protein 1 (MCP-1). MCP-1 protein levels also correlated with its transcript levels in the nose. MCP-1 is of interest, as MCP-1 has been identified as a key molecule in injury-induced inflammation response. The general decrease in cytokine expression associated with FA was surprising, as FA is known to induce local inflammation in nasal tissues resulting from longer exposure conditions. These results therefore provide novel insight into the temporal and tissue-specific responses to FA inhalation exposure.

910 Live Cell Imaging-Based Pathway of Toxicity Reporter Allows Identification of Chemical Specific Pathways Activation


Conventionally, cell based biomarkers have been sought after in the form of single or sets of genes, proteins or metabolites. However we propose a more heuristic approach to biomarkers, where cellular responses are viewed on the pathway or co-regulated gene set – level. Cells have evolved defense mechanisms such as DNA repair pathways (DDR), anti-oxidant pathways (OSR), ER-stress (UPR) mechanisms, rapid metabolic switching, metal stress response (MSR), Heat Shock Response (HSR) and inflammatory signaling that are critical for all essential cell types. We have GFP-tagged key proteins from all these major adaptive stress response pathways using BAC-cloning technology and targeting individual upstream “sensor” proteins, transcription factors and downstream target proteins. This platform now allows high content live single cell imaging of the dynamic responses induced by chemical exposure, including characterization of cell to cell variability and translation events. All the reporters were carefully characterized with targeted RNAi knock down. HCl cell imaging of these BAC reporter cell lines demonstrated that each adaptive stress response reporter is preferentially activated by their respective model compounds. We applied these HepG2 reporters to assess the activation of cellular stress responses by 150 compounds that are associated with drug-induced liver injury. All compounds were evaluated at 1, 5, 10, 50 and 100 Cmax and evaluated for the induction of Srxn1-GFP (Nef2 activation), CHOP-GFP (UPR activation), ICAM1 (NFkB signaling) and p21 (DNA damage). We identified specific sets of DILI compounds that strongly activate several, one or none of the pathway of toxicity reporters. In addition we see diverse time dynamics of individual adaptive stress responses, e.g. iodoacetamide induces an early reversible OSR (3-6 hrs)
in contrast to diethylmaleate which leads to a saturated OSR response for up to 24 hours. We anticipate that our cellular stress response reporters in combination with HCl may play a key role in future safety assessment of chemicals.

911 Validation of a Genomics-Based Hypothetical Adverse Outcome Pathway: 2,4-Dinitrotoluene Perturbs PPAR Signaling Thus Impairing Energy Metabolism and Exercise Endurance

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2,4-dinitrotoluene (2,4-DNT) is a nitroaromatic used in industrial dyes and explosives manufacturing processes that is found as a contaminant in the environment. Previous studies have implicated antagonism of PPARα signaling as a principal process affected by 2,4-DNT. Here, we test the hypothesis that 2,4-DNT-induced perturbations in PPARα signaling and resultant downstream deficits in energy metabolism, especially from lipids, cause organism-level impacts on exercise endurance. PPAR nuclear activation bioassays demonstrated inhibition of PPARα signaling by 2,4-DNT whereas PPARγ signaling increased. PPARα(-/-) and wild-type (WT) female mice were exposed for 14 days to vehicle or 2,4-DNT (134 mg/kg/day) and performed a forced swim to exhaustion one day after the last dose. 2,4-DNT significantly decreased body weights and swim times in WTs, but effects were significantly mitigated in PPARα(-/-) mice. 2,4-DNT decreased transcript expression for genes downstream in the PPARα signaling pathway, principally genes involved in fatty acid transport. Results indicate that PPARα signaling increased resulting in enhanced cycling of lipid and carbohydrate substrates into glycolytic/gluconeogenic pathways favoring energy production versus storage in 2,4-DNT-exposed WT and PPARα(-/-) mice. PPARα(-/-) mice appear to have compensated for the loss of PPARα by shifting energy metabolism to PPARα-independent pathways resulting in lower sensitivity to 2,4-DNT when compared to WT mice. Our results validate 2,4-DNT-induced perturbation of PPARα signaling as the molecular initiating event (MIE) for impaired energy metabolism, weight loss and decreased exercise performance. Toxicol Sci. 2014 Sep 1;141(1):44-58.

912 Sex-Dependent Metabolome Changes Depending on Age and Reproductive Cycle in Wistar Rats

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BASF and metanomics established a database (MetaMapTox) for toxicity modes of action, measuring metabolite profiles in rat plasma. Metabolome analysis in plasma was performed at different ages during development of Wistar rats of both sexes (childhood, pubescence, adulthood), and in females at late gestation and after lactation. Approximately 250 different endogenous metabolites were measured. Common metabolite changes in both sexes such as inorganic phosphate, 4-hydroxyproline, myo-Inositol, histidine, phenylalanine, dihydroxyphenylalanine, proline, arginine and allantoin were observed, those metabolites, are in general related with developmental characteristics in collagen synthesis, skeleton assembly, muscle and biogenic amine metabolism. In growing female rats, specific increases in creatinine, 2-hydroxybutyrate and citrate levels were noted, whereas in males in many complex lipids (as lysophosphatidylcholines), citrate and pseudouridine values decreased and xylitol levels increased over time. These results suggest a sex-dependent metabolite regulation regarding the cell and energy metabolism. Metabolome differences in females at the end of gestation and at the end of lactation compared to non-pregnant individuals revealed changes in metabolites such as complex lipids, triglycerides, fatty acids, energy metabolites and amino acids. These alterations can be explained with different demands of energy and structural components in these rats. In conclusion, different metabolite level regulations occur in male and female growing rats. In females the regulations depend also on the reproductive cycle.

913 Strain-Differences in the Proteome of Dioxin-Sensitive and -Resistant Mice Treated with 2,3,7,8-Tetrabromodibenzo-p-dioxin

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Dioxins cause various toxic effects through aryl hydrocarbon receptor (AhR) in animals with inter-species and strain differences in susceptibility. C3H/1pr and MRL/lpr are inbred mouse strains that are a dioxin-sensitive and -resistant type, respectively. However, the molecular mechanism underlying the different susceptibility still remains unclear. Here, we adopted a proteomic approach using two-dimensional electrophoresis and MALDI-TOF/TOF mass spectrometry to identify the difference in effects of 2,3,7,8-tetrabromodibenzo-p-dioxin (TBDD) exposure on hepatic proteomes between C3H/1pr and MRL/lpr mice. To confirm the induction of cytochrome P450 isozymes (CYPs) by TBDD treatment in each strain, we initially measured CYP1A1 and 1A2 protein levels. These results showed that at the dose of 10 μg/kg body weight, TBDD treatment increased CYP1A1 and 1A2 levels in both strains, but a more prominent induction was observed in C3H/1pr than MRL/lpr. The proteome analysis showed that based on more than 2-fold change in abundance by TBDD treatment, 40 up- and 17 down-regulated proteins in C3H/1pr and 7 up- and 10 down-regulated proteins in MRL/lpr were identified. Interestingly, the proteins induced in C3H/1pr were involved in the metabolism of tryptophan and its metabolites as endogenous AhR ligands, suggesting that AhR is more activated by accelerated production of endogenous AhR ligands in TBDD-treated C3H/1pr than MRL/lpr. We also identified proteins that underwent differential post-translational modifications between the two strains, e.g. peroxiredoxin 6 that shows adaptive responses to oxidative stress. The present study revealed that the high dioxin-susceptibility of C3H/1pr strain may be associated with more activation of AhR signaling pathway by endogenous AhR ligands and more efficient reduction of oxidative stress.

914 The Role of the Intestine in TCDD-Mediated Steatohepatitis in C57BL/6 Mice

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2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) elicits dose-dependent hepatic fat accumulation, inflammation, and progression to steatohepatitis with fibrosis. To investigate potential intestinal roles, we examined the effects of TCDD (0.01-30 μg/kg) on the jejunum, the segment predominantly responsible for fat absorption, in mice following oral gavage every 4 days for 28 days. Agilent 4x4k microarray analysis of the jejunal epithelium identified 439 differentially expressed genes ([fold change] ≥ 1.5, P1(t) ≥ 0.999) across one or more doses, many associated with lipid/cholesterol transport and metabolism, lipoprotein receptor activity, and immune cell activation. Phlp, CDS6, and Scarb1 induction suggests enhanced dietary fat hydrolysis and uptake consistent with increased hepatic fat accumulation. Using chamber studies indicated TCDD had no effect on fluoresceine-labeled 4kDa dextran flux across jejunal segments, suggesting lipid uptake is due to active transcellular rather than passive paracellular transport. Moreover, several major histocompatibility complex (MHC) class II genes (H2-As, H2-Aa, H2-Ab1, H2-DM1, Cβ4) exhibited dose-dependent suppression. The absence of dioxin response elements within MHC loci and a 32% decrease in F4/80+ stained lamina propria cells suggest migration of macrophages out of the proximal intestine. In contrast, hepatic RNA-Seq identified induction of MHC class II genes, chemokine ligands and chemokine receptors involved in macrophage recruitment (Ccr1, Ccr5, C5S, Cxcr7) consistent with increased F4/80 staining and macrophage infiltration into the liver. Collectively, these results demonstrate TCDD elicits intestinal changes consistent with hepatic lipid accumulation, inflammation and progression to steatohepatitis. Funded by SRF P42ES04911.
Environmental and occupational surroundings can result in exposure to different forms of metals. These metals include lead (Pb), cobalt (Co), and the widely studied element of metal intoxication, arsenic (As). Arsenic is found in the air, soil, and water, existing in one of two inorganic forms, arsenite (As(O2)3-) or arsenate (As(O4)3-). Extensive exposure to arsenic has been linked to a variety of pathological states including vascular diseases, metabolic diseases, and cancer and although the most studied mechanisms of arsenic toxicity are related to oxidative stress, many details about the mode of action of arsenicals and how these intersect with other pathways have yet to be elucidated. One pathway that has been shown to be misregulated is the autophagic protein quality control pathway, which leads to constitutively high levels of the receptor for advanced glycation end products (RAGE). RAGE is upregulated in cells in response to exposure to heavy metals, including arsenic. We have shown that arsenic exposure leads to increased autophagy, as measured by the expression of the autophagy marker Beclin 1. This increase in autophagy is associated with a decrease in cell proliferation and an increase in cell death. In this study, we investigated the mechanisms underlying the benefits of arsenic exposure, compromised autophagy, and increased Nrf2 levels. In this study, we show that arsenic can induce impairment of Nrf2 function. The ATPase activity of p97 in the presence of arsenate was assayed using a malachite green assay. Interestingly, p97 ATPase activity is increased in the presence of arsenite, mimicking autosomal dominant genetic lesions that also lead to compromised autophagy. We have also tested the effects of arsenic on p97 function using H9C2 cells that stably express UbG76VGF, a proteasome targeted fusion that requires p97 for efficient degradation. Cells exposed to arsenite showed an increase in GFP fluorescence compared to control, indicating a loss of p97 function. Collectively, our studies suggest a link between arsenic exposure, p97 misregulation, compromised autolysosome maturation, increased Nrf2 levels, and cellular transformation.

We evaluated the effects of cigarette smoke (CS) from a reference conventional cigarette (3R4F) and an aerosol from THS 2.2, a candidate modified risk tobacco product (cMRTP). ApoE-/– mice were exposed to an aerosol from 3R4F or from THS 2.2 for up to 8 months to a target nicotine concentration of 30 μg/L. After 2 months exposure to CS, cessation and switching groups were further exposed for up to 6 months to fresh air or THS 2.2, respectively. A battery of markers of disease was measured including vascular function, inflammation and emphysema. Exposure to CS induced time-dependent molecular, physiological and inflammatory responses in the lungs of ApoE-/– mice consistent with emphysematous changes. The size of athcerosclerotic plaques was higher in CS-exposed animals compared to both sham and THS 2.2-exposed animals. Micro-CT images of aortic arches after 8 months of exposure showed a larger plaque volume in 3R4F-exposed animals compared to sham, cessation or THS 2.2-exposed mice. Significant changes in the lung transcriptome of ApoE-/– mice were observed in response to 3R4F-exposure compared to the gene expression levels of sham-exposed mice, while smoking cessation and switching to THS 2.2 resulted in lower activation levels compared to 3R4F. Both smoking cessation and switching to the THS 2.2 aerosol halted the rate of disease development as assessed by inflammatory, histopathological and molecular endpoints. Our work demonstrates the power of using the ApoE-/– mouse model to study comorbidities associated with cigarette smoking and to investigate the mechanisms underlying the benefits of smoking cessation or switching to a cMRTP.
cohort of mice underwent in situ contractile testing. Muscle stem cells isolated from As(III)-exposed mice showed slower proliferation rate and myofibroblast formation compared to As(III) mice that showed altered cytoskeleton and a contracted phenotype. Contractile testing of the muscles revealed a significant decrease of muscle strength following As(III) exposure. Multi-photon imaging of the muscles showed that As(III) exposure increased the alignment of collagen fibers suggesting pathogenic matrix deposition after injury. In conclusion, these data indicate that As(III) disrupts muscle cell regeneration and extracellular matrix composition, thereby decreasing the regenerative ability after acute injury.

920 Chemoproteomic Profiling of Environmental Electro-philosophes Reveals Toxicity through Altered Cellular Metabolism


The large numbers of chemicals we are exposed to necessitate a better understanding of their interactions within complex biological systems to more accurately predict toxicities. Though many chemicals have incomplete toxic mechanisms due to unknown direct target interactions, of particular concern are reactive electrophilic chemicals that can interact covalently with metabolic enzymes, causing widespread changes to biochemical networks. Needed are in depth toxicological assessments that provide the direct protein targets of reactive chemicals in complex systems, as well as reveal the resulting biochemical effects of this interaction. To address this, we have developed a new chemoproteomic technology called reactivity-based protein profiling (RBPP) that employs bioorthogonal chemical probes that bind to reactive residues such as cysteines within complex proteins. Using RBPP, we first identify the direct and specific protein targets of reactive environmental chemicals in vivo, then examine the consequences of target engagement using functional metabolomics to reveal downstream metabolic changes. In this study we used RBPP to screen highly used environmental electrophiles and then identified novel targets of the highly reactive yet widely used fungicide chlorothalonil in vivo in mice. A bioorthogonal chlorothalonil probe revealed targets that include a network of hyperreactive cysteine-containing metabolic enzymes spanning multiple conserved metabolic networks. Functional metabolomic analysis revealed dramatic alterations in critical lipid and central carbon metabolic pathways, suggesting broad toxicity through previously undescribed mechanisms. Using techniques like RBPP to map proteome-wide interactions and biochemical effects of reactive chemicals is critical for prioritizing action on existing toxicants and for developing safer chemicals to minimize exposure-related health effects.

921 What Is Normal in Metabolomics?

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Over the last 10 years a data base containing toxicity and metabolome profiles of > 700 compounds from 28 day rat studies was developed. During development also data from > 80 control groups (with each 10 males and females) were obtained. For quality control some compounds inducing clear metabolic changes were investigated repeatedly. We have performed an in depth statistical analyses to obtain information on: 1) The distribution of statistically significant changes within the control populations 2) The graphical display of the distribution of changes in the control population for each of the 250 analyzed metabolites 3) The level of statistical significance at which the best balance between matches and mismatches in treatment groups is found. 1) We generated an empirical distribution for the number of significances under the null hypothesis by repeatedly dividing control groups of 10 animals into two groups at random and comparing them. This was done for each of the 80 control populations at three time points and both sexes. We noted a non-normal distribution of the frequency of statistically significant changes. The peak of the distribution curve was left of the center of the normal binomial distribution (the exact distribution under assumption of independence of each metabolite) but with an elongated tail to the right. 2) A graphical display was obtained by showing the relative change and the standard deviation of the control group under investigation versus the entire population of controls. 3) Comparing the exact repeats at different levels of statistical significance, the best balance between matches (metabolites regulated in the same direction) and mismatches (metabolites regulated in opposite directions) was obtained at a level of statistical significance of p = 0.1 (Welch t-test). Such statistical analysis helps to inform the quality of a study (relative to all other studies performed) and can be used to define control ranges (and hence to characterize metabolite regulations beyond simple statistics).
924 Inactivation of Fusarium by Neutral Electrolyzed Oxidizing Water in Tomato Seeds

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The presence of Fusarium oxysporum (F. oxysporum) and F. verticillioides in tomato represents an important economic problem. If contaminated seeds are planted, the risk of contaminated adult crops increases. Our objective was to evaluate the effectiveness of Neutral Electrolyzed Oxidizing Water (NEW) to reduce the presence of Fusarium in tomato seeds. Tomato seeds El Cid F1 were purchased from Harris Moran seed company, while Fusarium reference strains were donated by Facultad de Estudios Superiores, Cuautitlan. NEW at 10, 40, and 60 ppm of free chlorine were obtained from Esteripharma S.A. de C.V. Bleached and distilled water (DW) were used as controls. For infection of seeds, a conidial suspension containing 2-2.5x10^6 conidia per mL was prepared. Each treatment group (5 in total) consisted of 50 seeds with 4 replicates per treatment. Four different treatment times were tested (5, 10, 15 and 20 min). Seeds were immersed in conidial suspension for 20 min and then exposed to each specific treatment. Seeds were then incubated in plates containing potato dextrose agar for 7 days at room temperature. After that, 100 [0.1%] was added to dissolve the agar and proceed with counting of conidia. Data were analyzed with Kruskal-Wallis and Mann-Whitney methods. Significant differences between treatments (p <= 0.05) and controls were found. The most effective treatment was NEW60 with up to 64% inhibition for F. oxysporum, and up to 76% for F. verticillioides. Exposure time did not show significant differences (p > 0.05). These results suggest application of NEW can be an effective strategy to reduce Fusarium contamination in tomato seeds. Funding: CONACYT-PROINNOVA 196320.

925 Effects of Neutral Electrolyzed Oxidizing Water on Aspergillus Inactivation in Tomato Seeds

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Aspergillus flavus and A. parasiticus are mycotoxin producing pathogens that occur on tomato crops. Seed integrity is imperative to reduce the risk of contamination of adult crops. Neutral electrolyzed oxidizing water (NEW) is a promising strategy to reduce fungal contamination. In this study, we evaluated the efficacy of NEW to reduce the presence of Aspergillus in tomato seeds. For experiments, Aspergillus reference strains were donated by Facultad de Estudios Superiores, Cuautitlan, Mexico. NEW at 10, 40, and 60 ppm of free chlorine were obtained from Esteripharma S.A. de C.V. while bleach (6.5%) and distilled water were used as controls. Treatment groups included: bleach, water, NEW10, NEW40, and NEW60. Groups consisted of 50 seeds with 4 replicates per treatment. Treatments were tested at four different times (5, 10, 15 and 20 min). For infection of seeds (El Cid F1, Harris Moran seed company), a conidial suspension containing 2-2.5x10^6 conidia per mL was prepared. Seeds were immersed in conidial suspension for 20 min previous to treatments with NEW. Then seeds were incubated in plates containing Sabouraud media for 3 days at room temperature. After incubation, media in plates was dissolved with tween 80 [0.1%] to record conidia counts. Data were analyzed with nonparametric methods Kruskal-Wallis and Mann-Whitney. Exposure time did not show significant differences (p > 0.05). NEW60 was the most effective with up to 94% for A. flavus and 94% for A. parasiticus. Findings suggest that NEW can be effective to reduce Aspergillus contamination in tomato seeds. Funding: CONACYT-PROINNOVA 196320.

926 Development of a Zearealenone Entersorbent to Mitigate Toxin Exposures from the Diet

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Previous methods for the control of zearalenone (ZEN)-induced hyperestrogenism in animals have proven largely ineffective. The main objective in this study was to identify an entersorbet that has potential to decrease dietary bioavailability and subsequent estrogenic effects of ZEN. A variety of materials including talc, hectorite, and inulin were evaluated using in vitro and in vivo methods. Isotherms for ZEN adsorption were conducted at pH 2 and pH 6.5, mimicking pH conditions in the stomach and small intestine. A Hydro vulgaris toxicity study was performed to evaluate the potential safety of the additives. Computer-generated isotherm data were fit using the Langmuir model, and parameters of Qmax and Kd were estimated. An ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) method for quantification of ZEN and metabolite reduction was optimized for the detection of ZEN in urine. UPLC-MS/MS analysis of ZEN, α-zearalenol (αZOL), and β-zearalenol (βZOL) was conducted in ESI negative mode. Initial in vitro screening in aqueous solution (4 μg ZEN/mL) indicated that the most effective sorbents for ZEN at both pH conditions were talc, hectorite, and inulin. Average Qmax values for talc and inulin were 0.08 and 0.05, respectively. Average Kd values for talc and inulin were 1.1 x 10^1 and 7.3 x 10^6, respectively. The Qmax and Kd values for hectorite could not be accurately determined due to a partitioning phenomenon. Initial hydra bioassay results indicated that talc and hectorite afforded protection against ZEN without causing overt toxicity. The optimized UPLC-MS/MS method allowed for detection of ZEN and its metabolites down to levels as low as 0.015 ng/mL. Based on these results, pharmacokinetic screening in a rodent model is warranted to evaluate the bioavailability of talc and inulin following inclusion in the diet. Studies are ongoing to investigate similar entersorbet materials, including insoluble dietary fiber. This work was supported by BASF Corporation, Ludwigshafen, Germany.

927 Critical Control Point-Based Reduction of Deoxynivalenol and Zearalenone in Stored Adlay (Coix lachryma-jobi L.) Grains

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Improperly practiced postharvest procedures can pose mycotoxin-related risks in the production of medicinal herbs. As a health food with pharmaceutical supplements, cereal-based adlay has been broadly used in oriental medical practice. Compared with the standard production protocol, three provisional critical control points (CCPs) in the conventional procedure were identified and assessed for mycotoxin contamination in the adlay from small farms in Korea. Although various mycotoxins were present, the prevalence of deoxynivalenol (DON) or zearalenone (ZEN) was relatively high in the adlay. In terms of drying conditions, field drying in the conventional pathway was associated with more exposure to DON than heated-air drying. Moreover, the DON or ZEN levels in chaff were higher than the levels in the inner grain, suggesting that the hulling process as another CCP would reduce the DON or ZEN exposure. In particular, the DON or ZEN levels in adlay stored for protracted periods without dehulling were very high, but a lower storage temperature of 12°C was not effective at significantly reducing these mycotoxins. In this case, the inner grain was more contaminated with DON or ZEN than the chaff after protracted storage because surface fungi, which produce mycotoxins, can penetrate deep into grain with time. Heated-air drying and nonprotracted storage limited DON contamination in adlay. More importantly, an early dehulling point was identified as another CCP to reduce DON contamination in adlay. The Qmax and Kd values for hectorite could not be accurately determined due to a partitioning phenomenon. Initial hydra bioassay results indicated that talc and hectorite afforded protection against ZEN without causing overt toxicity. The optimized UPLC-MS/MS method allowed for detection of ZEN and its metabolites down to levels as low as 0.015 ng/mL. Based on these results, pharmacokinetic screening in a rodent model is warranted to evaluate the bioavailability of talc and inulin following inclusion in the diet. This is monitored as a central CCP for safer production of adlay from local farms (This work was supported by grants from the Basic Science Research Program, through the NRF Korea, funded 2012R1A1A2005387 and project no. PJ009435 of NIHHS, RDA, Republic of Korea).

928 Suppression of Arsenite-Induced Cytotoxicity and Activation of Nrfs by Aliphatic Electrophiles from Coriandrum sativum L. Leaf Extract in HepG2 Cells

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Leaves of Coriandrum sativum L. (C. sativum) are not only eaten as a spice, but have also been traditionally used as a medicine. Although a 70% ethanol extract of the leaves has been reported to activate Nrfs in HaGaT cells, the structures of the phytochemicals involved in Nrfs activation remain unknown. To address this issue, we isolated Nrfs activators from C. sativum leaf extract (CSLE). Exposure of HepG2 cells to CSLE caused covalent modification of Keap1 and activation of Nrfs. Preparative ODS column chromatography indicated that chemicals from CSLE can activate Nrfs. Subsequent UPLC-MS analysis with 2-diphenyle-
tly-1,3-iodo-1-hydrazine revealed that these phytochemicals possess a 2-alkenal group with a different carbon number and were identified as (E)-2-decenal, (E)-2-undecenal, (E)-2-dodecenal and (E)-2-tridecenal. Using (E)-2-decenal and (E)-2-dodecenal, we found that these 2-alkenals are covalently bound to recombinant mouse Keap1 and cellular Keap1 in HepG2 cells and activate the Nrf2/ARE signal transduction pathway. Overall, it is suggested that the 2-alkenal group of an α,β-unsaturated aldehyde is essential for Nrf2 activation. Consistent with this, (E)-2-butenal modified Keap1 and thus activated Nrf2, whereas butanal had little effect on Keap1 modification and Nrf2 activation. Interestingly, pretreatment with CSLE, (E)-2-decenal and even (E)-2-butenal significantly blocked arsenite-mediated cytotoxicity in HepG2 cells, presumably through Nrf2 activation, but not treatment with butanal. These results indicate that phytochemicals isolated from CSLE associated with Nrf2 activation are aliphatic electrophiles with a 2-alkenal group. Because (E)-2-decenal and (E)-2-dodecenal are abundant components found in CSLE, our findings may provide useful information concerning a potential intervention study using C. sativum in an endemic area of chronic arsenic poisoning in East Asia.

929 Prevalence of Enterobacteriaceae in Retailed Poultry Eggs and Their Zoonotic Relationship in South Africa


Food safety is an important public health issue and governments across the world are intensifying their efforts to improve the quality, quantity and also the safety of national food supplies. Salmonella pathogens in particular are the most common causes of food poisoning worldwide with negative economic consequences; and poultry meat, eggs and their products are major sources of salmonellosis. There is little information available on the risk factors for Salmonella infection in Africa. Therefore, this studies aims to establish the baseline epidemiology and the most prevalent serotypes of Salmonella for effective control and improved productivity. Eggs samples were pre-enriched in buffered peptone water and incubated at 37°C for 24 hours. One ml of the overnight broth sample was added to 9 ml of tetrathionate (selective medium) broth before plating them individually on XLD agar for 24 hours for bacterial growth. Bacteria were identified by Gram stain and confirmed by biochemical assays. The MALDI Biotyper was used to reliably identify a wide range of microorganisms. Preliminary results showed that out of 468 egg samples and 17 brands that were analysed during this survey, some egg brands had as high as 50% Salmonella positive samples, making them highly unsafe for consumption. Niced and the more expensive eggs had lesser Salmonella in them than the cheaper eggs and those not marketed as niced at this stage. 10% eggs tested positive for Salmonella in this study making them unsafe. Confirmation of presumptive enterobacteriaceae and Salmonella in particular was done by MALDI-TOF assay and the dendrogram showed Enterobacter cloacae 11%; Proteus mirabilis 7%; Stenotrophomonas maltophilia 7%; Klebsiella pneumoniae 4% and Salmonella spp 71%. These are potentially dangerous to health; therefore consumers are advised to avoid consuming raw eggs and their products to safe guard life. These results (Salmonella in particular) will be compared with data obtained from human cases and a correlation will be drawn to see their relatedness.

930 Inactivation of Pathogens on Contact Surfaces Using Decontaminating Substances Produced by Radiant Catalytic Ionization


Radiant Catalytic Ionization (RCI), is thought to be safe in humans as an organic form of treatment to disinfect food contact surfaces through the use of radical oxygen species. RCI has countless applications for reducing the number of bacteria on a variety of surfaces with varying conditions. The focus of this study is the inactivation of Escherichia coli, Listeria, and Staphylococcus, which were introduced to the tops of sterile cotton swabs, and food surfaces, using RCI. Our results indicate a 99.9% reduction in the recovery of bacteria with a 90 minute exposure to RCI, demonstrating that the oxidative gases produced with RCI is an effective surface disinfectant tool for use in food processing.

931 Does Nanostructured Synthetic Amorphous Silica in Industrially Manufactured Powdered Food Products Disintegrate after Oral Uptake?


Nanomaterials in consumer products are subject of an extensive discussion regarding potential risks. One question concerns the exposure to nanostructured performance additives in industrial food products. Synthetic amorphous silica (SAS) is used as a flow-agent, e.g. in soup products. SAS is a nanostructured material formed by flame hydrolysis or precipitation. Basic structural elements of the material (fused nanosized primary particles) are submicron aggregates that themselves form micrometer (or even larger) agglomerates. For risk evaluation it is important to understand whether structural changes may occur following processing and oral uptake. Previously we investigated potential effects of heating in water (modelled processing of soup powder before eating), acid environment (pH=1.3 like gastric juice, 37 °C) and fed-state simulated intestinal fluid (FeSSIF; acetate buffer, pH=5.0, sodium taurocholate, phosphatidylcholine. 37 °C) on structure and particle size distribution of food grade precipitated and pyrogenic SAS in vitro.

We, now addressed potential changes in a model food product. According to the composition of commercial soup powders we manufactured mixtures of potato starch and saccharose containing 1% (w/w) of SAS. Following suspension of the powder mixtures in the above media, samples (at defined intervals) were analyzed with Laser Diffraction, providing data on potential changes in volume weighted particle size distribution, and with (High Resolution) Transmission Electron Microscopy to evaluate possible particle degradation (size, morphology, and surface roughness).

We did not detect any signs of disintegration for precipitated as well as pyrogenic SAS in food powder mixtures following oral uptake. In addition, no structural changes (aggregates, agglomerates) or degradation (surface roughness) were observed. These results are consistent with our earlier studies where comparable data had been gained for pure SAS products in physiological media.

932 Autophagy and Senescence, Stress Responses Induced by the DNA-Damaging Mycotoxin Alternariol

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The mycotoxin alternariol (AOH), a frequent contaminant in fruit and grain, is known to elicit reactive oxygen species (ROS), DNA damage and cell cycle arrest. Cellular stress is often connected to autophagy, and we employed the RAW264.7 macrophage model to test the hypothesis that AOH induces autophagy. Following AOH treatment, the cell morphology was changed from round into more star-shaped. Acidine orange staining followed by flow cytometry analyses, revealed that AOH resulted in a ROS independent increase in acidic vacuoles (lysosomes and autolysosomes). Moreover, western and flow cytometry analyses showed increased expression of the autophagy marker LC3, and strong accumulation of LC3-positive puncta (fluorescence microscopy). Increased autophagic activity was verified by measuring the degradation rate of long-lived proteins (L-[14C] valine marked).

Furthermore, AOH induced DNA damage (comet assay), double strand breaks (DSB; γ-H2AX fluorescence staining). DNA damage was followed by increased p53 phosphorylation, expression of Sestrin2, and phosphorylation MAPK, as well as reduced phosphorylation of mTOR and S6 kinase (western), which are a common signaling pathway involved in autophagy. Transmission electron microscopy analyses of AOH treated cells not only clearly displayed structures associated with autophagy such as autophagosomes and autolysosomes, but also the appearance of lamellar bodies. Prolonged AOH treatment resulted in increased β-galactosidase activity (light microscopy), suggesting that the cells eventually entered senescence. In conclusion, our data identify AOH as an inducer of both autophagy and senescence. These effects are suggested to be linked to AOH-induced DSB (via a reported effect on topoisomerase activity), resulting in an activation of p53 and the Sestrin2-AKAP-mTOR-S6K signalling pathway.
Steviol glycosides have been identified as the compounds associated with the sweetness of Stevia plant extracts. The hydrolysis of the stevioside glycosides rebaudioside (Reb) A and Reb E, as well as steviolbioside (a metabolic intermediate) to steviol was evaluated in vitro using human fecal homogenates from healthy Caucasian and Asian donors. Incubation of each of the Reb in both groups resulted in a rapid hydrolysis to steviosid. Metabolism was complete within 24 h, with the majority occurring within the first 16 h. There were no clear differences in the rate or extent of metabolism of Reb E (% hydrolysis of 90.1 ± 2.1 at 16 h) relative to the comparative control Reb A (% hydrolysis of 86 ± 3.0 at 16 h). The hydrolysis of samples containing 2.0 mg/mL of stevioside Reb A and Reb E (60% hydrolysis at 16 h) tended to take longer than 0.2 mg/mL samples (88% hydrolysis at 16 h). There were no apparent gender and ethnicity differences in the rate of metabolism of any of the Reb, regardless of the concentrations tested. Steviolbioside, an intermediate in the hydrolysis of Reb E to steviol was also found to be rapidly degraded to steviol (% hydrolysis of 78 ± 5.0 at 16 h). These results demonstrate Reb E is metabolized to steviol in the same manner as Reb A. These data support the use of toxicology data available on steviol and on stevioside metabolized to steviol (i.e., Reb A) to underpin the safety of Reb E.
The market for medicinal plants covers 25% of the non-prescription drugs sold in Europe. Al has not been classified regarding carcinogenicity. However, Al production has been determined as carcinogenic to humans by the IARC, and its neurotoxic properties are well established. Grains, vegetables, legumes, and, particularly herbs and spices may exhibit important concentrations of Al. In general, Al uptake through food is approximately 1×10−4 mg/kg bw/day, and the oral bioavailability for Al from the diet is estimated to be 0.1 to 0.3%. A total Al intake of 10.17 mg/day was estimated for the Canary Islands population while in the USA is 7.2 and 8.6 mg/day for females and males respectively. Objectives: To determine and evaluate Al contents in 6 medicinal plants: Matricaria chamomilla, Tilia officinalis, Equisetum arvense, Valeriana officinalis, Salvia officinalis, and Asarum vulgare. Method: A total of 100 samples (26 organically grown) was analyzed, comprising the following species: M. chamomilla (35 samples, 7 of which organic), T. officinalis (29 samples, 6 of which organic), A. arvense (12 samples, 4 of which organic), V. officinalis (5 samples, 2 of which organic), S. officinalis (10 samples, 3 of which organic), and A. vulgare (9 samples, 4 of which organic). Metal content was determined by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES). Results: All samples presented detectable Al levels. Salvia officinalis had the highest mean Al level (110.35 mg/kg), followed by Valeriana officinalis (103.96 mg/kg). Mean Al contents in non-organic vs organic farming were: Matricaria chamomilla, 88.03 vs 55.84 mg/kg, Tilia officinalis, 59.89 vs 57.71 mg/kg, Equisetum arvense, 66.25 vs 24.07 mg/kg, Valeriana officinalis, 136.42 vs 71.90 mg/kg, Salvia officinalis, 130.23 vs 90.47 mg/kg, and Asarum vulgare, 35.33 vs 20.60 mg/kg. Conclusions: Medicinal plants contribute to dietary Al intake. Organically grown samples have lower Al levels than non-organic samples.

**Evaluation of the Food Additive Carrageenan in a Caco-2 Intestinal Absorption Model**

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Carrageenan (CGN; Mw 200,000-800,000) represents a group of polysaccharides that are extracted from certain species of red seaweed in the family Rhodophyceae. CGN has been widely used in food products because of its gelling, thickening, and stabilizing properties. It has been evaluated in standard animal safety studies and has been shown to be safe for human consumption. In vitro studies have reported that CGN may cause intestinal inflammation, ulcers, and cancer. CGN binding to Toll-Like-Receptor 4 in the gut and subsequent up-regulation of NF-κB is one hypothesis; absorption across the intestinal epithelium, followed by production of reactive oxygen species is another. The use of in vitro models to assess compound toxicity must be done with care. For example, the identity and purity of the CGN used must be known and the effects of protein binding on the availability of free CGN for cellular interaction should be considered. The aim of this study was to determine whether a food grade λ-CGN can be absorbed across a Caco-2 intestinal cell model. Caco-2 cells were seeded onto a transwell membrane at 20,000 cells/well. Tight junction formation was confirmed by TEER analysis. Food grade λ-CGN was fully characterized and prepared in media at final concentrations of 100, 500 and 1000 μg/mL. Ranitidine and Pindolol were included as controls for low and medium-high permeability, respectively. CGN was added to the apical chamber and incubated for 2, 4, or 6 hr. Samples were taken from the apical and basolateral chambers for assessment of CGN unidirectional permeability (Papp). Cytotoxicity was monitored by the MTT assay. Papp for CGN across the cell layer was undetectable and cell viability remained above 95%. This experiment was performed on two separate days, with three wells per treatment group, in compliance with Good Laboratory Practices. In conclusion, well-characterized food grade λ-CGN was not cytotoxic or absorbed across the Caco-2 cell layer.

**Aloe vera Extracts Induce Mutations and Oxidative Stress in Cultured**

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A 2-year cancer bioassay in rodents with a preparation of Aloe vera whole leaf extract administered in drinking water showed clear evidence of carcinogenic activity. To provide insight into the identity and mechanisms associated with mutagenic components of the Aloe vera extracts, we used the mouse lymphoma assay to evaluate the mutagenicity of Aloe vera whole leaf extract (WLE) and Aloe vera decolorized whole leaf extract (WLD). The WLD extract was obtained by subjecting WLE to activated carbon-adsorption. HPLC analysis indicated that the decolorization process removed many components from the WLE extract, including anthraquinones. Both WLE and WLD extracts showed cytotoxic and mutagenic effects in mouse lymphoma cells but at different concentration ranges, and WLD induced about 3-fold higher levels of intracellular reactive oxygen species than WLE. Molecular analysis of mutant colonies from cells treated with WLE and WLD revealed that the primary type of damage from both treatments was largely due to chromosome mutations (deletions and/or mitotic recombination). The fact that the samples were mutagenic at different concentrations suggests that while some mutagenic components of WLE were removed by activated carbon filtration, components with pro-oxidant activity and mutagenic activity remained. The results demonstrate the utility of the mouse lymphoma assay as a tool to characterize the mutagenicity of fractionated complex botanical mixtures to identify bioactive components.

**Crosstalk between Macrophage Inhibitory Cytokine 1 and Activating Transcription Factor 3 in Carrageenan-Exposed Enterocytes**

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Carrageenan (CGN), a widely used food additive, has been shown to injure the epithelial barrier in animal models. This type of damage is a clinical feature of inflammatory bowel disease (IBD) in humans. In the present study, the effects of CGN on pro-apoptotic responses associated with macrophage inhibitory cytokine 1 (MIC-1) regulation in human enterocytes were evaluated. CGN up-regulated the expression of MIC-1 that promoted epithelial cell apoptosis. Although MIC-1 induction was dependent on pro-apoptotic p53 protein, the pro-survival protein activating transcription factor 3 (ATF3) was negatively regulated by p53 expression. However, MIC-1 enhanced the expression of the pro-survival protein ATF3 in enterocytes exposed to CGN. Functionally, MIC-1-mediated epithelial cell apoptosis was counteracted by the pro-survival action of ATF3 in response to CGN exposure. These findings demonstrated that the counterbalance between MIC-1 and ATF3 is critical for deciding the fate of enterocytes under the food chemical stress (This work was supported by the Basic Science Research Program through the National Research Foundation of Korea, funded by Ministry of Education, Science and Technology Grant 2012R1A1A0205837).
Aflatoxin M1 (AFM1) is a metabolite that is found in milk and urine after consuming feed contaminated with AFB1 (AFB). Wherefore AFM1 is a biomarker for aflatoxin exposure. AFM1 is less toxic than AFB1; however, it has been considered as a potential carcinogenic to humans by the International Agency of Research on Cancer. In order to assess the AFM1 milk contamination, 52 dairy farms in Aguascalientes Mexico, were sampled. The presence of aflatoxin in feed in each dairy farm was also measured. Milk samples were analyzed via ELISA kit. The detection ranged from 5 to 80 ppt, and a 100% specificity. Our results show that 100% of the milk samples from the dairy farms tested had detectable levels of AFM1, but all of them were below The Mexican Official Norma (NOM) limits; nonetheless 29/52 dairy farms had AFM1 levels that exceeded The Commission Regulatory (EU) limits. The aflatoxin levels found in feed were below the NOM and EU. Even though there are local and International regulations to ensure foodstuffs having minimum levels of AFM1; the authorization of feed or food for human or animal consumption depends whether the NOM or EU regulations is applied. Could it be a public or animal health concern?

To evaluate the direct inorganic mercury-induced neurotoxicity and to study the mechanism underlying these effects, male and female rats were exposed to mercury chloride (0.5, 1.0, and 1.5mg/kg body weight) by oral gavage. The activity of acetylcholinesterase (AcChE) in plasma, erythrocyte, brain and erythrocyte membrane were determined: AcChE activity in all the compartments was inhibited to varying extents more profoundly in female. However, at highest dose of mercury; plasma AcChE activity was inhibited to the tune of 52% in the male and 84% in female animals. In the erythrocyte, the highest inhibition of 88% was achieved in the female rats. Erythrocyte membrane AcChE inhibition ranged from 55% to 84% in male while, and 37% to 88% in female. The lowest inhibition of 34% was obtained in the brain AcChE of the male animals. We observed negative associations between tissue mercury levels and AcChE activities in all the compartments except for brain where no significant relationship was found. The results call for further insight into the mechanisms of mercuric chloride-induced neurotoxicity.

**Phytochemical Screening of Various Lots of Echinacea for Use in Toxicity Studies**

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Echinacea is sold as a dietary supplement in the U.S. for its purported immune stimulating properties. There are many different products containing Echinacea including: tablets, extracts, capsules, and teas. Echinacea was nominated to the National Toxicology Program (NTP) for toxicity testing due to lack of safety data. There are up to 9 species contained with the genus Echinacea, and 3 are currently employed medicinally in the U.S. (E. purpurea, E. pallida and E. angustifolia). Although these 3 species belong to the same genus, they do not have the same therapeutic effect or chemical composition. NTP selected Echinacea purpurea for toxicity testing because it is the most commonly studied and utilized species on the market. Some Echinacea species have been adulterated with another closely related plant species, Parthenium integrifolium. The objective of this work was to screen several lots of Echinacea, purported to be E. purpurea, from multiple vendors in order to select an appropriate lot for use in NTP studies. Eight lots, sold as ethanolic extracts of Echinacea purpurea root or herb, were procured from multiple vendors and analyzed along with the reference materials for Echinacea species. Comparison of chromatographic profiles and analysis for various known Echinacea components (cichoric acid, caffeic acid, chlorogenic acid, echinacoside, cynarin, isobutylamides) showed significant lot-to-lot variation. Based on the presence of high levels of cichoric and caftaric acids and low levels (or the absence) of chlorogenic acid, echinacoside, and cynarin, two lots were identified as potential lots for testing. In conclusion, measuring 4 different endpoints of the so-called vector model (cytotoxicity, macropage activation, TNF release, oxidative burst), and converting these into a sum index, indicates inasmuch NMs interfere with particle clearance and basic immune function of the lung. A total of 27 previously characterized NM (pure and mixed oxides from Al, Ti and/or Zr; various CeO2; unmodified and surface-modified amorphous SiO2; coated ZrO2; graphite nanoplatelets; different organic and Fe2O3 pigments; Ag; ZnO) were tested against micron-sized quartz DQ12 and corundum. Results were compared to the outcome of 18 ITI and 18 STIS. The ranking of the NM in vitro ranged from ion- shedding silver or ZnO NM, to Al-doped CeO2, over SiO2 and ZrO2 modifications, to SrCo3 and BaSO4 as being least active. While the in vitro ranking in principle matched the in vivo results, a more detailed comparison of in vitro toxicity and STIS data also had to consider particle surface size. Thus, when STIS and vector model data were subjected into a 4 hazard categories each, 13 out of 17 in vitro tests matched the correct and 3 matched a neighbouring category. Overall, the in vitro approach with NR8383 cells appears promising and well suited to compare the hazard potentials of inhaled nanomaterials.
Methylmercury (MeHg) is a persistent environmental toxicant that poses significant health risk to the human population. Despite extensive research, the mechanism(s) by which it elicits toxicity have not been fully elucidated. It has been demonstrated that MeHg induces an oxidative stress response in the brain, which in part is coordinated by nuclear factor erythroid 2-related factor 2 (Nrf2). Nrf2 is a transcription factor that translocates to the nucleus in response to reactive oxygen species generation and upregulates phase II detoxifying enzymes and antioxidant proteins. It is well established that Nrf2 knockouts models are more susceptible to toxicity. The potential impact of altered Nrf2 gene expression on MeHg-induced oxidative stress in astrocytes, the primary site of MeHg accumulation in the brain, however, has yet to be investigated. Accordingly, we tested the hypothesis that Nrf2 knockdown in astrocytes will exacerbate MeHg toxicity, while overexpression will be protective. We first examined Nrf2 gene expression subsequent to MeHg exposure in astrocytes isolated from post-natal day 1 Sprague-dawley rats. Cells were exposed to MeHg (0, 0.5, 1, 5, 10 μM) for 24 hours. RNA was isolated, and qRT-PCR performed. We observed a significant decrease in Nrf2 gene expression between controls and 10 μM MeHg treated cells (0.592 +/- 0.04: p=0.05). We also examined Keap1, which retains Nrf2 in the cytoplasm in the absence of oxidative stress, but found no significant difference in gene expression from controls at any concentration tested. These results provide evidence that MeHg could be eliciting toxicity by down-regulating Nrf2 gene expression. They also support the possibility that MeHg functions upstream of Nrf2 in modulating the oxidative stress response. (supported by NIEHS R01ES020852)

Methylmercury (MeHg) toxicity is a continuous environmental problem to human health. Failure to protect cells against MeHg-induced early oxidative stress triggers subsequent endoplasmic reticulum (ER) stress and apoptosis. We previously reported that mild ER stress preconditioning is a useful therapeutic intervention against MeHg toxicity, the underlying mechanism being the induction of integrated stress responses. Further, our previous immunohistochemical and electron microscopic studies of MeHg-intoxicated rat cerebellum demonstrated that primary damage of astrocytes precedes damage of Purkinje cell dendrites and postsynapses, suggesting that astrocytes may play a role at the early stage of MeHg toxicity. Here, we investigated MeHg-induced stress responses in astroglial cells cultured from rat cerebral cortex. The involvement of oxidative stress and the phosphorylation of eukaryotic initiation factor 2a (eIF2α) pathway in the process of MeHg cytotoxicity were investigated. Quantitative real-time PCR analyses showed downregulation of glutathione peroxidase 1 mRNA and upregulation of mRNAs of manganese superoxide dismutase and thioredoxin reductase 1 after MeHg exposure. Upregulation of mRNAs of eIF4 and glucose regulated protein of 78 kDa (Grp78) were also observed after MeHg exposure. The induction of eIF4 accumulation is known to be mediated by translation inhibition of its upstream open reading frame (uORF) and translation facilitation of its protein-coding ORF of the phospho-eIF2α. Western blot analysis demonstrated that increased expression of phospho-eIF2α, Grp78 and eIF4 enhanced after MeHg exposure. Knockdown studies showed that eIF4 enhanced susceptibility to MeHg compared to non-silencing siRNA-transfectants. The results indicate that the same stress response pathways play a role in MeHg cytotoxicity of astroglia cells as previously reported in MeHg-susceptible myogenic cells. The investigation of specific roles of stress responses play a role in MeHg cytotoxicity were investigated. The investigation of specific roles of stress responses play a role in MeHg cytotoxicity.
Acute exposure to methylmercury (MeHg) disrupts internal calcium (Ca\(^{2+}\)) regulation, potentially leading to cytotoxicity. The receptors involved in the MeHg-induced increases in intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)) are not completely identified. We previously showed that activation of neuronal nicotinic acetylcholine receptors (nAChRs) may contribute to the increase of Ca\(^{2+}\), by either involvement of internal or external Ca\(^{2+}\) sources. Studies show that nAChRs appear to be involved during MeHg-induced cytotoxicity in differentiated sympathetic-neuron like PC12 (dPC12) cells and that application of the nonspecific nAChR antagonist, mecamylamine (MEC), increases cell viability after acute MeHg exposure. As different nAChR subtypes act in cell-type specific manner, and affect extra- and intracellular Ca\(^{2+}\) differently, we compared the role of the highly Ca\(^{2+}\)-permeable homeric α\(_7\) subtype nAChR with other heteromeric nAChRs, to the contribution to MeHg-induced [Ca\(^{2+}\)]\(_i\) increase. We used single cell microfluorimetry with fura2AM as the fluorophore. MeHg causes a kinetically-distinct biphasic increase in the fura2AM fluorescence ratio. The first phase results from release of Ca\(^{2+}\)-stores (P1) followed by extracellular Ca\(^{2+}\) influx (P2). dPC12 cells were exposed to 1, 2 or 5 μM MeHg in the absence and presence of the α\(_7\) specific blocker MLA (5μM) or MEC (5μM). MeHg treatment increased fura2AM fluorescence in dPC12 cells. The time-to-onset was inversely related to the concentration for P1 only. P2 was drastically hastened at high 5μM MeHg. MEC significantly delayed the time-to-onset of P1 (46%) and P2 (46%) at 1 μM MeHg. MLA delayed the time-to-onset of only P2 (43%) and only at 1μM MeHg. At higher [MeHg], neither MEC nor MLA slowed the response to MeHg. This project was supported by NIH grant R01ES03299 and R25NS065777.

The effect of methylmercury (MeHg) on spontaneous release of neurotransmitters such as dopamine (DA), glutamate, and GABA by both enhancing spontaneous release and impeding release following neuronal stimulation. Mechanisms responsible for this include unregulated increases in intracellular Ca\(^{2+}\) and complex effects on extracellular Ca\(^{2+}\) entry pathways. Previous studies from our lab revealed MeHg concentration- and time-dependent increases in DA release, but reduced levels, with and without MeHg, when extracellular Ca\(^{2+}\) was completely removed from treatment medium. As neurotransmitter release is highly regulated by extracellular Ca\(^{2+}\) levels, we examined the relationship between extracellular Ca\(^{2+}\) and MeHg effects on DA release from undifferentiated PC12 cells. Extracellular Ca\(^{2+}\) levels were varied from sub-physiological (0.6 μM) to supraphysiological (4.8 μM). A 60 min exposure to 2 μM MeHg was used for all studies for it’s ability to increase intracellular Ca\(^{2+}\) in PC12 cells. Extracellular DA levels were determined using HPLC coupled to electrochemical detection. Based on previous studies, we expected to see a concentration-dependent increase in MeHg-induced extracellular DA levels as extracellular Ca\(^{2+}\) concentrations were increased. However, results suggest that there was a Ca\(^{2+}\)-concentration-dependence relationship only up to a certain level. At 0.6 mM Ca\(^{2+}\), MeHg had little effect on DA release. As extracellular Ca\(^{2+}\) concentrations were increased, MeHg caused a significant concentration-dependent increase in extracellular DA levels. However at 4.8 mM Ca\(^{2+}\) MeHg was no longer able to elicit an increase in extracellular DA levels. This suggests that there may be an optimal range of extracellular Ca\(^{2+}\) levels that are required for MeHg’s action on spontaneous DA release. Research funded by a VICTER supplement to R01ES03299, T32ES007255, NSF DBI-1359302, and an SOT-sponsored Toxicology Summer Research Scholar award to YR.
Background: Methylmercury (MeHg) is a ubiquitous environmental toxicant that primarily targets the central nervous system (CNS) and has been associated with the development of the dopamine (DA) neurodegenerative disorder Parkinson's disease (PD). We have recently developed a Caenorhabditis elegans (C. elegans) model of MeHg toxicity that shows that low, chronic exposure confers DA neuron degeneration that is largely dependent on the transcription factor SKN-1/Nrf2 and the multidrug resistance protein MRP-7. Aims/Objectives: In this study we asked how SKN-1 and MRP-7 modulate whole animal and DA neuron vulnerability to MeHg. Methods: We utilized a reverse genetic screen, immunofluorescence, transgenic C. elegans, RT-PCR, Western analysis, and neuronal morphology to characterize the role that SKN-1, MRP-7, and post-translational modifications play in MeHg-associated toxicity. Results and Conclusions: Over 17,000 genes were screened for whole animal sensitivity to MeHg, and 92 genes were identified that affect whole animal and/or DA neuron pathology. These genes are strongly biased towards molecular mechanisms that affect the mitochondria and the ubiquitin-proteasome system (UPS). We also show that genetic knockdown of MRP-7 results in a 2-fold increase in Hg levels and a loss of DA neuron integrity. Chronic exposure to low concentrations of MeHg induces MRP-7 gene expression that affects transporter localization, organelle vulnerability, and specific post-translational modifications that modulate cellular toxicity. These studies show that a DA neuron-associated multidrug resistance and post-translational modifications play a critical role in cellular vulnerability. Support: NIEHS ES014459, ES003299, BRG and FNDR Fund to RN, IUCRG to RN and JP.
Manganese (Mn) is a ubiquitous trace element that is widely distributed in the environment and is essential for normal development and cellular homeostasis. However, chronic exposure to high levels of Mn is associated with Manganism, a clinical disorder characterised by symptoms and pathology similar to Parkinson’s disease. Significantly, patients with Manganism exhibit a progressive deterioration in motor and non-motor symptoms that resemble those seen in MPTP-induced Parkinsonism. There is evidence that Mn intoxication may result in neuronal cell repair and possible intervention of MnO exposure, and protein levels in the hippocampus and cerebral cortex were analyzed. Measurements showed significantly increased levels of CaMKII, GluR1, PSD95, synaptophysin and/or tau, compared to control mice, in the adult brains. The neurotoxic effects differed between brain regions. No changes in protein levels were seen in the neonatal brains. The data from the present study supports our previous study, where we observed a change in behavior in adult mice neonatally exposed to a mixture dose. Interestingly, effects were seen with mixtures of doses individually not resulting in effects. The changed protein levels may be a possible mechanism behind the previously observed aberrant behavior. This could also indicate that the cholinergic system is involved, as the compounds have shown to affect components of this neurotransmitter system. Further studies are needed to understand the mechanism behind developmental neurotoxic effects of environmental pollutants and potential mixture effects.

962 Activated Adult Neurogenesis in the Subventricular Zone following Intranasal Manganese Exposure in Rats

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Occupational exposure to Manganese (Mn) is mainly by inhalation, resulting in a disorder with symptoms similar to but distinguishable from idiopathic Parkinson’s disease. The olfactory pathway provides a direct route of entry for Mn into the CNS. Following intranasal exposure, our work has shown that Mn accumulates in the subventricular zone (SVZ) which is of particular concern, because this is one of only two neurogenic niches in the adult mammalian brain. This study was designed to test the hypothesis that intranasal Mn exposure cause Mn to accumulate in the SVZ which stimulates adult neurogenesis in this region. Adult rats were anesthetized and intranasally instilled with 0.2 mg Mn/kg or 0.8 mg Mn/kg (as MnCl2) or saline as control, daily for 14 days. Animals were euthanized 24 hours after the last dose. Brains from each group were then dissected to collect SVZ and other brain regions for quantification metal concentrations, or collected whole for IHC evaluation of BrdU+ and DCX+ cells, markers of cell proliferation and differentiation to migrating neuroblasts, respectively. Animals used for IHC also received twice daily intraperitoneal injections of 50 mg/kg BrdU during the last four days of the study and were perfused at necropsy. AAS analyses revealed a significant accumulation of Mn in the SVZ (9.40 ± 0.77 for high dose compared to control 0.68 ± 0.33 µg/g; p<0.01). Intranasal Mn exposure resulted in visibly significant increases in the intensity of DCX+ and BrdU+ fluorescent signals in Mn-treated animals compared to controls. Further quantification indicated that there was a 38% increase in DCX+ cells in the high dose group compared to control (p<0.001). For BrdU+ cells, there were 26% (p<0.001) and 90% (p<0.001) increases in the low- and high-dose groups, respectively, compared to controls. Our data provide evidence that adult neurogenesis is stimulated following intranasal Mn exposure. The implications of our findings are that neuronal cell repair and possible intervention in disease progression deserve further investigation. (Supported in part by NIH/NIEHS ROI-E008146)

963 Detection of Retinal Changes in Chronic Manganese-Exposed Nonhuman Primates by Optical Coherence Tomography

J. S. Schneider1, D. W. Anderson1, C. Williams1, M. Ault1 and T. R. Guilarte2.

Manganese (Mn) is a ubiquitous trace element that is widely distributed in the environment, due to natural biochemical processes or intentional dispersal. We have previously seen that a single exposure to a combination of Mn and chlorpyrifos (CPF) during a critical period of the developing brain induced a neurologic effect manifested as altered adult spontaneous behavior. The aim of the present study was to investigate if neonatal exposure to a mixture of Mn and CPF could affect the levels of proteins important for normal brain development in the mouse. Male mice were exposed to single oral dose of Mn (0.4 mg/kg/bw), CPF (5 or 10 mg/kg/bw) or a mixture dose (0.4 mg Mn·MeHg + 5 or 10 mg/kg/bw). The mice were euthanized 24h or 4 months after exposure, and protein levels in the hippocampus and cerebral cortex were analyzed. Measurements showed significantly increased levels of CaMKII, GluR1, PSD95, synaptophysin and/or tau, compared to control mice, in the adult brains. The neurotoxic effects differed between brain regions. No changes in protein levels were seen in the neonatal brains. The data from the present study supports our previous study, where we observed a change in behavior in adult mice neonatally exposed to a mixture dose. Interestingly, effects were seen with mixtures of doses individually not resulting in effects. The changed protein levels may be a possible mechanism behind the previously observed aberrant behavior. This could also indicate that the cholinergic system is involved, as the compounds have shown to affect components of this neurotransmitter system. Further studies are needed to understand the mechanism behind developmental neurotoxic effects of environmental pollutants and potential mixture effects.

964 Eogecentric Learning Deficits in Rats Produced by Developmental Manganese Overexposure or Adulthood 6-Hydroxydopamine Toxicity Are Exacerbated by the Combination

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Manganese (Mn) is an essential element in the human diet, however too much Mn is obtained neurotoxicity can result. In adults, Mn overexposure (MnOE) produces manganism, a neurological disorder with similarities to Parkinson’s disease. In an initial study Sprague-Dawley rats were exposed to Mn from postnatal day P9 until P28 and examined for a number of behaviors, including egocentric navigation in the Cincinnati water maze (CWM). The offspring exposed to Mn had longer latencies and more errors in the CWM than control animals. In a second experiment, we demonstrated that the dopaminergic neurotoxin, 6-hydroxydopamine (6-OHDA), produced learning and memory deficits in the CWM in adult animals following intra-neostriatal administration. Induction of subthreshold lesions using 6-OHDA to the neostriatum was used as a model of early Parkinson’s disease (motor deficits were not present), and showed that even in the absence of motor deficits, changes in egocentric learning and memory occurred. These results are similar to findings in patients with Parkinson’s disease who also show deficits in egocentric ability. The human literature suggests that MnOE throughout life may sensitize or exacerbate Parkinson’s progression. Therefore, we examined the effect of developmental MnOE in combination with a subthreshold 6-OHDA lesion of the neostriatum on cognitive ability in the CWM. Animals were exposed to Mn or saline from P4-P28 and in adulthood they were given subthreshold 6-OHDA lesions of the neostriatum. Animals that had been exposed to Mn developmentally had exacerbated deficits in the CWM compared with saline animals. These data provide preliminary evidence that early Mn exposure can sensitize dopaminergic neurons to later toxins and increase the probability for Parkinson’s disease. (Supported by NIH T32 ES07051, F30 ES006096).

965 Manganese Activates the NLRP3 Inflammasome in Microglia

P. Gordon1, D. C. Christie1, A. B. Robertson2, M. A. Cooper3 and T. Woodruff1.

Manganese (Mn) is an ubiquitous trace element that is widely distributed in the environment and is essential for normal development and cellular homeostasis. However, chronic exposure to high levels of Mn is associated with Manganism, a clinical disorder characterised by symptoms and pathology similar to Parkinson’s disease. Significantly, patients with Manganism exhibit a progressive deterioration in motor and non-motor symptoms that resemble those seen in MPTP-induced Parkinsonism. There is evidence that Mn intoxication may result in neuronal cell repair and possible intervention in disease progression deserve further investigation. (Supported in part by NIH/NIEHS ROI-E008146)

966 Detection of Retinal Changes in Chronic Manganese-Exposed Nonhuman Primates by Optical Coherence Tomography

J. S. Schneider1, D. W. Anderson1, C. Williams1, M. Ault1 and T. R. Guilarte2.

Manganese (Mn) is a ubiquitous trace element that is widely distributed in the environment, due to natural biochemical processes or intentional dispersal. We have previously seen that a single exposure to a combination of Mn and chlorpyrifos (CPF) during a critical period of the developing brain induced a neurologic effect manifested as altered adult spontaneous behavior. The aim of the present study was to investigate if neonatal exposure to a mixture of Mn and CPF could affect the levels of proteins important for normal brain development in the mouse. Male mice were exposed to single oral dose of Mn (0.4 mg/kg/bw), CPF (5 or 10 mg/kg/bw) or a mixture dose (0.4 mg Mn·MeHg + 5 or 10 mg/kg/bw). The mice were euthanized 24h or 4 months after exposure, and protein levels in the hippocampus and cerebral cortex were analyzed. Measurements showed significantly increased levels of CaMKII, GluR1, PSD95, synaptophysin and/or tau, compared to control mice, in the adult brains. The neurotoxic effects differed between brain regions. No changes in protein levels were seen in the neonatal brains. The data from the present study supports our previous study, where we observed a change in behavior in adult mice neonatally exposed to a mixture dose. Interestingly, effects were seen with mixtures of doses individually not resulting in effects. The changed protein levels may be a possible mechanism behind the previously observed aberrant behavior. This could also indicate that the cholinergic system is involved, as the compounds have shown to affect components of this neurotransmitter system. Further studies are needed to understand the mechanism behind developmental neurotoxic effects of environmental pollutants and potential mixture effects.
inhibitor Z-YVAD-FMK blocked inflammasome activation by Mn. Interestingly, Mn treatment did not prime the NLRP3 inflammasome for activation with either ATP or Nigericin. Collectively, these results demonstrate for the first time that Mn can activate the NLRP3 inflammasome and that pharmacological inhibition of this pathway could be a useful therapeutic strategy to mitigate chronic neuroinflammation and neurotoxicity following manganese exposure.

**966 Loss of pdr-1/parkin Alters Mn Homeostasis through Modulation of Ferroportin in *C. elegans***

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Environmental overexposure to the essential trace element manganese (Mn) can result in an irreversible condition known as mangasism that shares similar neuropathology with Parkinsonian disease (PD), with dopaminergic (DAergic) cell loss associated with motor and cognitive deficits. However, the mechanisms that mediate the pathophysiology of both disorders remain unclear. Many PD genes impart risk for autosomal recessive, early-onset PD, including the parkin/PARK2 gene that encodes for the E3 ubiquitin ligase parkin. Using Caenorhabditis elegans as a model that conserves the DAergic system, we previously reported significantly increased Mn accumulation in pdr-1/parkin mutants compared to wildtype (WT) animals. For the current study, we hypothesize that this increased accumulation is due to alterations in Mn transport in the pdr-1 mutants. Using a synchronous population of Mn-exposed L1 worms, we show no change in mRNA expression of the major Mn importers (smf-1,3), but a downregulation in mRNA levels of the Mn exporter ferroportin, fno-1.1 (p<0.0001). To test the role of ferroportin in altering Mn levels in pdr-1 mutants, we created a strain overexpressing FPN-1.1 in worms lacking pdr-1. The new strain showed attenuated Mn-induced lethality compared to pdr-1 mutants alone (p<0.0001), restoring survival to WT levels. These changes suggest a role of pdr-1 in modulating Mn export through altered transporter expression, implicating a novel role of the PD-associated gene in metal homeostasis (supported by NIEHS R01ES10565).

**967 Parkinsonism Due to a Hereditary Defect in a Manganese Efflux Transporter***

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Manganese (Mn) is an essential metal, but elevated cellular levels are toxic and may lead to the development of an irreversible and incurable parkinsonian syndrome. Mn-induced parkinsonism generally occurs due to exposure to elevated Mn levels under occupational or environmental settings. Recently, a new form of familial parkinsonism was reported in a high-throughput screen (HTS) of 40,167 small molecules that could modulate Mn transporter activity. To address this we took a novel approach to define cellular mechanisms of Mn biol-
Experimental de Enfermedades Neurodegenerativas, Instituto Nacional de Neurología and LAT1 were up-regulated. On PND 15 xCT and EAAC1 were significantly higher and xCT, EAAC1, GLAST and GLT1 part of the x-AG system, and LAT1 catalytic subunit were expressed as ratio to total creatine level (tCr). Comprehensive motor function was evaluated using the Unified Parkinson’s Disease Rating Scale (MDS UPDRS-III).

Introduction: Excessive exposure to manganese (Mn) has been associated with motor disorders resembling the symptoms of Parkinson’s disease (PD). However, Mn neurotoxicity shows a different pattern of perturbation of the basal ganglia circuit compared to PD. Our aim is to investigate the effect of chronic Mn exposure on the direct and indirect pathways of basal ganglia, by detecting *in vivo* changes of thalamic and striatal GABA levels non-invasively with Magnetic Resonance Spectroscopy (MRS), and exploring the relationship between GABA levels and motor performance. Methods: 32 welders and 23 controls from a truck manufacturer were recruited. GABA levels from thalamus and striatum were measured on a 3T GE MRI scanner, using a spectral editing sequence (MEGA-PRESS), and expressed as ratio to total creatine level (tCr). Comprehensive motor function was evaluated using the Unified Parkinson’s Disease Rating Scale (MDS UPDRS-III).

Group differences were examined using student t-tests and Spearman partial correlation controlled for age. Results: Average thalamic GABA/tCr levels in welders were significantly elevated compared to controls (0.29±0.120 v.s. 0.22±0.085, p<0.05). There was no group difference in striatal GABA/tCr levels. A significant correlation between thalamic GABA/tCr levels and UPDRS scores was found within the welders (R=0.500, p=0.01). Conclusion: The elevation of GABA levels in the thalamus is in line with an excitation of the indirect pathway as mechanism of Mn neurotoxicity, activating the GABA projection to the thalamus. In contrast, the lack of elevated striatal GABA levels speaks against an inhibition of the direct pathway without dopaminergic neurodegeneration. Equally, the correlation with the UPDRS score suggests that thalamic GABA levels directly influence motor disorders through inhibited thalamocortical projections.

**Neurological Effects of Gestational Arsenic Exposure**

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Chronic exposure to As has been associated to learning disabilities as well as to memory impairment. As biomethylation in tissues requires glutathione (GSH), GSH depletion causes oxidative and degenerative damage in the central nervous system. A mouse model was used to investigate effects of gestational As exposure that could lead to oxidative and excitotoxic damage impacting learning and memory. Membrane transporters related to GSH synthesis, glutamate transport and toxicity, such as xCT/4F2hc, the x -L-cysteine/glutamate antipporter system, EAAC1, GLAST and GLT1 part of the x-AG system, and LAT1 catalytic subunit of the L-system, were investigated. Protein expression was determined by western blots and immunofluorescence. To assess memory impairment, an object location task was used in rats on PND 90. N-methyl-D-aspartate receptor (NMDAR) subunits NR2A and B expression and the presence of As species in cortex and hippocampus were investigated. Animals were sacrificed on postnatal days (PNDs) 1, 15 and 90. On PND 1 GSSG levels were significantly higher and xCT, EAAC1 and LAT1 were up-regulated. On PND 15 xCT and EAAC1 were significantly up-regulated in cortex and hippocampus. This up-regulation was also observed using immunofluorescence in hippocampal cells. The transporters up-regulation were associated with NR2B down-regulation in cortex and hippocampus in both sexes. NR2A was down-regulated in males. On PND 90 the behavioral task showed significant and marginal spatial memory impairment in males and females, respectively. xCT continued up-regulated while GLT1 was down-regulated as well as NR2B only in the male hippocampus with gestational exposure. In *uro* exposure to As was associated to a negative modulation of NMDAR in the hippocampus that might explain the neurological effects observed in human populations.

**Investigation of Zinc Toxicity in Olfactory Neurons Using In Silico and Molecular Techniques**

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Zinc is both an essential and potentially toxic metal. It is widely recognized that oral zinc supplementation can reduce the effects of the common cold; however, there is strong clinical evidence that intranasal zinc gluconate gelment for this purpose causes anosmia, or the loss of the sense of smell, in humans. Using the rat olfactory neuron cell line, Odora, we have investigated the means by which zinc is toxic to olfactory neurons. Using RNAseq and *in silico* analyses, we identified pathways associated with oxidative stress, calcium regulation, and ATF generation as being up-regulated upon zinc exposure. Many genes in these pathways are polymorphic in humans, suggesting that individuals may be differentially susceptible to zinc toxicity. We are currently utilizing shRNA to reduce expression of polymorphic genes that are involved in zinc metal response (Mt1), oxidative stress (Gclm), and ATP generation (Akr1b) to demonstrate their critical role in mediating zinc toxicity and to identify adaptive pathways in response to reduction of expression of the respective genes. We have also established the time course of recovery in wild-type mice exposed to intranasal zinc gluconate. Future studies are planned using metallothionein knockout mice and glutamate-cysteine ligase knockout mice to test the hypothesis that these knockout mice will show increased olfactory mucosal damage and delayed histological and behavioral recovery in olfactory neurons in response to intranasal zinc gluconate administration.

**The Impairment of Learning Ability of Male F1 Tokai High Avoider Rats Exposed to Tributyltin via the Placenta, Their Dams’ Milk, and Their Food**

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Tributyltin (TBT) compounds have been known to be environmental pollutants. The impaired learning ability of rats by TBT was observed among Wistar-derived Tokai High Avoider (THA) rats, which achieved stable learning ability with the Sidman electric shock avoidance test, after the exposure via the placenta, their dams’ milk, and their food. In this study, we evaluated the neurotoxic effects of TBT on male F1 THA rats by the Sidman electric shock avoidance test, after the exposure to TBT via the placenta and their dams’ milk or their food. In this study, we evaluated the neurotoxic effects of TBT on male F1 THA rats by the Sidman electric shock avoidance test, after the exposure via the placenta, their dams’ milk, and their food. The pregnant THA rats were exposed to TBT at 0 and 50 ppm in their food. The F1 rats were exposed to TBT via the placenta and their dams’ milk. From 4 weeks after delivery (weaning time), the rats were exposed to TBT at 0 and 50 ppm in their food. From 6 weeks of age, the Sidman electric shock avoidance test sessions were performed for 60 min/day for 10 consecutive days for the male F1 rats. The rats could avoid electric shocks by pressing a lever. Avoidance rates for shock exposures were calculated for the first and second halves (30 minutes each) of each session. The mean body weight of TBT-exposed rats at 6 weeks of age was significantly lower than that of the control. Significantly lower mean values of the avoidance rate in the TBT group compared to the control were observed in the first 30 minutes of the session on days 6 through 10. The mean value of avoidance rates in the second 30 minutes of the session on day 10 was significantly lower than that in the control. The learning ability was impaired in the F1 THA rats exposed to TBT at 50 ppm in their dams’ food and their food after weaning.
Drug addiction is a public health problem that affects both brain and behavior. Evidence suggests that brain regions important in drug addiction can be disrupted by environmental neurotoxins and result in increased sensitivity to psychoactive substances later in life. Our goal was to investigate the impact of early life exposure to environmentally relevant levels of lead (Pb) on sensitization to cocaine. To determine if rats exposed to Pb early in life had increased sensitivity to the psychoactive properties of cocaine, we exposed rats to different levels of Pb from gestation to late adolescence resulting in blood lead levels (BLLs) averaging 0.6 ± 0.1, 4.4 ± 0.2 or 22 ± 0.7 μg/dL. Adolescent rats were injected with saline, 5 (low) or 15 (high) mg/kg cocaine-HCl, before being placed in activity chambers where total distance was measured for 60 minutes. Our data showed that male rats with the highest BLLs had increased cocaine-induced locomotor activity compared to rats fed control chow. In addition, male rats with BLLs averaging 4.4 μg/dL, just below the current CDC level of concern (5 μg/dL), had greater locomotor activity after exposure to 5 but not 15 mg/kg of cocaine compared to controls. Using the same paradigm, female rats with blood Pb levels of 4.4 μg/dL had the greatest behavioral response to cocaine compared to control rats. The D1-dopamine receptor (D1R) antagonist, SCH23390 blocked the Pb-induced increase in locomotor activity compared to control rats. The D1-dopamine receptor (D2R) antagonist, Raclopride. These data suggest that even at low levels of Pb exposure, below the current CDC level of concern, cocaine-induced responsivity is increased, and this increase seems to be mediated by D1R activation. In summary, early life Pb exposure may alter developing brain circuits involved in drug addiction, thus enhancing the liability for drug use in adolescence. [Supported by ES006189 to TRG and pilot project P30ES008098 to KHS]

**976 Lead Significantly Impairs Critical Processes for Adult Hippocampal Neurogenesis in Primary Cultured Adult Neural Stem Cells**

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Adult hippocampal neurogenesis is the process whereby adult neural progenitor/stem cells (aNPSCs) in the subgranular zone (SGZ) of the dentate gyrus (DG) lead to the generation of adult-born neurons in the hippocampus. These adult-born cells have been implicated in some forms of hippocampus-dependent learning and memory, and various extracellular and intracellular stimuli have been shown to modulate adult neurogenesis. However, little is known about the effect of neurotoxic agents on this process. The goal of this study was to determine whether the heavy metal lead impair critical processes in adult neurogenesis and to characterize the underlying signaling pathways using primary cultured SGZ-aNPSCs isolated from adult mice. Lead significantly reduced aNPSC total cell number and significantly increased apoptosis, starting at 0.1 μM. Lead also significantly decreased cell proliferation at concentrations ≥ 0.2 μM. We found that lead decreases Akt phosphorylation and increases phosphorylation of c-Jun NH2-terminal kinase (JNK), c-Jun, and p38. Furthermore, we found that inhibition of JNK or p38 activity via pretreatment with JNK or p38 inhibitors was sufficient to block the lead-induced decrease in cell number and increase in apoptosis. Pretreatment with the antioxidant N-acetylcysteine or supplementation with the antioxidant-rich B27 supplement significantly attenuated the effect of lead on total cell number, cell proliferation, and apoptosis, suggesting that oxidative stress and the activation of mitogen-activated protein kinase (MAPK) signal transduction pathways may underlie lead toxicity in SGZ-aNPSCs. Furthermore, lead significantly impaired spontaneous neuronal differentiation and neuronal maturation. Our data show that lead concentrations of lead significantly impair critical processes in adult hippocampal neurogenesis.

**975 Early-Life Lead Exposure and Sensitization to Cocaine: A Novel Pathway to Addiction**


Early life exposure to lead (Pb) and prenatal stress (PS) contribute to sex-specific alterations in brain development and modify the function of the hypothalamic-pituitary-adrenal (HPA) axis, the major stress response system. While low-level developmental Pb exposure can alter the adrenocortical responses to stress in adulthood, the mechanisms underlying effects of Pb and PS on developmental programming of the HPA system are not well understood. This study examined the effects of Pb or PS on expression of the glucocorticoid receptor gene Nr3c1 in the frontal cortex and hippocampus of male and female mice and examined potential epigenetic modifiers of Nr3c1 gene expression. Two-week-old female C57BL/6 mice were randomly assigned to receive drinking water containing 0 or 100 ppm Pb acetate for 2 months prior to breeding and through lactation. After mating, pregnant females were randomly assigned to a non-stress (NS) or Pb (i.e., restraint stress 3x/day at 45 min/day from gestational day 11-19) condition. This yielded 4 treatment groups: NS, 0-Pb, 100NS, and 100-Pb. Animals were euthanized at age 60 days and frontal cortex (FC) and hippocampus (HIPP) were dissected and frozen prior to analysis. mRNA was extracted from samples for qPCR processing while DNA was extracted from others for pyrosequencing. In FC, Pb and PS individually increased Nr3c1 expression in females while only combined Pb + PS had an effect in males. In HIPP, Pb and PS individually decreased Nr3c1 expression, while in males, Pb + PS resulted in downregulation in all conditions. Pyrosequencing analysis of CpG sites on the Nr3c1 gene proximal to the NGF-I binding domain suggest a potential role of DNA methylation in these observed effects. Additional work is underway to further explore a potential epigenetic basis for sex differences in outcomes from Pb and PS. Supported by grant ES021534.
loss in the groups treated with Pb alone or Cd alone. Correlating with the lack of cochlear pathology, these animals did not develop threshold shifts significantly different from controls. In contrast, animals in the groups exposed to noise, including those exposed to Pb, Cd, or Pb and Cd, in conjunction with noise, showed hair cell loss. These groups also experienced threshold shifts significantly different from the control group. Mean and standard deviation percentage threshold shifts at 32 kHz ranged from 3 ± 4 dB (control) to 42 ± 9 dB (noise alone). However, no potentiation or synergistic effects were found in any of the groups exposed to a combination of toxic agents. This study does not support Pb and Cd ototoxicity in adult mice. Data also do not suggest a synergistic potentiation of hearing loss with the combination of exposure to metals and loud noise in adult mice.

### 980 Age-Dependent Increase of Brain Cu Levels and Expressions of Cu Regulatory Genes in the Subventricular Zone and Choroid Plexus

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Our recent data suggest Cu is concentrated in the subventricular zone (SVZ) along the wall of brain ventricles. Anatomically, SVZ is in direct contact with cerebrospinal fluid (CSF), which is secreted by choroid plexus (CP) where the blood-CSF barrier (BCB) is located. Regulation of Cu transport across the BCB influences the Cu level in CSF, therefore, changes in Cu regulatory gene expressions in SVZ and CP may determine Cu levels in CSF and SVZ. This study was designed to determine Cu levels in SVZ, CP, and other brain regions, assess Cu-regulatory gene expression levels and investigate relationships between age, Cu levels, and Cu regulatory genes in SVZ and CP. The SVZ and CP were dissected from brains of 3- to 6-week-old rats and the 6-month-old rats were used for primary cultures of neural stem cells (NSCs), neuroblasts (NBs) and choroidal epithelial cells (CECs). Our atomic absorption spectroscopic analyses revealed that the SVZ of adult and old animals contained the highest Cu level as compared with selected brain regions. Significant positive correlations between age and Cu levels in SVZ and CP were observed; the SVZ Cu level of old animals was 7.5- and 5.8-fold higher than those of young and adult rats (p<0.01, respectively). The qPCR quantitaiton showed that in SVZ, the mRNA expression levels of Mt3, Mt2a and Mt1a were highest, while in CP, Ctrl was expressed the highest. Expressions of Mt3 and Mt2a in NSCs were lower than NBs, but higher than CECs (p<0.01). The Ctrl expression was inversely correlated with age and Cu levels in the SVZ (p<0.01), but positively correlated with age in CP (p<0.01). Dmt1 had significant positive correlations with age, Cu and Ctrl expression levels in the CP (p<0.01). These findings confirm the Cu accumulation in SVZ, Cu levels in all tested brain regions are increased as the function of age. The SVZ has a different expression pattern of Cu-regulatory genes from the CP. The age-related increase of Mt and decrease of Ctrl may contribute to the high Cu level in this neurogenesis active brain region.

### 981 A Gene-Metal Screen Reveals Enhanced Selenium and Cadmium Neurotoxicity in Dopaminergic Cells Expressing Human Alpha-Synuclein

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Alpha-synuclein(a-syn) is a small soluble protein expressed primarily in the central nervous system. Mutations in a-syn are known to cause Parkinson’s disease (PD) that is characterized by motor, cognitive, and psychiatric abnormalities. Despite the paucity of information on the function of wild-type a-syn protein, there has been evidence to support aggregation of a-syn as a pathological hallmark in PD. In fact, heavy metals are implicated in the pathophysiology of PD. However, the neuroprotective role of wild-type or mutant a-syn in metal transport and homeostasis dynamics are poorly understood. Equally unclear is the role of native a-syn in enhancing ROS-induced toxicity. Here, we utilized an established mesencephalic dopaminergic cell line expressing human wild-type a-syn (N27-syn) or empty vector (N27-vec) to conduct a gene-metal screen aimed at uncovering a-syn’s function in regulating metal-induced neurotoxicity in dopaminergic cells via a MITT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay. Herein, we report that expression of human wild-type a-syn enhances cadmium (Cd2+) and selenium (Se4+) induced dopaminergic toxicity in N27-syn compared to N27-vec cells in a dose-dependent manner. However, human wild-type a-syn protects dopaminergic cells from manganese (Mn2+) neurotoxicity in a dose-dependent manner. We report no statistically significant genotypic differences between N27-syn and N27-vec cells following exposure to copper (Cu2+), zinc (Zn2+), lead (Pb2+), cobalt (Co2+), nickel (Ni2+), iron (Fe3+), and aluminum (Al3+). We conclude that a-syn may, through a currently unknown mechanism, upregulate Cd2+ and Se4+ transporters to cause neurotoxicity. Alternatively, a-syn may interact with Cd2+ or Se4+ to induce oxidative stress in the dopaminergic cells. Ongoing experiments are to understand if the aforementioned differences in cell viability are directly related to a-syn neuromodulation of metal transporter systems and homeostasis.

### 982 Post-Traumatic Stress Disorder (PTSD) among Gulf War 1 Veterans Exposed to Depleted Uranium (DU): 22 Years of Follow-Up

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The Dept. of Veterans Affairs provides health surveillance for 80 Gulf War 1 cohort members (CMs) exposed to DU friendly fire. CMs with retained DU fragments excrete higher levels of urine uranium (uU) with a DU isotopic signature than do CMs with inhalation-only exposures. DU and other heavy metals are known to cross the blood brain barrier; thus this study evaluated whether CMs with greater DU body burden experience greater PTSD severity. Because in animal models DU accumulation in the Central Nervous System was dose and age-dependent we also evaluated PTSD severity over time. CMs attended on average 4.5 of 10 biennial visits. CMs completed a 39 item Questionnaire (MQ) at each visit. MQ items were scored from 1-5 (higher indicating greater PTSD severity). Each CM’s score was the mean of 39 items. DU was measured using urine Uranium concentration analyzed by ICP mass spectrometry. CMs were divided into high (uU>0.1 mcg/g creatinine) and low- (uU<0.1 mcg uUc creatinine DU groups. The impact of uranium exposure on Mississippi scores was analyzed using mixed model linear regression, accounting for within person correlation and adjusted for age and IQ. Slopes differed for the high vs. low uranium groups (p<0.02). PTSD severity was lower, not higher, with a higher DU body burden, despite this subpopulation having a greater likelihood of stress during a traumatic injury event. Both exposure groups had similar PTSD severity int 1995. By 2013, PTSD had declined slightly for the high uU group (coefficient= -0.010, p=0.24), while it rose slightly for the low group (coefficient= 0.02, p=0.03). Thus we see no evidence that DU body burden increasingly affects PTSD severity. Supported by the Dept. of Veterans Affairs and approved by the Baltimore VA Medical Center’s Office of Research and Development and University of Maryland’s School of Medicine Institutional Review Board.

### 983 Response of Erythrocyte Acetylcholinesterase Activity in Rats Subchronically Exposed to Low-Level Cadmium

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Acetylcholinesterase (ACHE) in the erythrocyte, is one of the typical extra-neural AChE enzymes that has an essential role in acetylcholine-mediated neurotransmission. Epidemiological studies suggest an association between cadmium in drinking water and neurotoxicity. However, the precise elements affected by cadmium remain unknown. In order to investigate the potential effects of cadmium (Cd) on ACHE, the animals were exposed to 100, 200 and 500 ppm cadmium dose for 12 weeks in their drinking water. Control animals received distilled water for the same period. At the end of 12 weeks, the activity of erythrocyte and erythrocyte ghost membrane acetylcholinesterase, as well as blood cadmium concentration, were determined spectrophotometrically. Dose-dependent significant (p < 0.05) reduction in both the erythrocyte and erythrocyte ghost membrane acetylcholinesterase activities characterized the effect of cadmium exposure. While erythrocyte ACHE activity was inhibited to the tune of 63 % compared to the control, the inhibition was 78 % in the erythrocyte membrane. The blood cadmium level increased dose-dependently in animals exposed to cadmium. Correlation, as calculated by Pearson’s method, revealed a significant (p < 0.05) dose-dependent inverse linear relationship between cadmium exposure, membrane erythrocyte ACHE activity and blood cadmium levels. The research findings suggest that ACHE activities in the erythrocyte and erythrocyte membrane could be used as a biomarker of cadmium-induced neurotoxicity.
Although the critical roles of microRNA (miRNA) in various diseases have been extensively studied, its dose- and time-dependent expression patterns in governing toxicity have not been well investigated. Understanding their patterns of miRNA expression in chemical treatment across different doses and time-points could provide insights into the roles of miRNA underlying toxicity. In this study, an integrated analysis of miRNAs expression with their target miRNAs was conducted to investigate the dynamic nature of miRNA-regulated modules in rat livers exposed to thioacetamide, a carcinogen, at multiple dose levels and treatment durations. At each dose- and time-point, both miRNA profiling and miRNA expression profiling of the same rat livers were generated with next generation sequencing and microarray, respectively. We observed that the expression patterns of miRNA and miRNAs were significantly different in a dose- and time-dependent manner. We found that two miRNAs, mir-34a and miR-100 were significantly upregulated over all doses and time-points examined. mir-34a is a known tumor-suppresser miRNA. mir-100 is thought to negatively regulate the mammalian target of rapamycin (mTOR), a central regulator of cell metabolism, growth, proliferation and survival. Also, the integrative analysis defined a p53-related network that is regulated by the differentially expressed miRNAs. These results suggest that mir-34a and mir-100 could potentially be used as biomarkers or therapeutic targets for liver cancer and drug-induced liver injury (DILI) and demonstrated the potential application of miRNAs to assess liver carcinogenicity and DILI at the early stage of drug discovery and development.

Arsenic enhances the genotoxicity of other carcinogenic agents such as ultraviolet radiation and benzo[a]pyrene. Inhibition of DNA repair is suggested as an important aspect of arsenic cocarcinogenesis. It is reported recently that DNA repair proteins such as poly(ADP-ribose) polymerase (PARP-1) are direct molecular targets of arsenic. Also, arsenic has been shown to generate reactive oxygen/nitrogen species (ROS/RNS), but little is known about the role of arsenic-induced ROS/RNS in the mechanism underlying arsenic inhibition of DNA repair. Here we report that arsenic-generated ROS/RNS inhibits PARP-1 activity in cells, and especially peroxynitrite (ONOO-), a free radical in ROS/RNS, may play an important role. Cellular exposure to arsenite, as well as hydrogen peroxide and NONOate (nitric oxide donor), decreased PARP-1 zinc content, enzymatic activity, and PARP-1 DNA binding. Furthermore, the effects of arsenite on PARP-1 activity, DNA binding, and zinc content were partially reversed by the antioxidant ascorbic acid, catalase, and the NOS inhibitor, aminoguanidine. Most importantly, arsenite incubation with purified PARP-1 protein in vitro did not alter PARP-1 activity or DNA binding ability, whereas exposure of cells to retained PARP-1 inhibitory activity. We also demonstrated that arsenite generates peroxynitrite in superoxide and nitric oxide dependent manner by utilizing HKGreen4, a fluorescent probe of peroxynitrite. These results strongly suggest that cellular generation of ROS/RNS plays an important role in arsenite inhibition of PARP-1 activity, leading to the loss of PARP-1 DNA-binding ability and enzymatic activity.

Gli2 is one of the key transcription factors in sonic hedgehog (Shh) pathway. Aberrant activation of Gli2 has been reported in several malignancies. However, the role of Gli2 in melanoma progression is unclear. Our results showed that knockdown of Gli2 in melanoma cells inhibited the proliferation as well as induced apoptosis in melanoma cells in vitro, which was associated with down-regulation of c-Myc. Melanoma cells with stable Gli2 knockdown demonstrated diminished tumor growth when injected subcutaneously in athymic nude mice. In addition, Gli2 inhibitors GANT58 and GANT61 significantly inhibited the growth of melanoma cells in vitro, down-regulated Gli2 and c-Myc expression, and induced apoptosis in melanoma cells. These results suggest that Gli2 is a potential therapeutic target for melanoma.

Arsenite Causes Zinc Loss and Inhibits the Activity of Poly(ADP-ribose) Polymerase-1 through ROS/RNS Generation and Peroxynitrite Production

986 Benzo(a)pyrene in Colon Carcinogenesis

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Colorectal cancer (CRC) is the second leading cause of cancer-related death in America. Both environment and genetics contribute to the pathogenesis of CRC. Epidemiological biomarkers show that ~70% of CRCs are attributable to dietary carcinogens. Exposure to benzo(a)pyrene (BaP), a ubiquitous environmental carcinogen, is associated with an increased risk of CRC. Dietary ingestion rather than inhalation is the major route of BaP exposure. Importantly, when mice are orally given BaP the highest DNA mutation frequency is found in the colon. Contrary to lung cancer, however, consensus has not yet been reached to ascertain the level of CRC risk posed by BaP and the underlying mechanisms of BaP-induced colon toxicity. Genome-wide association studies have identified ~20 common single nucleotide polymorphisms (SNPs) that are low-penetrance (increase CRC risk 1.1 to 1.6-fold) and yet occur in over 1% of the populations. A novel noncoding variant (SNP rs7837822) located in the p53 polyadenylation signal (PAS) was identified as a genetic risk variant associated with increased risk (1.39-fold) of colon adenoma. To mimic this naturally occurring human polymorphism, we generated a mutant mouse line carrying the equivalent p53 variant in the mouse genome (termed p5371755C) using zinc-finger nuclease technology. This mouse line is the only animal model available for individuals carrying low-penetrance CRC susceptibility alleles. Here we will present compelling preliminary data establishing a link between exposure to BaP and increased CRC incidence associated with low-penetrance alleles. First, we found that mice orally administered BaP and a tumor promoter developed dextran sulfate sodium (DSS)-induced colon tumors. Second, upon exposure to BaP/DSS, heterozygote p53s71755C and homozygote p53s71755C/mice developed more colon tumors than the wild-type (WT), p53+/+ controls. These data support that the interaction of environmental carcinogens and low-penetrance susceptibility alleles is a significant determinant of CRC pathogenesis. We will also report the underlying mechanisms for disease susceptibility at-risk individuals under the exposure of environmental carcinogens.
correlated well with the concentrations achieved in vivo. Melanoma cells treated with 2,5, 7 and 7.5μM PL for 48 hours induced significant apoptosis, with concomitant inhibition of G2 as well as G0-M. Mango juice overexpression and treatment with Shh ligand significantly blocked PL mediated inhibition of cell proliferation and induction of apoptosis, establishing the role of G2 in PL mediated growth suppression of melanoma cells. PL inhibited nuclear translocation as well as DNA binding of G2 as observed by analysis of G2 in nuclear fraction and Chromatin Immunoprecipitation (ChIP) assay indicating the importance of G2 transcriptional activity in melanoma cell proliferation. In conclusion, our results provide convincing evidence that G2 mediated induction of c-Myc plays an intra-cellular role in melanoma tumor growth and subsequently establishes that PL mediated inhibition of melanoma cells in vitro and in vivo is associated with inhibition of G2.

989 Comparison Study of Anti-Inflammatory Effects of Polyphenolics in Colitis Rat Model Targeting mTOR Signaling Pathway

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Background: Polyphenolics from pomegranate (ellagic acid, ellagitannins (including punicalagins and punicalins), flavonoids, and anthocyanins) and mango (gallic acid, galloyl derivatives, flavonol glycosides and benzophenone derivatives), have been shown to have potent anti-inflammatory activities. Ulcerative colitis, a chronic inflammation of the large intestine may increase risk of human colorectal cancer. Polyphenolics have been shown to suppress inflammation through different mechanisms, including inhibition of the mTOR signaling pathway. Methods: To determine the anti-inflammatory effects and possible mechanisms of mango and pomegranate juices in DSS-induced colitis in rats, SD rats were administered control juice (15.7g sugar and 0.05g citric acid/100ml), mango juice (Total phenolic content of 47.53mg/g L/GAE), or pomegranate juice (Total phenolic content of 2457.23mg/g L/GAE), and were exposed three cycles of 3% dextran sodium sulfate (DSS) in the drinking juice followed by a 2-week recovery period. Colon inflammation and injury scores were assessed, and cell proliferation was assessed in colon sections by Ki-67 staining. The mRNA and protein levels of pro-inflammatory proteins and cytokines were also analyzed. Results: Both Mango and pomegranate juice reduced DSS-induced colon inflammation and cell proliferation during chronic colitis in rats compared to control juice. Mango juice suppressed HIF1α levels and reduced mRNA and protein expression of pro-inflammatory cytokines. In contrast, Pomegranate juice suppressed HIF1β levels, and increased inhibitory responses of TGFβ. Conclusions: These results suggest that polyphenolics of different predominant structure may differentially regulate inflammation-involved pathways while attenuating DSS-induced colitis. Both mango and pomegranate polyphenolics seem to have potential in the prevention and mitigation of colon inflammation.

990 Mechanistic Studies of Cancer Cell Mitochondria- and NQO1-Mediated Redox Activation of Beta-Lapachone, a Potentially Novel Anticancer Agent

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Beta-lapachone (beta-Lp) derived from the Lapacho tree is a potentially novel anticancer agent currently under clinical trials. Previous studies suggested that redox activation of beta-Lp catalyzed by NAD(P)H:quinone oxidoreductase 1 (NQO1) accounted for its killing of cancer cells. However, the exact mechanisms of this effect remain largely unknown. Using chemiluminescence and electron paramagnetic resonance (EPR) spin-trapping techniques, this study for the first time demonstrated the real-time formation of ROS in the redox activation of beta-lapachone from cancer cells mediated by mitochondria and NQO1 in melanoma B16-F10 and hepatocellular carcinoma HepG2 cancer cells. ES936, a highly selective NQO1 inhibitor, and rotenone, a selective inhibitor of mitochondrial electron transport chain (METC) complex I were found to significantly block beta-Lp mediated redox activation. In B16-F10 cells, HepG2 cells ES936 inhibited beta-lapachone-induced oxygen radical formation by ~80% while rotenone exerted no significant effect. These results reveal the differential contribution of METC and NQO1 to beta-lapachone-induced ROS formation and cancer cell killing. In melanoma B16-F10 cells that do not express high NQO1 activity, both NQO1 and METC play a critical role in beta-Lp redox activation. In contrast, in hepatocellular carcinoma HepG2 cells expressing extremely high NQO1 activity, redox activation of beta-Lp is primarily mediated by NQO1 (METC plays a minor role). These findings will contribute to our understanding of how cancer cells are selectively killed by beta-lapachone and increase our ability to devise strategies to enhance the anticancer efficacy of this potentially novel drug while minimizing its possible adverse effects on normal cells.

991 Silencing KRAS Overexpression in Cadmium-Transformed Prostate Epithelial Cells Mitigates Malignant Phenotype

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Cadmium (Cd) is a potential human prostate carcinoma. Chronic Cd exposure malignantly transforms the control RWPE-1 human prostate epithelial line into CTPE cells by an unclear mechanism. Here, we assessed KRAS expression in CTPE cells, an oncogene often activated in prostate cancer. CTPE cells greatly overexpressed KRAS protein (2000% of control) together with increased phospho-ERK protein (211% control) indicating the RAS-ERK signaling pathway activation. This pathway controls cell survival, proliferation and motility, and if activated favors tumorigenesis. Since KRAS activation appears key to Cd transformation, we hypothesized that KRAS knockdown (KD) would reverse malignant phenotype. Thus, RNA interference using shRNAmirs for KRAS KD was used in CTPE cells. KRAS shRNAmir primarily expresses miRNA 30 transcript that targets KRAS to silence its expression. CTPE cells were transduced with KRAS shRNA, or non-targeting control shRNA. Stable KD cells were formed by growth in media with puromycin throughout the study. Post transduction, KRAS protein was below detection in CTPE KD cells, confirming stable KD. KRAS KD markedly decreased phospho-ERK (by 55%) and phospho-AKT (by 68%) in CTPE KD cells compared to control cells, indicating repression of stimulated RAS/ERK and basal PI3K/AKT signaling pathways. Elevated secreted metalloproteinase-2 (MMP-2) activity, an indicator of Cd-induced malignant transformation, was suppressed by 75% in KRAS KD to 68% of non-KD levels, and this suppression persisted throughout the experiment (12 weeks). Anchorage-independent growth (colony formation), typical of cancer cells, decreased by 57% by 6 weeks after KD and this persisted to the end of the experiment. Protein levels of p21, a cell cycle inhibitor that impacts proliferation, increased 343% with KRAS KD. KRAS KD also decreased BCL2 (61%), an anti-apoptotic protein often activated in cancers. Thus, KRAS silencing impacts Cd-induced malignant phenotype, inducing loss of multiple typical cancer cell characteristics.

992 Hematological Alterations and Markers of B Cell Activation in Workers Exposed to Benzene, Formaldehyde, and Trichloroethylene

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Benzene, formaldehyde (FA), and trichloroethylene (TCE) are ubiquitous chemicals present in workplaces and in the general environment. Benzene is an established leukemogen and probable lymphomagen. FA was recently classified as a myeloid leukemogen, while TCE is classified as a probable lymphomagen. Epidemiologic associations between FA and leukemia, and between benzene, TCE and lymphoma are, however, still under debate. Previously, we have shown that exposures to these chemicals are associated with hematotoxic effects in cross-sectional studies of factory workers in China. Here, we compare and contrast patterns of hematotoxicity and markers of B-cell activation across studies to further evaluate possible mechanisms of action and consistency of effects with observed cancer risks. Workers exposed to benzene and FA, but not TCE, showed declines in granulocytes, platelets, and/or monocytes, cell types derived from myeloid progenitor cells. Alterations in lymphoid cell types, including B-cells and CD4+ T-cells, and markers of B-cell activation, were apparent in workers exposed to benzene and TCE. Additionally, the frequency of chromosome 7 loss (monosomy 7) was increased in cultured circulating myeloid progenitor cells from both benzene- and FA-exposed workers. Given that alterations in myeloid and lymphoid cell types have been associated with hematological malignancies, our data provide biologic insight into the epidemiologic evidence that links exposure to benzene and FA with risk of myeloid leukemia, and exposure to TCE and benzene with risk of lymphoma.
993 Membrane Progesterone Receptor Alpha Signaling in Breast Cancer Cells
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Progesterin signaling is mediated via the nuclear progesterone receptor (nPR) and membrane progesterone receptors (MPR). Progestins include the parent compound progesterone (P4) and its biologically active metabolites, 5α-dihydroxyprogesterone (5αP) and 3α,5α-dihydroxyprogesterone (3αHP). Cell proliferation and detachment are stimulated by 5αP while 3αHP elicits opposing effects. While progesterin signaling through nPR has been extensively studied, events downstream of MPR activation remain to be elucidated. We hypothesize progesterin binding to MPR alpha (MPRα) activates MAPK signaling to modulate cell cycle progression. We established MPRα expression in eleven breast cancer cell lines using western blot analysis and qPCR, allowing us to characterize the expression status for nPR, MPRα, or both. We ascertained expression of P4 receptors to study signaling pathways initiated by progestins and determine the receptors responsible. We assessed whether the ERK, AKT, and/or JNK pathways are activated by progestin treatment. Our results suggest P4 stimulated MPRα signaling resulted in phosphorylation of AKT. To determine whether MPR-mediated AKT signaling could affect cell cycle progression and cellular proliferation, we conducted flow cytometry on propidium iodide stained cells and the CellTiter Glo assay, respectively. By treating breast cancer cells lacking the nPR with P4, 5αP, and 3αHP we aim to determine how progestins modulate cell cycle progression independent of nPR-mediated events. Our preliminary results indicate 5αP may block breast cancer cell cycle progression. In summary, MPRα-mediated signaling via AKT may play a vital role in cellular survival and proliferation of breast cancer. Understanding pathways mediated by progestins independent of the nuclear receptor may reveal treatment strategies for nPR negative breast cancer and inform breast cancer biology.

994 Gene Expression Changes in Human Endometrial Cells Exposed to the Tamoxifen Metabolite Endoxifen
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The selective estrogen receptor modulator tamoxifen (TAM), used for adjuvant therapy and chemoprevention of breast cancer, is predominantly metabolized to endoxifen (4-hydroxy-N-desmethyltamoxifen). Whereas the metabolite endoxifen is not directly on the pathway responsible for TAM-induced DNA damage, it may participate in other pathways important for TAM protective effects, as well as the TAM-induced endometrial cancers that sometimes appear in breast cancer patients. Previously we studied gene expression changes in human endometrial stromal (HESCs) and normal human mammary epithelial cells (NHMECs) exposed to TAM, and showed that induction of steroidal and proliferative pathways predominate in HESCs, while immune-response pathways are the major TAM-induced pathways in NHMECs. In this study we focused on gene expression changes induced by treatment of HESCs with 10 μM endoxifen for 48 hr. We used the Human Gene 1.0 ST Affymetrix expression array, and gene expression analysis was carried out using the mAdb tool and Ingenuity Pathway Analysis (IPA). Genes significantly overexpressed in the HESCs exposed to endoxifen include: some involved in lipid metabolism, FABP3, FADS1, FASN; some involved in humoral signaling suppressed the development of precancerous lesions and it also reduced the infiltration of mast cells, suppressed the immunostaining of TNF-α, COX-2, iNOS and VEGF. Zingerone treatment significantly attenuated the level of TNF-α and it also reduced the depletion of the mucous layer as well as attenuated the shifting of stalomucin to sulphomucin. Conclusion: Our findings suggest that zingerone has strong chemopreventive potential against DMH-induced colon carcinogenesis but further studies are warranted to elucidate the precise mechanism of action of Zingerone.

995 Regulation of Gene Expression Profiles in Clear Cell Renal Cell Carcinoma by Cadmium Exposure
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Clear cell renal cell carcinoma (ccRCC), the predominant subtype of kidney cancer, displays variability in risk for developing metastatic disease, and tissue-based prognostic biomarkers are urgently needed. Recently, we’ve elucidated two subtypes of ccRCC, clear cell A (ccA) and clear cell B (ccB). The subtypes convey a prognostic value, with tumors displaying the ccA signature associated with better survival compared to ccB. We developed and validated a 34-gene subtype predictor to classify clear cell tumors as ccA and ccB using RNA-sequencing data from ccRCC samples from The Cancer Genome Atlas (TCGA) and the NanoString platform using samples collected at the University of North Carolina. This novel tool can be used to analyze risk for developing metastatic disease and as a baseline metric to elucidate how ccRCC gene expression signatures are influenced by environmental toxicants. Heavy metals can induce nephrotoxicity altering gene expression in the kidney, and thus increasing the risk of developing RCC. In addition to transcript alterations, numerous studies have found that Cd exposure can result in epigenetic deregulation by changing DNA methylation levels. We have observed significant decreases in methylation sites associated with the 34-gene ccA/ccB algorithm among ccRCC tissue compared to normal tissue of smokers from TCGA. Smoking population is known for higher levels of cadmium exposure, as one cigarette may contain 1-2 ug cadmium. Interestingly, the majority of this cohort was either ccB subtype or had metastatic disease, suggesting a possible correlation between cadmium exposure and aggressive disease. Furthermore, higher Cd concentrations were detected in clinical RCC tissues collected at UNC from smokers compared to non-smokers, with the majority being of the ccB subtype. Exploring both acute and chronic cadmium induced changes in gene expression can provide insight into mechanisms that may enhance our understanding of the genetic interactions that drive the biological responses to these exposures and their influence on prognostic signatures of RCC.

996 Zingerone Suppresses the Development of Precancerous Lesions via Regulating the Hyperproliferation, Inflammation and Angiogenesis in the Colon of Wistar Rats
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Purpose: Colon carcinogenesis is a multistep process and it emanates from a series of molecular and histopathological alterations. Zingerone, a phenolic alkalone, one of the active components of ginger, possesses multiple biological activities, such as antioxidant and anti-inflammatory properties. In the present study, we investigated the chemopreventive potential of zingerone against 1,2-dimethylhydrazine (DMH)-induced precancerous lesions i.e., aberrant crypt foci (ACF) and mucin depleted foci (MDF), and its role in regulating the hyperproliferation, inflammation and angiogenesis in the colon of Wistar rats. Methods: Animals were divided into 5 groups. In group III, IV and V, Zingerone was administered at the dose of 25 mg/kg bw, orally while in group II, III and IV, DMH was administered subcutaneously in the groin at the dose of 20 mg/kg bw, once a week for first 5 weeks and animals were euthanized after 9 weeks. Results: Zingerone supplementation suppressed the development of precancerous lesions and it also reduced the infiltration of mast cells, suppressed the immunostaining of Ki-67, NF-kB-p65, COX-2, iNOS and VEGF. Zingerone treatment significantly attenuated the level of TNF-α and it also reduced the depletion of the mucous layer as well as attenuated the shifting of stalomucin to sulphomucin. Conclusion: Our findings suggest that zingerone has strong chemopreventive potential against DMH-induced colon carcinogenesis but further studies are warranted to elucidate the precise mechanism of action of Zingerone.

997 Analysis of DNA Methyltransferase Expression in a Transplacental Mouse Model with Indole-3-Carbinol Dietary Intervention during Exposure to Dibenzo[def,p]Chrysene
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Dibenzo[def,p]chrysene (DBC) is a polycyclic aromatic hydrocarbon (PAH) that results from the incomplete combustion of organic materials. Human exposure to DBC may occur through diet, inhalation, and/or skin contact. Indole-3-carbinol (I3C) is a dietary phytochemical derived from cruciferous vegetables. Our laboratory previously reported that maternal consumption of I3C was protective against DBC induced precarcinogenesis in the offspring of exposed dams. We therefore hypothesize that the chemopreventive effect occurs in part through alteration of DNA methyltransferase expression based on previous studies in cancer cell lines. In this study pregnant mice were fed AIN93G control diet or diet containing 500 ppm I3C beginning on gestation day 9 through birth. On gestation day 17, pregnant study pregnant mice were fed AIN93G control diet or diet containing 500 ppm I3C or both. We ascertained expression of P4 receptors to study signaling pathways leading to TAM-DNA damage may be also involved in the induction of TAM-induced endometrial tumors.

998 In Silico Prediction of Gene Expression Profiles in Clear Cell Renal Cell Carcinoma by Cadmium Exposure
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Clear cell renal cell carcinoma (ccRCC), the predominant subtype of kidney cancer, displays variability in risk for developing metastatic disease, and tissue-based prognostic biomarkers are urgently needed. Recently, we’ve elucidated two subtypes of ccRCC, clear cell A (ccA) and clear cell B (ccB). The subtypes conveyed a prognostic value, with tumors displaying the ccA signature associated with better survival compared to ccB. We developed and validated a 34-gene subtype predictor to classify clear cell tumors as ccA and ccB using RNA-sequencing data from ccRCC samples from The Cancer Genome Atlas (TCGA) and the NanoString platform using samples collected at the University of North Carolina. This novel tool can be used to analyze risk for developing metastatic disease and as a baseline metric to elucidate how ccRCC gene expression signatures are influenced by environmental toxicants. Heavy metals can induce nephrotoxicity altering gene expression in the kidney, and thus increasing the risk of developing RCC. In addition to transcript alterations, numerous studies have found that Cd exposure can result in epigenetic deregulation by changing DNA methylation levels. We have observed significant decreases in methylation sites associated with the 34-gene ccA/ccB algorithm among ccRCC tissue compared to normal tissue of smokers from TCGA. Smoking population is known for higher levels of cadmium exposure, as one cigarette may contain 1-2 ug cadmium. Interestingly, the majority of this cohort was either ccB subtype or had metastatic disease, suggesting a possible correlation between cadmium exposure and aggressive disease. Furthermore, higher Cd concentrations were detected in clinical RCC tissues collected at UNC from smokers compared to non-smokers, with the majority being of the ccB subtype. Exploring both acute and chronic cadmium induced changes in gene expression can provide insight into mechanisms that may enhance our understanding of the genetic interactions that drive the biological responses to these exposures and their influence on prognostic signatures of RCC.
tochime P450 1b1 (Cyp1b1). In addition, we examined gene expression of the DNA methyltransferases: Dnmt1, Dnmt3a, and Dnmt3b. Gapdh was used as a reference gene to normalize all six genes of interest. qRT-PCR analysis did not reveal statistically significant changes in the genes examined. These results can in part be attributed to the intra- and inter-litter variation in each treatment. In conclusion, neither DBC nor 13C alter Dnmt expression at the gene level in neonate thymus. Future studies will investigate other tissues as well as 13C inhibition of histone deacetylase (HDAC) expression and activity.

998 Ligand Activation of PPARδ Inhibits UVB-Induced Skin Cancer

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Previous studies show that ligand activation of PPARδ inhibits chemically-induced skin cancer. Since UVB is a more common factor causing NMSC, the hypothesis that ligand activation of PPARδ inhibits UVB-induced skin cancer was examined. Female SKH1 mice were irradiated with UVB (180mJ/cm²) 3 times/week followed by topical treatment with PPARδ ligands (GW0742: 1, 5 or 10 micromolar) or sodium oleate (25, 50 or 100 micromolar). Ligand activation of PPARδ delayed the onset of tumor formation, and this effect was more pronounced with 1 and 5 micromolar GW0742. After 25 weeks, the average tumor multiplicity per mouse was 23.8 in control mice as compared to 15.2, 15.4 and 15.0 in mice treated with 1, 5 or 10 micromolar GW0742, respectively. The average tumor multiplicity per mouse was 21.4, 18.2 and 13.2 in mice treated with 25, 50 or 100 micromolar sodium oleate, respectively. The average tumor size was also reduced in mice treated with 5 micromolar GW0742 and with 25 micromolar sodium oleate compared to control mice. To begin to determine the mechanisms for these chemopreventive effects, female SKH1 mice were irradiated with UVB (30mJ/cm²) 2 times/week followed by topical treatment with PPARδ ligands as described above. Cellular patches (> 8 adjacent cells/patch) containing wild-type and mutant p53 were detected in epidermal sheets using immunohistochemical methods after 20 weeks of treatment. Ligand activation of PPARδ inhibited p53 mutant patches by 55%, 63%, 75% in mice treated with 1, 5 or 10 micromolar GW0742 and by 64%, 77% and 78% in mice treated with 25, 50, 100 micromolar sodium oleate as compared with control mice, respectively. Further, cytochrome P450 1b1 (Cyp1b1) and pyrimidine (6-4) pyrimidine photodiomers caused by UVB irradiation were also reduced significantly by ligand activation of PPARδ. In both ligands. Combined, these results suggest that activation of PPARδ prevents UVB-induced skin cancer by targeting p53 mutant cells and inhibiting UVB-induced DNA damage. (Supported by CA124553).

999 Peroxisome Proliferator-Activated Receptor-δ (PPARδ) Inhibits Tumorigenesis by Interfering with RARα-Mediated MMP2 Activation and Inducing Cell Differentiation in Human Testicular Embryonal Carcinoma Cells

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Testicular germ cell tumors are most prevalent in young adult men ages 15-40 years, and arise as a result of abnonmal testicular development. Peroxisome proliferator-activated receptors (PPARδ) regulates a variety of biological processes but the function of PPARδ in carcinogenesis remains controversial. A stable human testicular cancer cell line, NT2/D1, that constitutively over-express PPARδ were produced to effectively examine the role of this receptor in human cancer models. Interestingly, over-expression of PPARδ suppressed MMP2 and MMP9 activities, and was associated with the decreased invasion and cell migration. Furthermore, the results of immunoprecipitation, electrophoretic mobility shift assay and chromatin immunoprecipitation indicated that this inhibitory effect was achieved by interfering with the formation of RAR-RXR complex, resulting in a decrease of RARα-mediated MMP2 activation. Therefore, by competing for binding with RARX, relatively higher expression of PPARδ may cause the attenuation of tumor progression. Indeed, over-expression of PPARδ reduced tumor volumes and tumor weights of ectopic xenografts from NT2/D1 cells compared to controls. Over-expressing PPARδ also caused decreased expression of OCT3/4 in xenograft tumors, suggesting that PPARδ induced cell differentiation. Combined, these novel observations demonstrate the inhibitory effect of activating or over-expressing PPARδ on tumorigenesis in human testicular embryonal carcinoma cells.

1000 PPARβ/δ and PARβ/γ Modulate UV-Induced Apoptosis and Cytokine Secretion in a Human Melanoma Cell Line

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Ultraviolet (UV)-induced skin tumorigenesis results from DNA damage, increased inflammation, and evasion of apoptosis. The peroxisome proliferator-activated receptor (PPAR) class of nuclear receptors have been shown to modulate these properties in non-melanoma skin cancers. However, limited data exists regarding the role of PPARs in melanoma. The present study examined the effect of PPARs on UV-induced apoptosis and cytokine secretion in the UACC903 human melanoma cell line. The effect of increased expression of either PPARβ/δ or PPARγ on UV-induced endpoints was examined by generating stable UACC903 cell lines that over-express PPARβ/δ and PPARγ. Ligand activation of each PPAR enhanced expression of the known PPAR target gene ANGPTL4 in cell lines with elevated PPAR expression, but did not alter the expression of the putative anti-apoptotic PPAR target gene PDK1. UV irradiation increased the secretion of TNFα, IL-6, and IL-8 in all cell lines. However, these levels were significantly reduced in cells over-expressing PPARβ/δ and PPARγ. TNFα secretion was further attenuated by ligand activation of PPARs prior to UV irradiation. In contrast, ligand activation of PPARs had no further effect on UV-induced secretion of IL-6 or IL-8. All cell lines exhibited time-dependent increases in the activity of the pro-apoptotic caspase enzymes following UV irradiation. Over-expression of PPARβ/δ and PPARγ was found to augment these changes in activity. Ligand activation of PPARs in cell lines over-expressing the respective PPARs further enhanced the receptor-dependent changes in caspase activity. Collectively, these results demonstrate that both PPARβ/δ and PPARγ modulate UV-induced cytokine secretion and apoptosis in a model of human melanoma. Further studies are therefore needed to delineate the mechanism(s) and therapeutic potential of PPARs in UV-induced cancer models.

1001 7-Cysteine-pyrrole Adducts Is a Potential Active Metabolite Leading to Pyrrolizidine Alkaloid-Induced Cytotoxicity and Tumorigenicity

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Pyrrolizidine alkaloid (PA)-containing plants are widespread in the world and are probably the most common poisonous plants affecting livestock, wildlife, and humans. PAs require metabolic activation to form pyrrolic metabolites, all of which contain a (±)-6,7-dihydro-7-hydroxy-1-hydroxymethyl-5H-pyrrolizine (DHP) base, to exert PA-induced cytotoxicity, genotoxicity, and tumorigenicity. We previously reported that metabolism of tumorigenic PAs produced a set of four DNA adducts, designated as DHP-2'-deoxyguanosine (dG)-3, DHP-dG-4, DHP-2'-deoxyadenosine (dA)-3, and DHP-dA-4, that are responsible for liver tumor initiation and are potentially common biological biomarkers of PA carcinogenesis and exposure (Xia et al., Chem. Res. Toxicol., 26, 213-255, 2013). 7-GSH-DHP, a PA metabolite formed in vivo and in vitro, can potentially be enzymatically hydrolyzed into 7-cysteine-DHP. In this study, 7-cysteine-DHP was synthesized by rehydration of dehydromonocotyline with cysteine and its structural was fully characterized by mass and NMR spectral analysis. We then determined that reaction of 7-cysteine-DHP with dG in water formed both DHP-dG-3 and DHP-dG-4 adducts in a concentration- and time-dependent manner. Besides, another two structural isomers, DHP-dG-1 and DHP-dG-2 adducts, were also formed as predominant products. The structures of these DHP-dG adducts were determined by comparison of their HPLC retention times, UV-visible absorption spectra, and LC/MS data with those of previously synthesized standards. This study represents the first report that 7-cysteine-DHP adduct can be an active metabolite leading to PA-induced cytotoxicity, genotoxicity, and tumorigenicity. (This article is not an official guidance or policy statement of the U.S. FDA. No official support or endorsement by the U.S. FDA is intended or should be inferred).
It is generally believed that cancer prevention is the most promising strategy for reducing both cancer incidence and cancer-related mortality. One promising approach to cancer prevention is an active intervention with agents that are expected to suppress or attenuate the initial phases of carcinogenesis. In previous studies, we demonstrated a potent chemopreventive effect of tributyrin, a butyric acid prodrug, on experimental hepatocarcinogenesis. This cancer-inhibitory effect of tributyrin has been linked to the suppression of sustained cell proliferation and induction of the apoptotic cell death driven by activation of the p53 apoptotic signaling pathway. The goal of the present study was to investigate the underlying molecular mechanisms linked to tributyrin-mediated p53 activation. Using in vivo and in vitro models of liver cancer, we demonstrated that treatment with tributyrin or sodium butyrate resulted in an increase in the level of nuclear/cytoplasmic p53, and, consequently, in an increase of the ratio of nuclear/cytoplasmic p53. Moreover, the treatment with tributyrin or sodium butyrate induced marked increase in the level of nuclear CRM1 protein, a major nuclear exporter protein. The results demonstrate that the chemopreventive activity of tributyrin on experimental liver carcinogenesis may be attributed to an inhibition of nuclear export and retention of p53 and CRM1 proteins in the nucleus.

The AHR is a ligand-activated and ultraviolet (UV)-B-sensitive transcription factor. In its inactive form, the AHR rests in a cytosolic multiprotein complex. Upon ligand binding, this complex dissociates and the AHR shuttles in the nucleus to modulate gene expression. Beside various drug-metabolizing enzymes, AHR target genes encode for proteins involved in proliferation and apoptosis. As apoptosis is probably the most important mechanism restraining photocarcinogenesis, we investigated the role of AHR in regulating apoptosis in human keratinocytes (KC) and mouse skin. We found that AHR inhibition in vitro as well as in vivo resulted in a significant elevation of UVB-induced apoptosis, which was due to a reduced expression of the cell-cycle regulators E2F1 and checkpoint kinase-1. The increase in apoptosis correlated with the removal of cells harboring UVB-specific DNA lesions, as determined by HPLC/MS/MS. To study the pathophysiological relevance of AHR inhibition, we performed a photocarcinogenesis study in AHR+/+ and AHR-/- SKH-1 hairless mice. Strikingly, we observed ~50% less skin tumors in AHR+/+ and AHR-/- and AHR+/+ and AHR-/- SKH-1 hairless mice. Strikingly, we observed ~50% less skin tumors in AHR+/+ and AHR-/- mice indicating that the AHR damps KC apoptosis in vitro and thereby contributes to skin carcinogenesis. Our results identify the AHR as a promising target for chemoprevention of non-melanoma skin cancer. We next asked if the inhibitory effect of AHR on apoptosis is also reversible in human A431 and SCL-1 cutaneous squamous cell carcinoma (cSCC) cell-lines. Exposure of the cells to different genotoxic stimuli (chemotherapeutic drugs, UV radiation) induced apoptosis, which was further elevated by a 3'-methoxy-4'-nitroflavone-mediated inhibition of AHR's anti-apoptotic action, we performed a photocarcinogenesis study in vivo and in mouse skin. We found that AHR inhibition resulted in a significant elevation of UVB-induced apoptosis, which was due to a reduced expression of the cell-cycle regulators E2F1 and checkpoint kinase-1. The results demonstrate that the chemopreventive activity of tributyrin might be of therapeutic relevance, e.g. it may allow reducing dose and/or duration of chemotherapy.
CArG-box binding factor-A (CBF-A) is a member of the heterogeneous nuclear ribonucleoprotein (hNRNP) family of RNA-binding proteins involved in a variety of cellular functions, including transcriptional regulation. CBF-A is overexpressed in multiple tumor types and is also a key regulator of epithelial-mesenchymal transition (EMT), an important process in cancer progression. CBF-A has two highly conserved isoforms that have opposing effects on promoter activity, suggesting that the ratio of the protein isoforms play a critical role in carcinogenesis. Previously we showed that CBF-A regulates the expression of the H-a-ras oncogene by binding to enhancer elements (ets-like) in the promoter region. It has been shown that CBF-A is a transcriptional inhibitor of osteopontin, which is implicated in tumor invasion, progression or metastasis in cancer originating in many tissues due to its interaction with cell surface receptors, growth factor/receptor pathways, and proteases. To evaluate the role of CBF-A in carcinogenesis, we generated knock-out mice and then exposed wild-type mice, as well as CBF-A null and heterozygous mice to a single dose of the mutagenic, DNA-alkylating agent N-ethyl-N-nitrosourea (ENU) (10 mg/mL, 9-10 weeks of age). While administration of ENU decreased the survival rate in all genotypes and sex compared to vehicle control mice, vehicle control CBF-A null male mice had a decreased survival rate compared to the wild-type male mice. CBF-A null mice exposed to ENU had an increased incidence of tumors and a decreased latency compared to the wild-type ENU exposed mice. Taken in concert, these data suggest CBF-A plays an important role in maintaining normal growth control. Support DOD(BC)710145, NIEHS Toxicogenomics Research Consortium (ES011387), NIEHS Center Grants (ES005022 and ES007033) and pilot funding Rutgers Cancer Institute of New Jersey (CA072720).

Several gene polymorphisms of xenobiotic/drug metabolizing enzymes and TP53 have been studied for the possible association with response to chemotherapy and survival rates of patients with non-small cell lung cancer (NSCLC). However, the studies in this regard are limited and the results are contradictory. In this study, CYP2E1*5B, CYP2E1*6 and CYP2E1*7B, GSTT1 (A140D) and TP53 (Arg72Pro) polymorphisms and response to platinum based chemotherapy and survival in 137 (125 men and 12 women) advanced stage NSCLC patients have been investigated. Although no significant associations were noted between the gene polymorphisms alone and response to chemotherapy, patients with combined variant genotypes of TP53 (Arg72Pro, Pro72Pro) and CYP2E1*6 (1*A/*6) responded significantly better than those carrying wild type genotypes to platinum based chemotherapy, (p<0.04). However, we observed that only the patients who had both variant genotypes of TP53 (Arg72Pro, Pro72Pro) and CYP2E1*6 (1*A/*6) had shorter survival (median, 17.9 months) compared to wild type genotypes (median, 28.1 months) with marginal significance (p=0.086). Multivariate analysis also revealed that adjusted hazard ratio of death (HR) of only the combined variant genotypes of TP53 (Arg72Pro, Pro72Pro) and CYP2E1*6 (1*A/*6) increased 51-fold, although not significant, as compared to wild type genotypes (HR, 50.61 ; 95 % CI, 0.44-5789.77, p=0.105). These results show that, among the studied genotypes, only the combination of TP53(Arg72Pro, Pro72Pro) and CYP2E1*6 (1*A/*6) is likely to be associated with response to chemotherapy and survival in the patients with advanced NSCLC. (Supported by the grants from Research Fund of Ankara University Nos: 10A3336002 and 2008-08-03-006HPD).
Metabolism of styrene (STY) by mouse lung CYP2F2 to cytotoxic metabolite(s) has been postulated as an essential step for mouse lung toxicity and mouse-lung specific tumorigenicity (Cruzan et al., RTP, 2012, 2013). The purpose of this study was to use whole-lung genomic analyses to further investigate potential modes of action (MoA) of STY in C57BL/6 wild-type (WT), CYP2F2 knockout (-/-) KO) and CYP2F2F1 humanized (2F2-KO + 2F1, A13, B16, transgenic, TG) male mice. Mice were exposed to 0, 40 or 120 ppm STY 6 hr/day 5 days/week for 1 or 4 wk. Five biological replicates for each treatment group of mice were analyzed for relative gene expression using Affymetrix whole genome HT-MG430_PM Titan arrays. 287 genes were significantly differentially expressed in WT mice at both STY concentrations. Gene ontology enrichment showed a strong dominance of cell cycle regulatory pathways consistent with cell proliferation. No genes were significantly differentially expressed in KO mice. Only a single gene was significantly differentially expressed at 120 ppm in TG mice after 1 week and a different single gene at 40 ppm after 4 weeks. This study supports the conclusion that the MoA of STY mouse lung toxicity requires CYP2F2 metabolism (but not by human CYP2F1) as a key gateway event, and also indicates that alternative MoAs mediated by either parent STY or non-CYP2F2 generated STY metabolites (e.g., styrene oxide) are unlikely. Supported by Styrene Information and Research Center (SIRC).

Exposure to cookstove emissions (CE) has been linked to significant increases in morbidity and mortality, with current estimates attributing CE exposure to over 4 million deaths annually. The development of several new cookstoves (CS) designs has led efforts to reduce CE with relative success, yet data supporting potential health benefits from the implementation of such devices remain limited. Since CE contain numerous redox-active components including VOCs, PAHs, metals, particulates, and radicals, oxidative stress is likely a key mechanistic feature of CE toxicity. Exposure from four CS: 3-Stone (3S), Natural Draft (ND), Forced Draft (FD), and Propane (PR); were previously determined to have substantial influence on the alteration of the lung redox balance in female CD-1 mice. As an extension, the current study examines the impact of CE on the intracellular oxidation of glutathione using fluorescent reporters expressed in both murine and human-derived lung epithelial cell lines. In brief, roGFP2, a reporter of intracellular glutathione redox potential (Egsh) was stably transduced into LA-4 (murine) or BEAS-2B (human) cells. Cells were exposed to methanol-derived extracts obtained from filters collected during the original in-vivo study. Using live-cell imaging, intracellular responses to each CE extract were observed in real-time. The CE extracts caused potent increases in the Egsh in both cell lines across equal- and compensated-dosing schemes, yielding the following relative potencies: 3S>ND≈FD>PR. Importantly, validation experiments confirmed the response of roGFP2 to be indicative of the oxidation status of glutathione. Together, these data indicate that exposure to various CE induces a substantial increase in intracellular Egsh in both human and murine epithelial cells, which is indicative of an oxidant-dependent impairment of redox homeostasis. Moreover, the use of cleaner CS appears to attenuate the oxidative changes observed. (Does not necessarily reflect USEPA policy)

Chronic alcohol consumption leads to increased fracture risk and an elevated risk of osteoporosis by decreasing bone mineral density through increasing os- teoclast activity, decreasing osteoblast activity, and increasing senescence. Our lab has shown this to be mediated by reactive oxygen species (ROS) produced by NADPH oxidases (NOX). We hypothesized that different dietary antioxidants, Curcumin (120mg/kg/d), N-acetyl cysteine (NAC) (1.2mg/kg/d), and Vitamin E (alpha-tocopherol) (60mg/kg/d) would attenuate osteopenia due to chronic alcohol consumption in female mice exposed to 3S-Stove (3S) and/or an adequate diet supplemented with an antioxidant with or without EtOH at 30% of total calories for 8 weeks. MicroCT analysis showed protection from trabecular bone loss in the EtOH+NAC and EtOH+alpha-tocopherol groups compared to the EtOH group (P<0.05). A significant decrease in bone volume (BV/TV) and trabecular number was detected in the EtOH group. These findings suggest that dietary antioxidants can prevent the negative effects of chronic alcohol consumption on bone health.
Loss of Mrp1 Potentiates Doxorubicin-Induced Cardiotoxicity in Mice

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Doxorubicin (DOX) is an effective cancer chemotherapeutic agent that induces dose-dependent cardiotoxicity in part due to its ability to induce oxidative stress. In the present study, we investigated the role of multidrug resistance-associated protein 1 (Mrp1/Abcc1) in DOX induced cardiotoxicity in mouse. C57BL (WT) and Mrp1 null (Mrp1-/-) littermates were administered intraperitoneal DOX (2 mg/kg body weight), or equivalent volume of saline, twice a week for 5 weeks, resulting in a cumulative DOX dose of 20 mg/kg; animals were examined 48 h or 2 weeks after the last DOX treatment. Chronic DOX treatment induced body weight loss and hemototoxicity, and these adverse effects were significantly exaggerated in Mrp1-/- mice. Mrp1-/- hearts also exhibited more DOX-induced cell apoptosis (measured by Terminal deoxynucleotidyl transferase dUTP nick end labeling assay) compared with WT hearts (p < 0.05). Moreover, cardiac function, assessed by measurement of fractional shortening (FS) and ejection fraction (EF) with M-mode transthoracic echocardiography, was significantly decreased in Mrp1-/- than WT hearts (p < 0.05 vs 20, 23, 29.5% and 95% CI of EF: 41.5 – 48.4% vs 47.7 – 56.7%). After DOX treatment, there was a comparable increase of 4-hydroxynonenal glutathione conjugate (GS-HNE) concentration in WT vs Mrp1-/- mice heart tissue. Despite the significantly higher GSH (1.4±0.27 fold) and GSSG (1.35±0.16 fold) concentration in heart from saline-treated Mrp1-/- mice compared to WT mice, no differences in the GSH/GSSG ratio were detected between genotypes in either saline or DOX treated mice hearts. Taken together, these data indicate that Mrp1 protected the mouse heart against DOX induced cardiotoxicity.

Ozone Effects on Protein Carbonyl Content in the Frontal Cortex and Cerebellum of Young Adult, Middle Age, and Senescent Brown Norway Rats

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Oxidative stress (OS) plays an important role in susceptibility and disease in old age. Understanding age-related susceptibility is a critical part of community-based human health risk assessment of chemical exposures. There is growing concern over a common air pollutant, ozone (O3), and adverse health effects including dysfunction of the pulmonary, cardiac, and nervous systems. The objective of this study was to test whether O3 plays a role in the adverse effects caused by O3 exposure, and if so, if effects were age-dependent. We selected protein carbonyl as a indicator of OS because carbonyl content of cells is a useful indicator of oxidative protein damage and has been linked to chemical-induced adverse effects. Male Brown Norway rats (4, 12, and 24 months) were exposed to O3 (0, 0.25 or 1 ppm) via inhalation for 6 h/day, 2 days per week for 13 weeks. Frontal cortical (FC) and cerebellum (CB) were dissected, quick frozen on dry ice, and stored at -80°C. Protein carbonyls were assayed using commercial kits. Hydrogen peroxide, a positive control, increased protein carbonyls in cortical tissue in vitro in a concentration-dependent manner. Significant effects of age on protein carbonyls in FC and a significant effect of age and O3 dose on protein carbonyls in CB were observed. In control rats, there was an age-dependent increase in protein carbonyls indicating increased OS in 12 and 24 month old rats compared to 4 month old rats. Although O3 increased protein carbonyls in both brain regions and in all age groups, O3 effects were statistically significant only in the 4 month old rats. These results indicate that FC and CB of 4 month old rats are more susceptible to oxidative damage caused by O3 when compared to those from 12 month and 24 month old rats. (This abstract does not necessarily reflect USEPA policy).

Determining Adaptive and Adverse Oxidative Stress Responses in Human Bronchial Epithelial Cells Exposed to Zinc

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Zinc is a ubiquitous contaminant of ambient air that presents an oxidant challenge to the human lung in environmental settings, which has been linked to various adverse health effects. Here, we further elucidate both the adaptive and adverse cellular responses of a normal human bronchial epithelial cell line (BEAS-2B) to zinc exposure in vitro. BEAS-2B cells were exposed to Zn2+ with 1 μM pyrithione, an ionophore that facilitates cellular uptake, for up to 48 h. Cytotoxicity was characterized by examining ATP levels with the CellTiter-Glo assay. Additionally, intracellular levels of Bak and Lamin B, markers of apoptosis, were quantitatively determined. Zn2+ exposure elicited a dose and time-dependent reduction in ATP levels compared with unexposed cells. After 24 h, markedly reduced viability was observed in cells treated with 5 (71.3%) and 7 (92.4%) μM Zn2+, but not cells exposed to 1 (88.2%) or 3 (94.2%) μM Zn2+ when compared with unexposed cells. Viability was further reduced in cells exposed to 3 μM Zn2+ after 48 h to 31±5%, however, cells exposed to 1 μM Zn2+ were not affected. After 24 hours, Bak and Lamin B levels increased from 17 and 10 ng/mL, respectively, in control cells to 38 and 25 ng/mL in cells exposed to 3 μM Zn2+. These levels increased further after 40 h to 67 and 30 ng/mL, but decreased to 49 and 26 ng/mL after 48 h of exposure. In agreement with the Bak and Lamin B levels, a portion of the cells treated with 3 μM appeared to recover. Visual inspection indicated that cells returned to a flattened, attached morphology between 40 and 48 h. These data suggest that the switch between adaptation and apoptosis in our model begins to occur at exposures of approximately 3 μM Zn2+ and as early as 24 h after exposure. Future work will determine the genomic response that mediates this switch, including the characterization of NRF2 and p53 pathway activation. This abstract does not necessarily reflect the policy of the US EPA.

An Imaging-Based RNAi Screen Identifies Novel Regulators of Nrf2 Activation

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The Nrf2 pathway is a key mechanism in protecting cells against Reactive Oxygen Species (ROS)-mediated toxicity. The activity of Nrf2 is controlled through KEAP1-mediated ubiquitination and subsequent proteasomal degradation. Accumulated Nrf2 translocates to the nucleus targeting the expression of a multitude of anti-oxidant genes, including Srxn1, HMOX1 and NQO1. To unravel the entire KEAP1/Nrf2/Srxn1 signaling pathway, we combine live cell imaging with GFP-tagging of individual pathway components. Using high throughput confocal imaging, we have quantified the concentration time course dynamics of Nrf2 pathway activation by, amongst others, CDDO-me. To provide full understanding of the upstream regulators of Nrf2, we applied a Dharmacon Smartpool siRNA-based knock-down screen using the Srxn1-GFP reporter as a downstream readout of Nrf2 pathway activation. We screened all individual kinases, phosphatases, ubiquitinases and transcription factors for their involvement in Nrf2 activation, a total of ~3300 candidates. After knock-down, CDDO-me was used to activate Nrf2 followed by quantification of Srxn1-GFP expression using automated high throughput imaging and analysis. Candidate Nrf2 pathway regulators that upon KD enhanced (58 genes) or inhibit (19 genes) the CDDO-me-induced Srxn1 upregulation were validated with single siRNAs. These hits were further validated with additional compounds which are known to activate the Nrf2 pathway: diethylmaleate, acetaminophen and diclofenac. Furthermore, most of the candidate genes were confirmed using two other independent GFP-BAC reporters for Nrf2 activation: NQO1-GFP and HMOX1-GFP. Based on these results 10 activating and 10 inhibiting hits were chosen to be studied in more mechanistic detail. Candidate hits included BRD4 and XRRA which have previously been associated with Nrf2 regulation. Interestingly, some of the novel hits also directly affected Nrf2 stability. This work contributes to more elaborate understanding of the signaling networks that control Nrf2 signaling in the context of xenobiotic-induced cytotoxicities.
Oxidative Stress Alters Global Histone Methylation and Acetylation

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The JmJc-domain-containing histone demethylases (JHDMs) can remove histone lysine-methylation in proximal promoter regions and thereby regulate gene expression. The JmJc-domain of the TET protein and histone demethylases uses iron (Fe(II)) and α-ketoglutarate (tKg) as cofactors in an oxidative demethylation reaction that produces hydroxymethyl-lysine and demethylates of various methylated lysines mostly on Histone H3. We hypothesize that reactive oxygen species will oxidize Fe(II) to Fe(III), thereby attenuating the activity of JmJc-domain-containing dioxygenases. To minimize secondary responses from cells, extremely short oxidative stress period (3 hours) were utilized to answer this question. Cells exposed to hydrogen peroxide (H2O2) for 3 hours exhibited increases in several histone methylation marks including H3K4me3 and decreases of histone acetylation marks including H3K9ac and H4K8ac pretreatment of ascorbic acid reduced these alterations. The oxidative stress level was measured by generation of 2',7'-dichlorofluorescein (DCF) and GSH/GSSG ratio. A cell free system indicated H2O2 inhibited histone demethylase activity while increased Fe(II) rescued this inhibition. Cells exposed to a low dose and long term (3 weeks) also showed increased global levels of H3K4me3 and H3K27me3. However, these global methylation alterations were not sustained after 3 to 6 days washout. The cells exposed to short term oxidative stress also appeared to have higher activity of class II histone deacetylase (HDAC) instead of class I HDAC. In conclusion, we have found that short term oxidative stress affects global levels of several histone marks through modulating the activities of their modifiers.

Potential Role of NQO1 As a Redox-Sensitive Molecular Switch

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NAD(P)H quinone oxidoreductase 1 (NQO1) has been considered a xenobiotic metabolizing enzyme as well as an anti-oxidant enzyme for its ability to reduce quinones directly to hydroquinones, detoxify superoxide and generate reduced pools of lipophilic antioxidants. In recent years, however, NQO1 has been shown to play a role in the stability of many proteins including p53, cFos and PGC-1α. The mechanism whereby NQO1 modulates the stability of these proteins appears to occur through protein–protein interactions leading to protection from 205 protein-protein interaction.

A key feature in the interaction of NQO1 with the target proteins was the ability to modulate the binding of NQO1 depending upon intracellular levels of NAD(P)H or following treatment with the NQO1 inhibitor dicumarol. In support of these data, we observed that NQO1 undergoes a conformational change upon binding of NAD(P)H or dicumarol leading to loss of immunoreactivity to antibodies targeting the C-terminus of NQO1. In cell-free experiments with purified rhNQO1, antibodies targeting the C-terminus of NQO1 could efficiently pull-down rhNQO1 in the oxidized state, but after the addition of NAD(P)H or dicumarol, no NQO1 could be immunoprecipitated. Similarly, when cells were subjected to more oxidizing conditions greater amounts of NQO1 could be immunoprecipitated by antibodies targeting the C-terminus of NQO1. The treatment of cells with β-lapachone, which has been shown to cause rapid oxidation of NAD(P)H, resulted in the pulldown of significantly greater amounts
of NQO1 when compared to nontreated cells. Immunochemical studies using antibodies targeting the C-terminus also showed more intense staining for NQO1 following treatments with β-lapachone when compared to nontreated cells. These data suggest that in cells NQO1 exists primarily in the reduced state, however, following oxidative stress which depletes NAD(P)H greater amounts of NQO1 are in the non-reduced conformation. These data support a role for NQO1 as a redox sensitive molecular switch that may alter its protein conformation depending upon the redox state of the cell.

1025 Molecular Mechanisms of All-Trans-Retinoic Acid-Mediated Selective Cytoprotection against Renal Injury

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Chemical-induced nephrotoxicity is a major cause of acute kidney injury. Pretreatment of LLC-PK 1 cells with all-trans-retinoic acid (ATRA, 25 μM, 24 hr) affords cytoprotection against p-aminophenol (PAP), iodoacetamide (IDAM) and 2-(glutathion-S-yl)hydroquinone (MGHQ)-induced necrosis. In contrast, pretreatment of cells with ATRA provides no protection against cisplatin-induced apoptosis. Oxidative stress is a major contributor to cellular damage. To investigate the mechanism by which ATRA affords cytoprotection, we determined its effects on ROS generation using a DCFDA assay. ATRA did not alter PAP or MGHQ-induced ROS levels. In contrast, N-acetylcysteine (10 mM) completely abolished ROS generation and concomitantly increased cell viability following PAP and IDAM treatment. Moreover, ATRA had no effect on Nrf2 protein expression levels. These findings suggest that ATRA protection occurs independent of ROS alterations. Elevated ROS disrupt endoplasmic reticulum protein folding guided by the molecular chaperone Grp78. Grp78 is a pivotal contributor to the adaptive response to oxidative stress. During ATRA-mediated cytoprotection, Grp78 was inactivated in a time-dependent manner, with a 2-fold increase at 24 hr. Moreover, significant induction of Grp78 was evident at a low dose of ATRA treatment (1 μM). Further supporting a role for the unfolded protein protective response, ATRA increased p-eIF2α 2-fold at 4 hr. Thus, signaling proteins downstream of ROS production (i.e., Grp78; eIF2α) might be critical in the biological mechanism of ATRA-mediated protection. An additional mechanism of ATRA protection toward IDAM appears to be a direct interaction between ATRA and IDAM, as a similar level of protection was found with or without ATRA pretreatment. However, this interaction/selection is protective for IDAM and is not involved in protection against PAP. Further investigations are ongoing. Thus, ATRA and/or analogs thereof, may serve as an effective therapeutic intervention in acute renal injury (ES006094, T32ES007091, ES016578).

1026 Plant Extracts from Nordic Fruits and Vegetables Activate a Nuclear Factor-Erythroid 2-Related Factor (Nrf2) Pathway and Exert Protective Effects on Human Cells In Vitro

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Phytochemicals in fruit and vegetables have been shown to be antioxidants and a high intake is associated with prevention of certain diseases. Emerging evidence support a novel view on antioxidants via a biological mechanism to stimulate the cellular defense system for oxidative stress through release of detoxifying enzymes. The nuclear factor erythroid 2-related factor 2 (Nrf2) is a key regulator in the cellular defense against oxidants. We have investigated if polar and non-polar extracts of Nordic fruits and vegetables of a wide variety of species (strawberry, raspberry, blackberry, blueberry, carrot, elderberry, blackthorn, hawthorn, sea-buckthorn, broccoli, apples, and rose hips) can act as biological antioxidants. To investigate the potential of each extract to activate the Nrf2 pathway, human MCF-7 and RWPE cells were transfected with a reporter plasmid where the expression of luciferase is under the control of a promoter containing Nrf2 responsive elements. The transduced cells were then treated with the extracts. We found that a large number of the extracts were able to increase the Nrf2 activity in the cells. Further, we investigated if the extracts exerted protective effects on cells exposed to oxidative stress induced by hydrogen peroxide. Pretreatment with several extracts was found to protect MCF-7 and RWPE cells from cytotoxicity induced by hydrogen peroxide. In conclusion, we have shown that extracts from Nordic fruits and vegetables are able to induce the Nrf2 pathway, indicating that they act as biological antioxidants. We have also shown that the extracts contain substances that prevent the cytotoxicity induced by hydrogen peroxide. These findings are important for the understanding of biological effects of antioxidants and human health effects of consumption of fruits and vegetables.

1027 Oxidative Stress Response in Samples from Surface Water and a Drinking Water Treatment Plant

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Water may be contaminated by e.g. pesticides, pharmaceuticals and other chemicals emitted from industrial/urban activities and consumer products. Oxidative stress response is a sensitive indicator of toxicity, responding to a wide variety of chemicals. The nuclear factor erythroid 2-related factor 2 (Nrf2) is a key regulator in the cellular defense against oxidative stress through release of detoxifying enzymes. To investigate the potential of water samples to activate the Nrf2 pathway, human NCI-H295R cells were transfected with a reporter plasmid where the expression of luciferase is under the control of a promoter containing Nrf2 responsive elements. Water was sampled from the entire water chain; upstream of rural areas, water streams in areas with heavy agricultural activity, downstream of a sewage treatment plant, lake water, a drinking water treatment plant intake, different steps in the drinking water treatment plant, and consumer tap water using both passive and active sampling. The water samples were extracted, concentrated and then analyzed for cytotoxicity by MTS test and oxidative stress response in the transfected NCI-H295R cells. Surface water samples from agricultural areas were analyzed for a total of 131 pesticides, with 87 of these being detected. No cytotoxic effects were revealed, but a large number of the samples exerted oxidative stress, even in the samples where all the detected pesticides were below the water quality standards as defined by the Swedish Chemicals Agency. In the drinking water samples, there was a tendency of higher oxidative stress response in the final steps of water treatment compared to the raw water. In conclusion, we have established a reporter gene assay to measure oxidative stress response in concentrated water samples and detected effects in samples not displaying general cytotoxicity.

1028 Mn Superoxide Dismutase (MnSOD) Attenuates Hypoxia-Induced Cell Death by Altering ERK Activation

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Supraphysiological concentrations of oxygen are often required to treat patients with respiratory failure. However, prolonged exposure to hypoxia results in severe lung injury and pulmonary cell death. Hypoxia-induced lung injury is mediated by overwhelming generation of reactive oxygen species (ROS) in mitochondria, leading to the damaged structures and functions of pulmonary cells. MnSOD, which converts superoxide into less toxic hydrogen peroxide (H2O2), is a significant antioxidant enzyme in mitochondria. Here, we tested whether overexpressing MnSOD in human lung epithelial cells and human lung fibroblasts would protect them against oxygen toxicity. Both human lung epithelial cells (A549 cells) and human lung fibroblasts (HFF-1 cells) were cultured, were exposed to 95% O2 for up to three days. The percentage of live cells was determined by Trypan Blue Exclusion assay. Most epithelial cells and fibroblasts with overexpressed MnSOD in mitochondria survived after exposure to three days of hypoxia, while many cells expressing vector controls died. Accompanied with higher cell survival in hypoxia, human lung epithelial cells and fibroblasts over-expressing MnSOD have elevated levels of H2O2, analyzed using flow cytometry, and exhibited higher basal levels of ERK activation compared to the controls. On the other hand, the survival advantage was blunted when these cells treated with ERK inhibitor, PD98059. These data suggest that moderate overexpression of MnSOD in mitochondria significantly protects against hypoxia-induced cell death, perhaps via H2O2-induced ERK activation in response to hypoxia.

1029 Oxidative Stressors Induce Differential Cellular Responses between Human Primary and HaCaT Keratinocyte Cells

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Oxidative stress reflects the status of imbalance between production of reactive oxygen species (ROS) and the ability of the system to cope with ROS. Nuclear factor E2-related factor 2 (Nrf2) plays a critical role in ROS defense through regulation of a battery of genes coding antioxidant proteins and detoxification enzymes by binding the antioxidant response element (ARE) and initiating gene transcription. We studied the Nrf2-response pathway and determined doses of prototype chemicals (curcumin, hydrogen peroxide; H2O2) associated with sub-threshold,
adaptive and adverse oxidative cellular responses in two cell types: immortalized keratinocytes (HaCat) and primary neonatal keratinocytes (HEKn). Our goal is to determine the key biomarkers in the pathway and identify regions of safety for oxidative stressors. In HaCat cells, markers of ROS and adaptive antioxidant responses were used to identify the effects of these stressors. Prolonged use of chloramphenicol induced non-regenerative anemia. This study compared the effects of different antioxidative supplements on oxidative stress and anemia in broiler chickens. The anemia was corrected in all groups, and GGT values also increased significantly. Those birds that received Livestovit had the lowest GSH, H2O2, and MDA levels, indicating that HEKn cells are more sensitive to oxidative damage than HaCat cells, and may therefore be the more conservative in vitro model for oxidative stress response. In an attempt to advance the transition to in vitro toxicity testing described in the National Academies of Sciences report, “Toxicity Testing in the 21st Century (TT21C): A Vision and A Strategy”, these time- and dose-response data are being used to support development of a computational model for oxidative stress response and, ultimately, provide proof of concept in vitro based safety assessments.

1030 Prolonged Use of Chloramphenicol Induces Aplastic Anemia and Oxidative Stress in Broiler Chicken
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In an attempt to combat bacterial infections in domestic poultry production in developing countries, poultry producers use antibiotics without recourse to the effects they might have on the animals. We investigated the effects of prolonged use of Chloramphenicol in the broilers and the antioxidant potential of four commercially available antioxidants—multivitamin—Selcon Forte®, Stressroak®, Superliv® and Livestovit® and their activities compared to that of Vit C. Two hundred and ten-day-old broilers were purchased from a local hatchery and randomly divided into seven groups. Group A (control) received Chloramphenicol (12.5 mg/kg body weight) for the first six days while all the other groups received chloramphenicol for six weeks. Group B did not receive any antioxidant. Group C received Vitamin C, Group D Selcon Forte Group E, Stressroak Group F, Superliv while Group G received Livestovit in drinking water. All birds received the same broiler starter feed, ad libitum for six weeks and were dully vaccinated and given prophylactic coccidiosis medication. Blood samples were collected via the jugular vein after six weeks in three replicates for evaluation of haematological parameters and erythrocyte osmotic fragility. Plasma ALT, AST and GGT were also determined. Five birds were culled from each group for the determination of the total GSH, H2O2 and lipid peroxidation MDA in the liver, kidney and the cerebrum. Those birds that received Chloramphenicol alone presented with aplastic anemia, elevated erythrocyte osmotic fragility and evidences of liver damage seen as increased AST, ALT and GGT values. The levels of GSH, H2O2 and MDA also increased significantly in the Group compared to the control. The anemia was however corrected in those groups that received the antioxidants. The oxidative stress induced by chloramphenicol was ameliorated in those birds that received antioxidants. This study demonstrated that prolonged use of chloramphenicol induced non-regenerative anemia and oxidative stress, even at normal recommended dosage.

1031 Role of GDF15 (Growth and Differentiation Factor 15) in Pulmonary Oxygen Toxicity
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GDF15 (Growth and Differentiation factor 15) a secreted cytokine, is a direct target of p53, and is known to play a role in cell proliferation, apoptosis, and angiogenesis. GDF15 is a part of the in vivo gene expression signature of oxidative stress and has been shown to have anti-inflammatory, pro-angiogenic, and anti-apoptotic effects. The role of GDF15 in hyperoxic lung injury and repair is unknown. We tested the hypothesis that GDF15 will be induced in vivo and in vitro in a hyperoxic lung injury model and will play a crucial role in decreasing apoptosis and oxidative stress in vitro. Wild type (WT) (C57BL/6) mice (8-10 wk-old), were exposed to hyperoxia (FiO2=95%). GDF15 expression was quantified in the lungs at the mRNA level at room air and after 48 hours of hyperoxia exposure. BEAS-2B (human bronchial epithelial cells) and human pulmonary vascular endothelial cells (HPMEC) were exposed to hyperoxia and expression of GDF15 measured at the mRNA and protein level. Using siRNA, we achieved knockdown of GDF15 and we measured the effect on cell viability, oxidative stress and apoptosis. There was a significant induction in GDF15 expression in vitro, in the lungs after 48 h of hyperoxia exposure. In vitro, both BEAS-2B and HPMEC cells showed a significant increase in GDF15 expression both at the mRNA and protein level. After siRNA mediated GDF15 knockdown, there was a significant decrease in cell viability, increase in oxidative stress and apoptosis compared to control cells transfected with siRNA with a scrambled sequence. Thus, we show for the first time, the induction of GDF15 in a hyperoxic lung injury model both in vivo and in vitro, and demonstrate increased susceptibility of BEAS-2B and HPMEC cells under hyperoxic conditions when GDF15 is silenced. This shows that GDF15 plays a crucial role in maintaining cell viability and decreasing oxidative stress in this model. Further studies to elucidate the mechanistic role of GDF15 in the modulation of hyperoxic injury could lead to the development of novel strategies to prevent or treat acute lung injury in humans.

1032 C66 Ameliorates Diabetic Nephropathy by Upregulating Nrf2 Function via Enhancing miR-200a and Downregulating miR-21 via CBP Inhibiting Activity
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Oxidative stress contributes to the pathogenesis of diabetic nephropathy (DN). Nuclear factor (erythroid-derived 2)-like 2 (Nrf2) plays a central role in antioxidant-redox signaling. Previously we found the prevention of DN by a novel curcumin analog C66 with up-regulation of Nrf2. Here we tested streptozocin (STZ)-induced diabetic Nrf2-null and their wild type mice with C66, showing that Nrf2 deletion partially abolished the renal protection by C66, indicating the partial dependence of Nrf2 for C66 renal prevention from diabetes. To define how C66 induces Nrf2, we tested our hypothesis that C66 induces Nrf2 via up-regulating miR-200a by treating diabetic mice with C66 in the presence of LNA-anti-miR-200a (LNA-200a), an in vivo miR-200a inhibitor, for 6 months. Diabetic mice, but not C66-treated diabetic mice, developed significant albuminuria, renal oxidative damage and fibrosis. C66 up-regulated miR-200a to inhibit Kelch-like ECH-associated protein 1 (KEEP1), resulting in the induction of Nrf2, effects of which were reversed by LNA-200a along with partial attenuation of DN. Following studies focused on additional protection by C66. In support of previous reports that curcumin down-regulated miR-21 and miR-21 accelerated DN, we showed here that diabetes-induced miR-21 expression was decreased by C66. To define whether down-regulated miR-21 by C66 plays a protective role on DN besides Nrf2 induction, diabetic Nrf2-null mice were treated with either C66 or LNA-200a, both of which decreased miR-21 with the attenuation in albuminuria and fibrosis. In line with the fact that curcumin is a histone acetylase (HAT) inhibitor, our mechanistic study further revealed that C66 inhibited the activity of HAT with decreased occupancy of CREB binding protein (CBP) that belongs to HAT and transcriptional factor p-Smad3 at the promoter of miR-21. Thus the present studies indicate for the first time that C66 ameliorates DN by up-regulating Nrf2 function via enhancing miR-200a and down-regulating miR-21 via CBP inhibiting activity.

1033 Unique Natural Product Compounds Activate Nrf2 and Autophagy
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Nuclear factor (E2) related factor 2 (Nrf2) is a critical transcription factor that regulates a battery of genes responsible for maintaining ideal redox homeostasis. While kept at low basal levels through Keap1-Cull-C-Rbx-1-mediated ubiquitylation and subsequent proteosomal degradation, Nrf2 activation has proven to diminish oxidative stress, a key feature in the pathological progression of many diseases, including neurodegenerative diseases such as Parkinsons disease (PD) and Huntington disease (HD). Because Keap1 is redox sensitive, a multitude of Nrf2 inducers are highly electrophilic and possess many off target effects, leading to protein dysfunction and cellular toxicity. This notion calls for highly selective Nrf2 inducers capable of avoiding covalent modification of Keap1 in order to achieve Nrf2 induction. An ARE-luciferase cell-based reporter screen to detect such molecules yielded the diterpenoid, geopyxin A, from a fungal extract. Further analysis of geopyxin A derivatives revealed molecules capable of Nrf2 induction via covalent or non-covalent modification of Keap1. In addition to the Nrf2 inducing effects, three small geopyxin derivatives were found to induce autophagy, a bulk degradation process, through an unknown mechanism. Recently, autophagy has emerged as a therapeutic target in order to enhance the clearance of mutant protein species, such as (A53T) α-synuclein and mutant huntingtin, which have strong aggregation potential. Because aggregation of mutant proteins resulting in cytotoxicity is a hallmark of PD and HD, these molecules are currently being tested in order to assess their neuroprotective effects against (A53T) α-synuclein and mutant huntingtin toxicity.
1034 ATM-14, a Novel Antioxidant Radio-Mitigator Targeting Mitochondria
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ATM-14 and SMA-1 (mitochondria-protecting antioxidants) were developed by our team and the cytotoxic and proliferative effects were compared to Mito-tempo (known antioxidant) in three different cell lines using MTS assay. Cytotoxic effects were evaluated in a lung carcinoma (A549) cell line. ATM-14 demonstrated enhanced cytotoxic effects at 100 nM dose at 48 hours incubation as compared to SMA-1 and Mito-tempo. The proliferative effect was performed in Human Fibroblast cell lines (PCS 201-012); these results exemplified that ATM-14 stimulated cell proliferation at 100 nM at 48 hours incubation as compared to SMA-1 and Mito-tempo. Primary jejunal epithelial cells were isolated from small intestine tissue of rhesus macaques. Cells were irradiated at 6 Gy using a Cs 137 irradiator. The cells were divided into three groups: Non-Irradiated Control (C), Irradiated (IR) and Irradiated plus drug (IRD) groups, Post irradiation cells were kept in an incubator for twenty four hours. Twenty four hours after irradiation, the ATM-14 (100 nM) drug was added to the IRD group and incubated with the drug for forty eight hours. A Cell Viability test was performed using trypan blue in primary small intestinal epithelial cells. There was a significant difference in the cell density and cell viability between the C, IR and IRG groups. Cells in IRG groups were much smaller and with a greater amount of debris/fibrin compared to C and IRD groups. Cell count was 40% of the control in the IR group but was increased to 60% of control levels in the drug-dosed (IRD) dose group. These results confirm ATM-14 at 100 nm as a potent radio-mitigator in the primary small intestine epithelial cell line after 48 hours of incubation and its potential selective cytotoxicity for cancer cells but not for primary cells when compared to SMA-1 and Mito-tempo. ATM-14 will be further tested in clonogenic assay and in vivo mouse models.

1035 Protective Role of Methionyl Dipeptide against Hypochlorous Acid Toxicity Depends on Sequence
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Hypochlorous acid (HOCl) is a powerful oxidant and its toxicity is predominantly through the oxidation of proteins and peptides. Oxidation of methionine is related to many protein misfolding diseases such as Alzheimer’s and Parkinson’s. In order to understand the role of methionyl peptide sequence in oxidation we used three dipeptides containing either methionine or alanine (Met-Ala, Ala-Met and Met-Met). All the reactions were performed with 0 to 2 μmol of hypochlorous acid in 2.0 mL of 0.1 M phosphate buffer, pH 7.0, and 0.1 mM DTPA, while keeping the concentration of methionyl peptide (0.5 μmol) constant. The reactions were monitored from 200-800 nm wavelength using a Varian Cary 50 Bio UV/Vis spectrophotometer. All the methionyl dipeptides showed a strong absorption between 200-210 nm. However, only Met-Met was oxidized in the presence of HOCl. The absorption at 205 nm was found to decrease with increasing concentration of oxidant. The amount of oxidation was 5.1% (±2.8%), 12.9% (±0.9%) and 13.3 % (±5.6%) for 0.5, 1 and 2 μmol of oxidant, respectively. The results suggest that the oxidation of methionyl methionine can occur even at lower doses than the physiological concentrations of HOCl (25 molar), and necrotic cell death. During TGHQ-mediated cell death, PARP-1 hyperactivation and elevations in iCa2+ are reciprocally coupled, amplifying cell death. PARP-1 hyperactivation increases intracellular levels of poly(ADP-ribose) (PAR). Additionally, siRNA knockdown of poly(ADP-ribose) glycohydrolase (PARG), a protein responsible for the breakdown of PAR polymers to ADPR monomers, does not protect against TGHQ-induced cell death in HK-2 cells. Thus, increases in iCa2+ appear independent of PARG metabolism, suggesting PAR plays a role as a cell death signal. We speculate that PARylation of cytotoxic proteins triggers downstream signaling, activating store-operated Ca2+ entry, ultimately increasing iCa2+ concentrations, and leading to PARP-1-dependent cell death. To further elucidate the role of PARylated proteins in cell death, we identified PAR-proteins following exposure of HK-2 cells to TGHQ. We immunoprecipitated using a pADPR antibody, and resolved the PARylated proteins by SDS-PAGE. PARylated proteins were identified in vitro using an in vitro PARP1 IRD assay where protein identification was accepted at ≥95.0% probability and with at least two identified peptides. Using this proteomics-based approach, we identified PARylated proteins that were solely present in TGHQ-treated samples. Of particular interest, we identified several Ca-related proteins containing the pADPR-binding motif, including the Sarcoplasmic/Endoplasmic Reticulum Calcium ATPase (SERCA) pump, a subunit of KIAA1967, and several isoforms of heat shock protein (HSP)90. Currently, we are investigating the possible role of PARylated proteins in Ca-signaling, and their coupling to PARP-1 hyperactivation in necrotic cell death.

1036 Mutated in Colorectal Cancer (MCC) Interacts with Keap1 and Activates Nrf2 Signaling
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Mutated in colorectal cancer (MCC) is a protein that was originally thought to be the cause of familial adenomatous polyposis (FAP) since it is frequently methylated in colorectal tumors and is in close proximity to the adenomatous polyposis coli (APC) gene. Nevertheless, discovery of APC as the main contributor to APC devi-

1037 Identification of Poly(ADP-Ribose)-Associated Proteins in Response to ROS Stress
2,3,5-Tris-(glutathion-S-yl) hydroquinone (TGHQ) is a nephrotoxic and nephrocarcinogenic metabolite of hydroquinone. TGHQ generates reactive oxygen species (ROS), which cause DNA strand breaks, leading to hyperactivation of poly(ADP-ribose) polymerase (PARP)-1, increases in intracellular calcium concentrations (iCa2+), and necrotic cell death. During TGHQ-mediated cell death, PARP-1 hyperactivation and elevations in iCa2+ are reciprocally coupled, amplifying cell death. PARP-1 hyperactivation increases intracellular levels of poly(ADP-ribose) (PAR). Additionally, siRNA knockdown of poly(ADP-ribose) glycohydrolase (PARG), a protein responsible for the breakdown of PAR polymers to ADPR monomers, does not protect against TGHQ-induced cell death in HK-2 cells. Thus, increases in iCa2+ appear independent of PARG metabolism, suggesting PAR plays a role as a cell death signal. We speculate that PARylation of cytotoxic proteins triggers downstream signaling, activating store-operated Ca2+ entry, ultimately increasing iCa2+ concentrations, and leading to PARP-1-dependent cell death. To further elucidate the role of PARylated proteins in cell death, we identified PAR-proteins following exposure of HK-2 cells to TGHQ. We immunoprecipitated using a pADPR antibody, and resolved the PARylated proteins by SDS-PAGE. PARylated proteins were identified in vitro using an in vitro PARP1 IRD assay where protein identification was accepted at ≥95.0% probability and with at least two identified peptides. Using this proteomics-based approach, we identified PARylated proteins that were solely present in TGHQ-treated samples. Of particular interest, we identified several Ca-related proteins containing the pADPR-binding motif, including the Sarcoplasmic/Endoplasmic Reticulum Calcium ATPase (SERCA) pump, a subunit of KIAA1967, and several isoforms of heat shock protein (HSP)90. Currently, we are investigating the possible role of PARylated proteins in Ca-signaling, and their coupling to PARP-1 hyperactivation in necrotic cell death.

1038 Vitamin A (VA) Prevents Oxygen-Induced Retinopathy (OIR) in Newborn Rats via Transcriptional Regulation of VEGF-A and HIF-1alpha (HIF-1α)
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Oxygen administration is used for the treatment of preterm infants with respira-
tory distress, and contributes to the development of retinopathy of prematurity (ROP). The purpose of this study was to test the hypotheses that exposure of newborn rats to a combination of VA and hyperoxia would (i) alleviate OIR with abnormal neovascularization compared to those exposed to hyperoxia alone and (ii) these effects would be via the induction of VEGF-A and HIF-1α. Newborn Fisher 344 rats were maintained in room air or exposed to hyperoxia (95% O2) for 7 days. Some animals were treated i.p. with VA (2 mg/kg) or corn oil (CO), once daily for the first 5 days of hyperoxia. Animals were sacrificed at selected time points after termination of hyperoxia. Retinal vascular densities were assessed. Chromatin Immunoprecipitation (ChiP) assays were performed using 6 retinas in each group from 3 different rats with anti-RAR alpha (RAR-α) antibody. The DNA fragment that corresponded to the RAR-α binding site was quantified by qPCR, using primers for the HIF-1α or VEGF-A promoter regions. Seven to 30 days after termination of hyperoxia, the animals displayed formation of abnormal retinal vessels and large avascular areas only in the hyperoxia group. Animals given VA+hyperoxia showed significantly less abnormal neovascularization compared to those exposed to hyperoxia alone and VEGF-A promoter regions. Seven to 30 days after termination of hyperoxia, the animals displayed formation of abnormal retinal vessels and large avascular areas only in the hyperoxia group. Animals given VA+hyperoxia showed significantly less abnormal neovascularization. The i.p. administration of VA upregulated the transcriptional activation of HIF-1α and VEGF promoter via the RAR-α response elements, and prevented the abnormal retinal neovascularization at the later time points. Our study supports the hypotheses that VA protects retinas from OIR with abnormal neovascularization, and that

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augmentation of the transcriptional activation of VEGF-A and HIF-1α promoters contributes to the retinoprotective effects of VA. These results encourage development of further studies to elucidate molecular mechanisms of VA action and clinical trials in infants at risk of developing ROP.

**1039 Impaired Nrf2 Signaling and Mitochondrial Dysfunction Mediate Hypoglycemia-Induced Oxidative Stress and Toxicity at Blood-Brain Barrier Endothelium**

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Oxidative stress-induced endothelial cell toxicity is central to the pathophysiology of hypoglycemia-associated cerebrovascular complications including loss of blood-brain barrier (BBB) function and integrity. However, molecular mechanisms underlying hypoglycemia-induced endothelial oxidative stress are little understood. Given the evidence that Nrf2 is a master regulator of various cytoprotective genes implicated in anti-oxidant defense and mitochondrial bioenergetics, we investigated the role of Nrf2 in regulation of BBB endothelial function/integrity and its responses to hypoglycemia, using hCMEC/D3 cell line (established in vitro model of human BBB). Confluent monolayers of D3 cells were exposed to normal or hypoglycemic media (5.5 or 2.2 mM D-glucose, respectively) for 3-24 h. BBB integrity was assessed by labeled dextran permeability (4-70 kDa). Gene silencing was performed by specific siRNA transfection. Nrf2 knockdown induced a significant downregulation/re-distribution of functional proteins (claudin-5, ZO-1 and VE-cadherin) at cell-cell contacts, reduced glucose uptake (~30%) and significantly increased permeability to all dextrans (160-180% of control). Interestingly, Nrf2 knockdown significantly suppressed mitochondrial transporters ABCB8 and B10 (implicated in mitochondrial homeostasis and ROS suppression). Moreover, Nrf2 or ABCB10 knockdown potentiated monocyte-endothelial interactions, indicating oxidative stress-induced endothelial activation. Notably, hypoglycemia (3-24 h) induced progressive downregulation of Nrf2 (without affecting mRNAs) and its target, NQO-1. ABCB10 expression was also significantly suppressed with a parallel increase in mitochondrial ROS, following 12 h hypoglycemia. Overall, this study suggests that Nrf2 is critical for normal BBB endothelial function. We provide a novel mechanistic insight in which Nrf2 regulates mitochondrial ABC transporter expression. Importantly, Nrf2 suppression potentiates hypoglycemia-induced oxidative stress at BBB endothelium, possibly through mitochondrial dysfunction.

**1040 PFOA Activates the Unfolded Protein Response in Pancreatic Acinar Cells**

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Perfluoroalkyl and polyfluoroalkyl substances (PFASs), such as perfluorooctanoic acid (PFOA), are widely used in consumer and industrial applications due to their unique hydrophobic properties. PFOA does not readily decompose in the environment and detectable levels are found in 98% of the American population. Human epidemiologic and animal studies suggest that PFOA exposure elicits adverse effects of PFOA in this organ. We have previously shown that exposure of mice to PFOA for 7 days triggers oxidative stress in the pancreas, which is associated with focal ductal hyperplasia, inflammation, and increased pancreatic enzyme secretion. Increased secretion of pancreatic enzymes by the acinar cells of the exocrine pancreas can trigger the unfolded protein response (UPR) leading to endoplasmic reticulum (ER) stress. The goal of this study was to investigate whether the oxidative stress observed in the mouse pancreas following PFOA exposure is mediated by ER stress. Using the mouse acinar 266-6 cell line, we observed that PFOA treatment resulted in a time and dose-dependent phosphorylation of eIF2α that led to upregulation of the mRNA and protein levels of ATF4 and C/EBP homologous protein (CHOP). Activation of the ER stress response by PFOA was blocked by pretreatment with the chemical chaperone 4-phenyl butyrate, but not the general antioxidant N-acetyl cysteine. We also observed that PFOA treatment led to an increase in cytosolic Ca2+ levels, similar to the long chain fatty acid palmitoleic acid which is known to trigger ER stress. mRNA levels for CHOP were increased in the pancreas of mice exposed to PFOA for 7 days, indicating that ER stress also occurs in vivo. Our findings demonstrate that PFOA activates the double stranded RNA-dependent protein kinase-like endoplasmic reticulum kinase (PERK)-eIF2α-ATF4 arm of the ER stress pathway, both in vitro and in vivo, and may be the trigger for oxidative stress observed following PFOA exposure.

**1041 Role of Plasma Antioxidants for Assessing the Response to Oxidative Stress by Systemic Inflammation**

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Exploration of the role of antioxidants in inflammation-mediated oxidative stress is a field of ever-increasing attention, both in science and in commerce. One common approach used in assessing the systemic inflammatory response and oxidative stress is to measure the decreases of endogenous antioxidants. The goal of the present study was to test whether acutely exposing Göttingen mini pigs to the endotoxin lipopolysaccharide (LPS) results in a loss of antioxidants from plasma. We set as a criterion that a significant effect should be measured in plasma and seen at both doses and at more than one time point. Animals were injected with two doses of LPS at 2.5- and 5 μg/kg i.v. Control blood/plasma samples were collected from each animal before the LPS injection. Experimental samples were collected at 2 h, 16 h, 48 h and 72 h post-LPS dosing. Compared with the controls, statistically significant losses were not found for either dose at multiple time points in any of the following antioxidants: ascorbic acid, tocopherols (α, δ, γ), ratios of GSH/GSGG, cysteine/cysteine (Cys/CySS), mixed disulfides and total antioxidant capacity. However, uric acid, total GSH, and total Cys were significantly increased, probably because LPS had harmful effect on liver. Indeed, our histopathology findings and other studies have demonstrated that LPS induces apoptosis and massive necrosis in liver tissue. The leakage of substances from damaged cells into the plasma may have increased plasma antioxidants concentrations making changes difficult to detect. The experimental porcine model is certainly a better model of inflammation than rodent models since the porcine physiology and organs are very similar to humans and have been successfully used in earlier studies to evaluate systemic inflammation and oxidative stress. We conclude that measurements of antioxidants in plasma are not sensitive markers for oxidative damage induced by inflammation and are not the right choice for the assessment of oxidative damage in vivo.

**1042 Cerium Dioxide Nanoparticles: Pro- or Antioxidant Potential In Vivo?**

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Cerium dioxide nanoparticles (CeO2) are being developed as an anti-oxidant that may protect against tissue damage from stroke and radiation treatments by reacting with elevated reactive oxygen species (ROS). However, the current literature suggests that CeO2 may be both pro- and anti-oxidant. Based on previous observations, we predict that in a high ROS environment, CeO2 will act as an anti-oxidant and may improve microvascular function by decreasing the overall ROS level. Spontaneously hypertensive rats (SHR) were selected as a model of high ROS because it has been established that in this model, increased ROS levels negatively affects microvascular function via nitric oxide synthase (NOS) uncoupling and NO scavenging. SHR were intravenously injected with CeO2 (100 μg) suspended in saline and 5% fetal bovine serum. Mesenteric arteries were examined 24 h later via intravital microscopy. Endothelium-dependent dilation was evaluated with acetylcholine (Ach) that was iontophoretically applied to the arteries (20, 100 and 150 nA). Superoxide and hydrogen peroxide were scavenged in the presence of the superoxide dismutase mimetic 2,2,6,6-tetramethylpiperidin-N-oxyl (TEMPOL, 10-5M) and catalase (50 U/mL). The contribution of NO was evaluated during NOS inhibition with N G-monomethyl-L-arginine (L-NMMA, 10-5M). Finally, dihydroethidium (10-4M) staining was performed to visualize changes in local ROS production. The SHR responded minimally to Ach (12±11%, of maximal dilation). This response was partially restored during incubation with TEMPOL and catalase (44±6%); as well as incubation with L-NMMA (47±15%). Following CeO2 exposure, SHR microvascular function was significantly improved (33±6%). Endothelium-dependent dilation was further increased during TEMPOL and catalase incubation (47±9%), as well as with L-NMMA (48±11%). These results indicate that in a high ROS environment CeO2 may have anti-oxidant activity that improves microvascular function. R01-ES015022(TRN), NSF-IGERT(VCM), F32-ES023435(PAS).
1043 Exposure to 1, 2-Naphthoquinone Induces Protein Sulfenylation in Human Bronchial Epithelial Cells

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Oxidant stress is involved in the toxicity of many xenobiotics, including environmental electrophiles such as the diesel exhaust component 1,2-naphthoquinone (1,2-NQ). In addition to directly forming adducts with biomolecules, 1,2-NQ also participates in single electron redox reactions that generate H$_2$O$_2$. Sulfenylation, the H$_2$O$_2$-catalyzed oxidation of cysteinyl thiols (-SH) to the sulfenic (-SOH) derivative, is a pivotal regulatory posttranslational modification involved in signaling. We investigated whether 1,2-NQ-induced H$_2$O$_2$ promotes the formation of protein sulfenylation in BEAS-2B human bronchial epithelial cells. We utilized the genetically-encoded fluorogenic sensor HyPer to monitor H$_2$O$_2$ levels and determined that a 10 min exposure to 30 µM 1,2-NQ induced a robust increase in intracellular H$_2$O$_2$. Cells were treated with 0-1000 µM 1,2-NQ for 10 min and then labeled with dimedone, a small cell-permeable compound that specifically and irreversibly adducts cysteinyl sulfinic groups on proteins. Protein sulfenes were then detected in cell protein extracts by immunoblotting using an anti-body raised against 2-thiodimethone. BEAS-2B cells exposed to 1,2-NQ showed a dose-dependent increase in levels of sulfenylation for proteins ranging from 30 to 250 kDa. Overexpression of catalase effectively suppressed intracellular H$_2$O$_2$ concentrations and blunted 1,2-NQ-induced protein sulfenylation. To our knowledge, this is the first report of protein sulfenylation induced by exposure to an environmentally relevant oxidant. Furthermore, this work demonstrates the utility of protein sulfenylation as a functional marker of xenobiotic-induced oxidative stress. This abstract of a proposed presentation does not necessarily reflect EPA policy.

1044 Investigating Sulforaphane As a Protective Agent against Benzoquinone Toxicity in Mouse Fetal Liver Cells

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Benzoquinone is a ubiquitous environmental pollutant associated with childhood leukemia and lab studies have shown that it is an effective inducer of cell death between environment exposure and tumor incidence in CD-1 mouse offspring. While the mechanism of benzoquinone’s carcinogenicity has not been fully elucidated, an increase in ROS and increases in DNA damage have been implicated. Sulforaphane (SFN) has been shown to be a potent inducer of Nrf2, which is thought to contribute to SFN’s protective effects against carcinogenesis. Using benzoquinone (BQ), a reactive metabolite of benzoquinone, we used cultured CD-1 mouse fetal liver cells to investigate mechanisms of benzoquinone’s toxicity and whether SFN was protective in this model. Cultured cells were exposed to 0-25 µM BQ for 0.5-24 hours and flow cytometry was used to detect ROS levels. Separately, cells were exposed to 0-5 µM SFN for 2-24 hours, and Nrf2 protein expression was measured using qRT-PCR for downstream target genes. Subsequently, cells were treated with BQ (+/- SFN pretreatment) and levels of ROS, 8-OHdG (a marker of oxidative DNA damage; via colorimetric assay), and expression of DNA repair genes Brca1, Brc2a, Oggl, and Parp-1 were measured. Increases in ROS following 30 minute BQ exposure were not protected by pretreatment with SFN, despite induction of Nrf2 target gene expression following 24 hours of SFN exposure. Interestingly, while BQ treatment alone did not result in a significant increase in 8-OHdG levels, adding a 24 hour SFN pretreatment led to a significant increase in levels of 8-OHdG. Preliminary results show BQ exposure decreased DNA repair gene mRNA levels, which was not reversed by SFN treatment. While SFN was used in this experiment to examine its role as a protective agent against benzoquinone’s toxicity, in this study, SFN was not protective, and in some cases exacerbated the toxicity associated with BQ exposure.

1045 Persistent Antiretroviral Nucleoside Reverse Transcriptase Inhibitor (NRTI)-Induced Mitochondrial Compromise in Transplacently Exposed Patas Monkey Offspring


Mitochondrial dysfunction has been implicated as a potential contributor to developmental toxicity following transplacental exposure to antiretrovirals (ARVs). Recent data suggest that ARVs can induce cellular metabolic deficiencies in multiple species, including non-human primates (NHPs). Patas monkeys (Erythrocebus patas) are unique in that they are primates that can be virally transmitted with HIV-1. Here, we measured mitochondrial dysfunction in hearts collected at birth, 1 yr and 3 yr of age, compared to untreated controls (P<0.05 at each time point). In order to evaluate mitochondrial function, mesenchymal cells isolated from bone marrow of patas at birth, 1 yr and 3 yr were cultured and subjected to Seahorse analysis. Maximal oxygen consumption rate (OCR), observed under uncoupling conditions, was reduced in cells from 3 yr old patas exposed to AZT/3TC/NVP, compared to unexposed controls (P<0.05). However, 3 yr old patas infants exposed to AZT/3TC/NVP had no OCR alterations. Overall, based on findings in the patas, we conclude that when mitochondrial dysfunction is observed in children born to mothers receiving NRTI therapy it is not likely to be readily reversible. However, the data shown here also suggest that manifestations of mitochondrial damage may vary with the drug combination used.

1046 Microsampling in Juvenile Rats


Juvenile toxicity studies are a requirement by FDA and EMA, prior to performing clinical trials in pediatric populations. An essential part of each toxicity study is the assessment of the toxicokinetic behavior of a test compound. This requires repeated sampling of blood/plasma for bioanalysis, which is a limiting factor especially in juvenile rodents. In order to obtain a full pharmacokinetic plasma profile, many pups are necessary. Capillary Microsampling (CMS) is a technique for obtaining small amounts of blood (~30 µl per sample), thereby significantly reducing the number of animals that is needed for pharmacokinetic assessment. In this study, the usefulness of CMS for sampling pups was investigated, how much samples could be taken per animal, which route of sampling was most successful and what the quality of the samples was. Adult Wistar Han were weaned in-house and allowed to deliver their offspring for use in study. Blood samples were taken from pups at PND 4, 11 and 18, via three different routes: jugular vein, submandibular vein and lateral tail vein. Two samples per animal could be taken at PND 4, which is roughly 10% of the total blood volume; however, sampling via the jugular vein resulted in better quality samples and a higher percentage of success, compared to tail vein or submandibular sampling. For pups at PND 11 or 18, more samples could be drawn, up to 3 or 4 serial samples per animal. As for PND 4, the jugular vein was the preferred route of sampling. When the animals get older, the tail vein becomes an easier route of sampling. When the animals get older, the tail vein becomes an easier route of sampling. Overall, with capillary microsampling, it is possible to perform (limited) serial sampling in rat pups from PND 4 onwards; the jugular vein is the preferred route of sampling. Therefore, using CMS in juvenile studies significantly reduces the number of animals needed for kinetic assessment.

1047 The Postnatal Development and Growth of the Cardio-Respiratory System in Sprague-Dawley Rats

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The purpose of this study was to investigate the histomorphological changes of the cardio-respiratory system in rat pups over the first month of life. The heart weight and the heart weight relative to body weight ratio were calculated for 51 rats over 13 timeponts, ranging from postnatal day 1 (PND1) to PND30. For each timepoint, whenever possible, equal number of females and males were used. The aortic wall progressively increased in thickness throughout the duration of our study as a result of an accumulation of extracellular matrix (collagen and elastic fibers). Postnatally, the cardiac volume essentially increased by cardiomyocyte hypertrophy, which showed a more mature appearance around PND21. In the newborn myocardium, mitotic figures and apoptotic bodies were frequently seen, increased at PND21 and showed a diffuse pattern. At birth, the trachea was lined by immature columnar ciliated epithelial cells, and submucosal glands became visible around one week after birth. The newborn rat has no alveoli and breathes with smooth, large gas exchange units termed “primary sacules”. An intense interstitial cellular proliferation was observed in the lungs between PND6 and PND14, when the majority of alveoli are separated by new secondary septa, formed from the
primary walls. These changes were accompanied by few mitoses and/or individual apoptotic cells. The findings described herein suggest that the cardio-respiratory system of rats is immature at birth. The data generated will serve as background historical database that will be valuable when performing postnatal developmental toxicity studies.

1048 Histomorphologic Features of Neonatal and Juvenile Uro-genital Development in Sprague-Dawley Rats

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This study describes the key histomorphologic postnatal developmental events occurring in the uro-genital system of rat pups from birth to postnatal day 30 (PND30). Tissues were collected from 51 rats, using equal numbers from each sex whenever possible, at PND1, 2, 4, 6, 8, 10, 14, 17, 21, 24, 26, 28, and 30. Our study revealed that nephrogenesis in rats ceased by PND14, while few mitoses were still observed at the cortico-medullary junction at PND21. Individual cell death and mitoses were abundant at birth and followed a centriplotal pattern (from outer cortex to medulla), accompanying developing renal structures. At PND26, the ovary exhibited a sharply demarcated cortico-medullary junction. Between PND21-PND26, numerous apoptotic ova were present. The first uterine glands became visible around PND14, whereas scattered apoptotic epithelial cells were still present up to PND26. By PND24, the superficial layer of the vaginal epithelium was multifocally composed of large cells containing a mucinous material mixed with few apoptotic cells. At birth, the seminiferous tubular epithelium was 1 to 2 layers thick and composed of spermatogonia and Sertoli cells. A seminiferous lumen was rarely seen and the interstitium was abundant and hypocellular (<1049 Exposure to Particulate Matter Increases the DNA Methylation of Replicase Genes in Urban Children

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Particulate matter (PM) is a complex mixture with adsorbed compounds such as metals and polycyclic aromatic hydrocarbons (PAHs). PM10 exposure in the metropolitan area of Mexico City (MAMC) is frequently above the Mexican standard (65 µg/m³/24 h). Exposure to air pollutants is associated with genetic damage and alterations in DNA methylation. An increase in DNA methylation is associated with a repressed transcription, and a hypermethylation of DNA-repair genes can contribute to DNA damage. Children are susceptible to air pollutants since the risk of diseases increases if the exposure occurs at early age. A cross-sectional study was conducted in 150 schoolchildren (7-10 years) from MAMC, and PM10 and its metal and PAHs contents were determined by air sampling to evaluate their association with DNA methylation: global (LINE-1) and DNA-repair genes: OGG1, APEX, PARP1, XPA, XPC and ERCC1 in blood cells by pyrosequencing, and the DNA damage by the comet assay (OTM parameter). The geometric mean of OTM was 33.6, the mean PM10 concentration was 106.7 µg/m³/24 h, the metal content in PM10 was as follows: Fe>Zn>Mn>Pb>Cu>V and the most abundant PAHs were: indene[1,2,3-cd]pyrene (IcdP) and benzo[b]fluorantene (BbF). LINE-1 methylation was positively associated with PM10 (p<0.05) and the multielement analysis showed that IcdP was positively associated with the methylation in ERCC1 and XPA enzymes with XPA methylation (p<0.05), and V with APEX methylation. Additionally, the DNA damage was modified by the interaction between the DNA-repair gene methylation and PAHs exposure, suggesting that PAHs were the main modulators of the DNA damage. Therefore, DNA modifications may be an early step to develop diseases such as leukemia, an important cause of mortality in children from MAMC (Supported by CONACyT-Grant #151797).

1050 The Impact of Prenatal Parental Tobacco Smoking on Risk of Diabetes Mellitus in Middle-Aged Women

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The purpose of this study was to identify whether parental tobacco smoking during gestation influences risk of diabetes mellitus. This is a prospective study of 44 to 54 year old daughters (N = 1,801) born in the Child Health and Development Studies pregnancy cohort between 1959 and 1967. Their mothers resided near Oakland California, were members of the Kaiser Foundation Health Plan, and reported maternal smoking status during pregnancy. Tobacco smoking was ascertained up to an early pregnancy interview. Daughters reported physician diagnoses of diabetes mellitus and provided blood for hemoglobin A1C measurement. Prenatal maternal smoking had a stronger effect than prenatal paternal smoking on diabetes mellitus risk, and the former persisted after adjustment for paternal race, diabetes, and employment (aRR = 2.4 [95% confidence 1.4 - 4.1] p < 0.01 and an RR = 1.7 [95% confidence 1.0 – 3.0] p = 0.05, respectively). Estimates of the effect of prenatal smoking were unchanged when further adjusted by daughters’ birth weight or current BMI. Maternal smoking was also a significant predictor of self-reported type 2 diabetes diagnosis (2.3 [95% confidence 1.0 - 5.0] p < 0.05). Women with parents who smoked during pregnancy had increased risk of diabetes mellitus independent of known risk factors, providing further evidence that prenatal environmental chemical exposures independent of birth weight and current BMI can contribute to adult diabetes mellitus. While other studies seek to confirm our results, caution toward tobacco smoking by or proximal to pregnant women is warranted in diabetes mellitus prevention efforts.

1051 Juvenile Toxicity Testing in Nonhuman Primate Models: Challenges and Experiences

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Testing the safety of new medicines for childhood indications is meanwhile an integral component of drug development and an ICH-related guideline is currently under development. In the past years, the need for using nonhuman primate (NHP) models has been encountered. Whilst juvenile toxicity testing is comparatively standardized in rodent models, challenges can arise if neonatal or very young animals are required since transport of these animals without maternal animal presence is not permitted or very limited. This work reviews our experience from 15 juvenile toxicity studies using NHPs (14 studies in cynomolgus monkeys, one study in marmosets). Approximately a third of test items were biopharmaceuticals. Animal ages at dosing start ranged from one months to 2.5 years for cynomolgus monkey and 2-4 days for the marmosets. Study size varied from 3 to 50 animals per study and study duration ranged from 1 day to a 53 weeks in-life plus 26 weeks recovery study period. Routes of administration were oral, intravenous, subcutaneous and intrathecal dosing. In one study, some infants from an enhanced pre- and postnatal study were used for juvenile toxicity examination at six months. When using very young animals, no issues were encountered regarding survival and maternal tolerance. In general, all dosing routes were well tolerated. Study parameters generally included clinical signs, clinical pathology and assessment of general behavior. However, typically, study designs were purpose-tailored and included additional specific organ system evaluations such as adrenal and gonadal hormones, immune system (e.g immunophenotyping), central nervous system (e.g. learning and memory test), bone density and development, and in-vivo imaging (computed tomography, magnetic resonance imaging). In conclusion, the conduct of juvenile toxicity testing in NHPs is generally feasible but requires a number of specific considerations. Experimental protocols mainly reflect a case-by-case rather than a standardized approach.

1052 Clinical Chemistry Sampling and Assessment in Juvenile Rats—Reduction in and/or Elimination of the Need for Additional Subsets of Pups


Clinical chemistry parameters are routinely used for assessing toxicity in adult animals and are also used to assess function in juvenile animals. However, in juvenile animals there is a limit to the amount of blood that can be taken from each pup and for these studies multiple subsets of additional animals with terminal endpoints are required. In contrast, microsampling has helped in the reduction of the volume of blood samples required for TK analysis in juvenile studies (Powles-Gilmer et al.). To continue to reduce the number of animals needed for these studies, the sample volume required for clinical chemistry parameters was assessed but limited by the volume needed by the analyzer for assessment and the accuracy of the assay if the
sample is diluted. In view of this sufficient sample for a full clinical chemistry profile could not be obtained but a selection of key parameters was chosen to reflect clear changes over the lactation period (from neonate to weaning), assessed key target organs (heart, liver and kidney) and were parameters that were suitable for quantification of the following dilution. Those selected were urea, aspartate aminotransferase, alanine aminotransferase, total protein, albumin, globulin and AG-ratio. Electrolytes were also analysed in non-dilute samples. Samples were successfully collected from only 3-5 pups per group per sex (although more animals were sampled). The samples were collected from pups that had been dosed with water from postnatal day (PND) 5. Samples of 0.15 ml were collected from the jugular vein on PND 10, 0.30 ml was collected on PND 14 and 0.5 ml on PND 21. Body weight for these animals was also assessed. The initial results in a limited sample set demonstrate clear expected patterns over the time assessed. The numbers of animals used on juvenile studies could be reduced further if clinical pathology samples could be taken from main study animals via the jugular vein successfully, but further investigation of the sampling technique and the effects on other toxicological endpoints would be needed.

1053 Microsampling-Coupled Bioanalysis in the Neonatal and Juvenile Göttingen Minipig and Beagle Dog

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Microsampling-coupled bioanalytical techniques are becoming more established in pre-clinical studies, particularly for rodents, to try and avoid or reduce the need for satellite animals for toxicokinetic evaluations. However, the same can apply for large animal species, such as the minipig and dog, when newborns are used in juvenile toxicity studies. Two litters each of newborn Göttingen Minipiglets and Marshall Beagle puppies were allocated to 4-week oral (gavage) pilot repeat dose toxicology and toxicokinetic studies with dosing from postnatal day (PND) 1. A series of 5 blood microsamples (60 μl each) was taken at specific time-points after dosing on each of PND’s 1, 8, 15, 22 and 28. Blood beads were drawn by capillary action into Microvette® tubes following needle puncture of the selected vein (minipig: saphenous or ear; dog: cephalic or jugular) from each non-anesthetized piglet or puppy. Plasma samples (target of 20 μl) were obtained following direct centrifugation of the Microvette® tubes and stored frozen (between -15 and -25°C) in cryotubes. In total, 25 samples were taken from each animal during the studies, however, on several occasions the target plasma volume of 20 μl was not attained so the blood sample was increased to 80 μl. For quantifying the test item in the plasma, a qualified LC-MS/MS method was used at the Sponsor. Only 10 μl of plasma was diluted in blank plasma and subjected to a protein precipitation step. The remaining plasma was retained frozen as a contingency for additional sample preparation. The remaining plasma was diluted frozen as a contingency for additional sample preparation.

1054 Sprague-Dawley Rat Juvenile Toxicity Studies: Control Data (Part 1)

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Juvenile Toxicity studies assist in identifying postnatal developmental toxicities that are not adequately assessed in standard reproductive toxicity assessments and that may not be adequately and safely tested in pediatric clinical trials. Over the years these studies have been performed with many different study designs, therefore large control data sets are not readily available. The study designs used to generate this data were based on guidelines/guidances from the European, Japanese and United States Regulatory Agencies, were GLP-compliant, and most of the protocols were reviewed by the regulatory agency prior to commencement of the study. Juvenile Toxicity studies can have a single or multiple components (general toxicity, reproductive toxicity and developmental neurotoxicity, and recently, developmental immunotoxicity). Juvenile toxicology is complicated due to the dynamic anatomic and physiological changes that occur during growth and development of an individual. Non-clinical juvenile toxicity studies are logistically complicated due to their size, the number, diversity and interdependence of endpoints based on target organ development, the myriad modes of actions that need to be evaluated, and constraints due to the physical size of the animal (e.g., route of administration, necropsy techniques, blood collection, behavioral assessments, etc.). The Juvenile Toxicity Study data presented were from studies performed between 2011 and 2014 and contain data from over 1700 control juvenile Sprague-Dawley rats. The following data will be presented: body weights and body weight changes (pre- and post-weaning), pre-weaning reflex ontology, food consumption, motor activity (PNDs 59, 65, 80, 90, 96 and 124), Functional Observational Battery (PNDs 59, 80, 96 and 124), auditory startle habituation (PNDs 24, 60, 83, 97 and 125), learning and memory (Biel Maze; PNDs 60, 90, 84, 103 and 131), sexual maturation, estrous cyclicity and fertility.

1055 Selection of a Positive Control in the Modified Comet Assay for Crosslinking Agents

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Cross-linking agents cannot be reliably detected utilizing experimental conditions in the standard comet assay. However, under certain modified conditions such as inclusion of X-ray irradiation in the comet assay (hereafter termed modified Comet assay), DNA-DNA and DNA-protein crosslinks may be detected. X-ray irradiation is a well-recognized modification to increase background DNA damage and, thereby, more readily detect potential cross-linking agents. In order to identify a cross-linking agent that can be included as an in vivo positive control in the modified comet assay, we tested three known chemicals that are considered to be cross-linking agents by prevailing scientific knowledge, namely, mitomycin C, chlorambucil, and Hexamethyl phosphoramide (HMPA). In the definitive assay, groups of ICR mice were dosed for 3 consecutive days with each of the three test articles and organs were harvested on day 3. Three organs, liver, stomach and duodenum, were tested in the standard and modified Comet assays. Mitomycin C was tested up to 16 mg/kg/day, chlorambucil at 40 and 80 mg/kg/day, and HMPA up to 2000 mg/kg/day. Standard Comet assay procedures were followed and slides were prepared. For the modified Comet assay, a portion of the cell suspensions, after preparing slides for the standard Comet assay, were X-ray irradiated and scored in a similar manner to that for the standard Comet assay. In slides from MMC- and chlorambucil-treated mice, no significant change in % Tail DNA was observed for any of the organs either with or without X-ray irradiation. In slides from HMPA-treated mice, no significant change in % Tail DNA was observed in liver or stomach cells with or without X-ray irradiation. However, in duodenal cells, there was a statistically significant and dose-dependent decrease in % Tail DNA (vehicle – 17.26, doses low to high – 12.77, 9.68 and 6.49) compared to the vehicle control in samples with X-ray irradiation. Thus, HMPA was considered validated as a cross-linking agent and in vivo positive control to detect cross-linking activity in the modified comet assay.

1056 Testing of 14-Hydroxycodeinone (14HC) in a Combined Modified and Standard Comet Assay

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14-hydroxycodeinone (14HC) is an impurity found in oxycodone drug substance which contains an alpha, beta-unsaturated ketone structural alert and tested positive in the in vitro chromosome aberration assay. This information has historically led to controlling the impurity to the default threshold of toxicological concern (TC) level resulting in ultra-low specifications. Recently, 14HC was tested in a combined modified and standard comet assay to determine if the slight decrease in % Tail DNA noted in stomach cells in a previously conducted standard comet assay with 14HC could be magnified to clarify if the response was due to crosslinking activity. 14HC was administered at doses of 80, 160, or 320 mg/kg/day in the definitive (GLP) combined modified and standard comet assay. ICR mice were dosed for three consecutive days and on day 3, liver, stomach and duodenum were harvested 3 to 4 hours after the last dose. Standard Comet assay procedures were followed (JaCVAM protocol 14.2 and OECD guideline 489). For the modified Comet assay, a portion of the cell suspensions were X-ray irradiated to increase background DNA damage, as it is recommended as a modification to the standard Comet assay, and scored in a similar manner to that for the standard Comet assay. The experiment employed a vehicle and two positive controls, methyl methane-sulfonate for the standard comet assay and Hexamethyl Phosphoramide for the modified comet assay. In the standard Comet assay, 14HC did not elicit a significant change in % Tail DNA in liver, stomach or duodenum cells relative to the concurrent vehicle control. However, in the modified Comet assay (X-ray irradiated arm), oral administration of 14HC resulted in a dose-dependent decrease in the % Tail DNA in duodenal cells with statistical significance attained at the highest dose tested and maximum tolerated dose of 320 mg/kg/day (vehicle control – 27.6, 320 mg/kg/day – 11.6). Thus, it was concluded that 14-Hydroxycodeinone, although negative in the standard Comet assay as previously reported, is a DNA cross-linking agent in the modified Comet assay.
The effects of genotoxic substances on fish genomes have been the theme of many studies, especially when seeking to establish the response of genes to environmental stimuli. To assess the genotoxicity and cytotoxicity effects of cadmium, mercury and their mixture on catfish, three hundred Juvelines of C. gariepinus were divided into control and experimental groups. With respect to experimental groups, a set of three groups (A,C) were respectively exposed to cadmium (15.3 mg/L, 7.6 mg/L and 5mg/L), another set of three groups (D,E,F) were respectively exposed to mercury (0.2 mg/L, 0.1 mg/L and 0.06 mg/L) and the last experimental set of three groups (G,H,I) were exposed to combined cadmium and mercury doses [15.3mg/L Cd + 0.2mg/L Hg]; (7.6 mg/L Cd + 0.1mg/L Hg) and (5mg/L Cd + 0.06mg/L Hg)]. The three different cadmium, mercury and combined cadmium and mercury concentrations correspond respectively to 1/3, 1/6 and 1/9 of LC50 of cadmium and mercury values determined in previous studies (Guedenon et al. 2011 and 2012). At the end of the first, second and third week five fish were randomly selected from each group and venous blood was collected and was immediately smeared. The study of the slides at 1000 x magnification revealed highly significant frequencies in MN and NAs reaching their peak at the end of the first week followed by a gradual decrease over the end of two and three weeks. The data obtained in the present work demonstrate that the environmental contamination with cadmium, mercury and their mixture can represent a great threat for the fish populations and also a serious problem for the aquaculture. Keywords: Clarias gariepinus, blood erythrocytes, heavy metals, MN, NAs

An in vivo mammalian erythrocyte micronucleus test was conducted to evaluate the potential o-methoxy cinnamaldehyde (CAS No. 1504-74-1), a material used in fragrances and flavors, to induce micronuclei in polychromatic (PCE) and normochromatic (NCE) erythrocytes in the bone marrow of the mouse. The study was conducted in compliance with GLP and OECD Test Guideline 474 (Mammalian Erythrocyte Micronucleus Test). Male albino Hsd:ICR (CD-1) strain mice were administered a single oral dose of o-methoxy cinnamaldehyde orally at dose levels of at 250, 500 and 1000 mg/kg body weight in arachis (peanut) oil at a dose volume of 10 ml/kg body weight and sacrificed at 24 or 48 hour after dosing. Control groups received the vehicle alone or cyclophosphamide (50 mg/kg body weight) as a positive control. Bone marrow was extracted and smear preparations were made and stained at 24 and 48 hours after administration and examined microscopically for the presence of micronuclei. At least 2000 PCE per animal were scored for micronuclei. In addition, the number of normochromatic erythrocytes associated with 1000 erythrocytes was counted. The ratio of PCE to NCEs was not substantially decreased in o-methoxy cinnamaldehyde treated groups compared to the mean value PCEs of the vehicle control indicating that o-methoxy cinnamaldehyde did not produce any cytotoxic effects in the bone marrow. There was no significant increase in the incidence of micronucleated PCEs in o-methoxy cinnamaldehyde treated groups relative to their respective vehicle controls in the mice regardless of dose level or bone marrow collection time. The mean values of micronuclei observed in the o-methoxy cinnamaldehyde treated groups were below or near vehicle control values. It was concluded that under the conditions of the test, o-methoxy cinnamaldehyde is not genotoxic.
seed extract caused a reduction in bone marrow suppression induced by aflatoxin B1 treatment. Prior administration of grape seed extract ahead of aflatoxin B1 reduced the aflatoxin B1 induced bone marrow reactive oxygen species generation significantly. Grape seed extract have a protective role in the abatement of aflatoxin B1-induced DNA damage in somatic cells of rats that is dose-dependent, at least in part, in their radical scavenger activity. Therefore, grape seed extract can be a promising chemopreventive agent to avert carcinogenesis risks in persons exposed to the dietary carcinogens, aflatoxin B1.

1062 In Vivo Genotoxicity Studies with Six Different Titanium Dioxide Materials (Three Pigment-Grade and Three Nanostructured) Demonstrate Negative Effects in Orally-Exposed Rats


Six pigment-grade (pg) or ultrafine (uf) /nanostructured (anatase and/or rutile) titanium dioxide (TiO2) particulates were evaluated for in vivo genotoxicity (OECD 474 Guidelines) in male and female rats by two different laboratories. All materials were robustly characterized. The BET surface areas of the pg and uf samples ranged from 7 – 17 m²/g and 50 – 82 m²/g, respectively. The materials were assessed for induction of micronuclei and toxicity in bone marrow by analyzing peripheral blood reticulocytes (RETS) by flow cytometry. Single oral intubation doses of 0, 500, 1000 or 2000 mg/kg body weight (bw) of each material were implemented with concurrent negative (water) and positive (cyclophosphamide) controls. Approximately 48 and 72 hours after exposure, blood samples were collected and 20,000 RETs per animal were analyzed. For each of the six tests, there were no biologically or toxicologically relevant increases in the micronucleated RET frequency in any TiO2 group at either time point. In addition, there was a lack of biologically relevant decreases in %RETs among total erythrocytes. Accordingly, all 6 TiO2 test substances were negative for in vivo genotoxicity effects. Additionally, one pg and one uf material each were evaluated for potential systemic exposure/uptake by analysis of TiO2 in blood and liver. No dose dependent increase in TiO2 was observed in blood (48 or 72 h) or liver (72 h) following exposures to 2000 mg/kg bw TiO2.

Table 1. Physicochemical characteristics of TiO2 Test Samples

Test sample, crystal structure, BET value, Calc. size (XSDC)* number % D50
uf-1, 80.3% anatase - 16.5% rutile* 50.4 m²/g, 43 nm
uf-2, 100% anatase 82 m²/g, 42 nm
uf-3, 100% rutile, 59 m²/g, 47 nm
pg-1, 100% anatase, 81.2 m²/g, 153 nm
pg-2, 100% rutile, 71 m²/g, 195 nm
pg-3, 100% rutile, 17.1 m²/g, 213 nm
* X-ray Scanning Disk Centrifuge

1063 TiO2 Nanoparticles Are Genotoxic on Lung, Blood, and Liver Cells after Repeated Respiratory Exposure in Rats


Titanium dioxide (TiO2) nanoparticles (NPs) can cause negative health effects, such as respiratory tract cancer in rats. However, mechanisms involved in TiO2-induced carcinogenicity have not been clearly defined and are poorly studied in vivo. The present study investigated TiO2 NPs-induced genotoxicity, oxidative stress and inflammation in rats exposed with TiO2 NPs (P25) at 0.5, 2.5 and 10 mg/kg final doses, administrated in 3 endo-tracheal instillations every 4 days. Endpoints were assessed at two time points, 2 hours and 35 days after the last instillation. In lungs, 2 days post-exposure all of TiO2 administrated was measured at all doses, but 90 days post-exposure only 1/3 of the small dose, 2/3 of the medium dose and the entire strong dose were measured. Two hours post-exposure, TiO2 NPs were equally genotoxic (comet assay) in lung cells at all doses and in liver cells at the 2 high doses (3-fold). In contrast, 35 days post-exposure TiO2 NPs were genotoxic in a dose-dependent manner in lung cells at the 2 high doses and in blood cells at all tested doses, but in a dose-independent manner in liver cells only at the 2 high doses. In peripheral blood TiO2 NPs caused chromosome damages on erythrocytes regardless of tested doses in the same order of magnitude in both time points. These genome alterations were associated with lung neutrophilic influx in a dose-dependent manner, but at higher levels 2h post-exposure. However no significant oxidative stress was evidenced on lung, blood or liver cells at both time points. Thus, in tested conditions TiO2 NPs showed significant genotoxic effects on lung, blood and liver cells, and inflammation in a dose-dependent or -independent manner depending on the time after last exposure. These results are the first to show clear genotoxic effects of TiO2 NPs on several organs and times of recovery after repeated respiratory exposure, which should help to understand toxicity mechanisms of NPs.
1066 Genotoxic Effect of Temephos in Human Liver Carcinoma Cells (HepG2)

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Temephos (O,O,O′,O′-tetramethyl-O,O′-thiodiphenyldiphosphorothioate) is a non-systemic Organophosphorus (OP) pesticide used in public health mainly as a larvicide and ectoparasiticide to control mosquitoes and other insects. Because few studies have reported the effects of Temephos on mammalian cells, the aim of this study was to investigate the genotoxicity exerted by Temephos (g.r.) in human Hepatoma (HepG2) cells. Cytotoxicity was evaluated using fluorescent dyes Fluorescein diacetate (FDA) and Ethidium bromide (EBr). Genotoxic effects were determined by Comet assay in its alkaline version and Cytokinesis-block micronucleus assay (CBMN). Three independent experiments were performed, each in triplicate. The viability and cytostaticity of HepG2 cells exposed to 0.5-10 μM of Temephos were not affected. Regarding genotoxicity measured by Comet assay, treatment with 10 μM of Temephos increased the parameters of tail length, tail moment, and percentage of DNA, compared with the control. In addition, in HepG2 cells, metabolic competent cells, Temephos increased the frequency of MN 1.5 more than times that in control cells, but not the frequency of apoptotic cells, the percentage of binucleated cells, and the remaining CBMN parameters. Our results suggest that Temephos biotransformation might generate genotoxic metabolites in the HepG2 cells. This study was supported by CONACyT-C. Básica, México (#156673).

1067 Cytotoxicity, Genotoxicity, and Mutagenicity Tests with HepG2 Cells Shown Negative Results for the Azo Dye “Acid Black 210”

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The textile industry counts with more than 2,000 different available dyes. Some of these dyes have the capacity to release certain aromatic amines, which pose cancer risk. European Union (EU) Regulation 1907/2006 on the registration, evaluation and authorization of chemicals prohibited the use of some aromatic amines in azo dyes, and the German Consumer Goods Ordinance (GGCO) defined azo dye-stuffs which can be cleaved to release carcinogenic amines, including “Acid Black 29”, “209”, “232”, and “94”. Investigations showed that “Acid Black 131” and “132” can be cleaved to release carcinogenic amines and they are not listed in the GGCO. No data about the genotoxicity of “Acid Black 210” (CAS No. 99576-15-5) is available in literature, but there are suggestions that this dye could generate the cleavage product sulfanilic acid, that may cause sensitization by skin contact, and 4-nitroaniline, that the Dutch Health Council recommended to classify as suspected human carcinogen in 2008. The present study aimed to identify the cytotoxicity, genotoxicity and mutagenicity of this dye with the classics in vitro MTT, comet and micronucleus tests, respectively, with HepG2 cells. The MTT test verified the azo dye “Acid Black 210” between the concentrations of 0.5 and 5,000 μg/mL and showed that no cytotoxicity occurred with less than 3,000 μg/mL for 24 and 48 hours when compared to the negative control. Micronucleus test were done between the concentrations of 0.05 and 1,000 μg/mL and the Comet assay with concentrations between 0.5 and 5,000 μg/mL of the azo dye “Acid Black 210” with no statistically significant differences between each concentration and the negative control. Our results suggests that exposure to the studied dye does not induce genotoxicity or mutagenicity in HepG2 cells in the conditions tested, and future findings can confirm the safety of using “Acid Black 210” by the leather industries. Financial Support: FAPESP (Process numbers 2013/09317-7, 2013/06172-8 and 2008/10449-7).

1068 Prediction of Mutagenicity and Carcinogenicity Using SAR Softwares for Caffeine and Epinephrine

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Epinephrine, commonly known as adrenaline occurs naturally, but is used as a stimulant in cardiac arrest, anaphylactic shock and asthma attacks. Caffeine, also produced naturally, stimulates the central nervous system and provides a boost of energy. However, when consumed excessively causes headaches, dizziness, and abnormal heart rhythms. To understand the mutagenic and carcinogenic potential of caffeine and epinephrine, they were subjected to three commonly used structure-activity relationship (SAR) software. The three softwares used were DEREK, ToxTree, and VEGA. Publically available procedures and/or manuals were utilized to generate data outputs and the data was evaluated in comparison with available toxicological data on epinephrine and caffeine. The results of this study show that for epinephrine, no carcinogenicity or mutagenicity data output from either DEREK or ToxTree. The VEGA software predicted epinephrine to have possible carcinogenic effect, however not mutagenic. Similarly, there was no predictable output data for caffeine with either DEREK or ToxTree. Again, VEGA predicted no mutagenicity or carcinogenicity for caffeine. These results indicate that, based on the structure activity relationship software, neither compounds are carcinogenic or mutagenic with the exception of prediction of VEGA for epinephrine. It should be noted that additional toxicological studies are required to augment the prediction of SAR softwares.

1069 Mitochondrial Fission- and Fusion-Deficient C. elegans Display Hypersensitivity to Environmental Mitotoxicants

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Genetic variation within the human population can alter sensitivity to various toxicants, including some that target the mitochondria. For example, the antibiotic gentamycin, which targets the bacterial ribosome, can also cause hearing loss. The risk of this side effect is worsened in individuals carrying a point mutation that renders the mitochondrial 12S rRNA more bacteria-like. Many environmental pollutants are also known mitotoxicants; however, whether these toxicants pose an increased risk to individuals suffering from mitochondrial disease is unknown. Since larval mitochondrial function is dependent on mitochondrial fusion function in Caenorhabditis elegans, we screened larval C. elegans carrying deletions in fzo-1, eat-3, drp-1, pink-1, and pdr-1, which are orthologs of human MFN2, OPA1, DRP1, PINK1, and PARK2, respectively, for exacerbation of growth inhibition by exposure to several mitotoxicants. MFN2 and OPA1 control mitochondrial fusion, and when mutated cause Charcot-Marie-Tooth neuropathy type 2A and dominant optic atrophy, respectively. DRP1 is a mitochondrial fission protein reported to cause neurodegeneration and early death when mutated, while PINK1 and PARK2 are mitochondrion genes linked to familial Parkinson’s Disease. We found that fzo-1, eat-3 and pdr-1 mutants are hypersensitive to chronic arsenite exposure, compared to wild-type worms. fzo-1 and eat-3-deficient nematodes also demonstrated sensitivity to alloxan, B1 and acetaldelyde. Interestingly, fzo-1 mutants display mild resistance to cadmium, and no phenotypes were observed in pink-1 and pdr-1 mutants. To further test the role of mitochondrial dysfunction in these sensitivities, we are measuring oxygen consumption, ATP production, and mtDNA damage and copy number. This research aims to help fill a knowledge gap that may leave an already affected population at a higher risk to certain environmental exposures. Funded by NIHES I1R01-EI017540-01A2.

1070 In Vivo Genotoxicity Study of NNK in Sprague-Dawley Rats

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The tobacco-specific nitrosamine NNK [4-(methylnitrosamino)-1-(3-pyrrolidyl)-1-butanoic acid] occurs naturally in cigarette smoke. It is a potent genotoxin and is considered to be human liver carcinogenic. Unfortunately, in vivo genotoxicity data of NNK following inhalation exposure are currently limited. To fill this gap, dose of 5x10-5 (Very Low), 5x10-3 (Low), 0.1 (Mid), and 0.5 (High) mg/kg bw NNK and the propylene glycol and water formulation (vehicle control article), together with filtered air ( sham control) were administered to 30 male Sprague-Dawley rats (5 rats per dose group) via nose-only inhalation exposure. The same doses of NNK were administered to SD rats via intraperitoneal (i.p.) injection and oral gavage for reference. Rats were sacrificed 3 hours post-dosing: lungs, liver, pancreas, kidneys and bladder were collected for Comet assay analysis as well as histopathological evaluation. Peripheral blood and bone marrow were collected 24 and 48 hours post for micronucleus analysis. After inhalation exposure, NNK induced a significant increase in DNA damage in rat liver. DNA adducts at the Mid and High doses, and in rat kidneys and pancreas at the High dose. No DNA damage was detected in rat bladders. In comparison, NNK induced significant increases in DNA damage in all organs in the i.p. and gavage studies, mainly at doses lower than inhalation study. The histopathological evaluation of Comet organs and...
micronucleus analysis of peripheral blood and bone marrow were mainly negative. We hypothesize that the different organ-specific genotoxic profiles are mainly due to the different effective doses of NNK resulting from the route-of-administration.

1071 Genotoxic Screening of Hospital Wastewaters from University of Port Harcourt Teaching Hospital (UPTH), Nigeria, Using the Allium Cepa Test

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The potential toxicity and genotoxicity screening of the University of Port Harcourt Teaching Hospital (UPTH) effluent was investigated using the Allium cepa assay. Toxicity was measured by macroscopic parameters (e.g. root growth inhibition) where the degree of root growth inhibition was used to assess the toxic status of the effluent tested, and genotoxicity was measured by microscopic investigation of the chromosomal aberrations. Heterogeneous samples of untreated wastewaters from four sections comprising the laboratories, mortuary, laundry and the kitchen were collected twice daily for six months and designated as COMPLEX MIXTURES. The physico-chemical parameters of the test liquid were determined in accordance with standard methods. The results of some of the parameters like BOD, COD, TSS and pH showed levels of physico-chemical properties above the maximum permissible limits for discharge of hospital effluent into the environment. Toxicity to root growth and genotoxicity were analysed at concentrations of 1%, 5%, 10%, 25% and 50% of the effluent samples. The onion bulbs were exposed to the effluent samples in the dark for 72 hours before measuring the root length of individual roots, there was inhibition of root growth of A. cepa at all concentrations compared to control. Root growth inhibition was concentration dependent for most of the samples. An effective concentration (EC50) amounting to 50% root growth inhibition by the effluent was deduced from the growth curve at 82.4%. Various morphological defects of the onions roots were also observed. In the in vivo genotoxic assay, cytological analysis of root tips after 48 hrs exposure to the different concentrations showed reduction in frequency of mitosis in the meristematic zones of the root tips. Various types of structural chromosomal aberrations were induced in the treated cells. UPTH wastewaters samples are believed to contain potent toxic substances that provoked the toxic and genotoxic responses herein.

1072 Cyto- and Genotoxic Potentials of Some Carbamate Pesticides in Human Lymphocytes In Vitro

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Pesticides are widely used for against undesirable pests, plants and diseases which cause loss of the product quality and safety in agriculture. Pesticides are low cost, easy to apply and have beneficial effects; however, incorrect usage may cause harmful effects on people. People are exposed to pesticides indirectly by accumulation on food and by drinking the water. In the present study, some pesticides have mutagenic, teratogenic and carcinogenic effects besides chronic toxic effects such as affecting nervous system, disrupting endocrine system. Carbamates are one of the widely used pesticides in agrochemical market due to their broad effects such as affecting nervous system, disrupting endocrine system. Carbamates reversibly inhibit acetylcholine esterase and cause acetylcholine accumulation in synapses. Although it is well known their acute toxicity occurred in nervous system, knowledge of their chronic toxic effects is limited. Especially, there are controversies about their DNA-damaging properties. In the present study, carbaryl, aldicarb, methiocarb and propoxur, widely used carbamates, have been investigated about their cytotoxic and genotoxic potentials. MTT, LDH, NRU assays and comet assay were used for determining their cyto- and genotoxic activities, respectively, in human lymphocytes in vitro. The studied carbamates were disturbed on membrane permeability whereas they were not harmful on the mitochondrial and lysosomal functions in human lymphocytes. The half maximal inhibitory concentration (IC50) values of carbaryl, aldicarb, methiocarb and propoxur were 0.724, 0.796, 0.651 and 2.895 μg/mL, respectively, by LDH assay. Also, four carbamates caused increase of tail carbaryl, aldicarb, methiocarb and propoxur were 0.724, 0.796, 0.651 and 2.895 μg/mL, respectively, by LDH assay. Also, four carbamates caused increase of tail

1073 Use of a Derived Centrosome Primary Cilia Model to Detect Aneugens

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A primary cilium arises from the centrosome in a quiescent or post-mitotic cell, serving as a sensory organelle that communicates chemical and mechanical stimuli from the environment to the interior of the cell. Cilium formation may, therefore, become a useful end point signalling exposure to genotoxins or anegens. Here we have used the anegens, Zoladex, Zolodine (AZT), an antiretroviral drug that induces DNA replication arrest and centrosomal amplification (CA, >2 centrosomes/cell), to evaluate cilium formation in K562 immortalized retinal epithelial cells. For 4 days, RPE cells growing in labtek chambers, were exposed continuously to 20 μM AZT, the drug was then removed, and cells were cultured for 24 hr to allow the centrioles to develop cilia. Slides were then stained to visualize cilia (anti-acetylated tubulin antibody, green), centrosomes (anti-pericentrin antibody, magenta), and appendages in the mature centriole (anti-Cep 170 antibody red). DNA in nuclei was stained with DAPI (blue). Slides were analyzed by immunofluorescence, and 100 cells were scored per treatment. Scoring revealed 0% and 21% of RPE cells with CA in 0 or 20 μM AZT treated cells, respectively. Ciliogenesis was evaluated on the same chambers, revealed that 5.6% of cells in the control culture were ciliated while 33% of the cells in the treated wells had cilia. Multiple cilia were observed only in 16% of the cells in the treated group, although most cilia were large and robust, short cilia were also present. These preliminary observations suggest that extranumery centrosomes are able to generate cilia. The integrity and functionality of these incipient organelles has yet to be determined.

1074 Using Human Cell Panel-Based Real-Time Cell Analysis Method to Detect and Differentiate Genotoxic Chemicals

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Chemicals damaging DNA structure or replication process cause a cascade of events in mammalian cells. Capturing the dynamic responses could help to understand the signaling pathways involved and to predict the genotoxicity. An impendence-based Real Time Cell Analysis method utilizing the sCELLigence instrument was developed to understand DNA damaging mechanisms. Three human cell lines of different tissue origins were used to test 20 chemicals with five different DNA damaging mechanisms: DNA intercalation, DNA crosslinking, nucleotide analogs, topoisomerase inhibitors and histone deacetylase (HDAC) inhibitors. The bronchial epithelial cell line BEAS2B showed the most consistent kinetic response profiles among all 20 chemicals. The renal adenocarcinoma cell line ACHN was the most sensitive to DNA damaging reagents, and displayed distinctive kinetic re- response profiles toward topoisomerase inhibitors and HDAC inhibitors. HepG2 cell line showed certain metabolic activity which enabled detection of cytotoxicity from chemicals requiring metabolic activation, such as benzopyrene. The most dramatic kinetic profiles were observed in ACHN cells responding to HDAC inhibitors including valproic acid, SAHA and (S)-HDAC-42. Within 40 hours exposure, cell index went up for more than two folds than controls. Responses to these chemicals were observed at low uM to nM range, which are at least comparable to other sensitive cell based assays. The study demonstrated that dynamic response profiles generated in human cell panel are useful to distinguish different genotoxicity mechanisms. Further detailed analysis and thorough testing with more chemicals may provide new insights to differentiate chemicals in the other three classes of compounds directly targeting DNA, and chemicals with other epigenetic mechanisms.

1075 Evaluating the Inflammatory and Genotoxic Effects of Smokeless Tobacco Using a Human Organotypic Model of Oral Epithelium

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In addition to the well-known effects of tobacco use on the causation of lung and cardiovascular disease, tobacco use is also implicated as a major cause of oral cavity disease that leads to thousands of deaths per year. Snus, a smokeless tobacco applied to the oral cavity, has been proposed as a less harmful alternative to smoking although its safety has not been adequately evaluated. The objective of this study was to evaluate the cytotoxic, genotoxic and inflammatory effects of snus using an in vitro model of human oral mucosa (EpIoral). EpIoral tissues were treated topically with 5 or 25 milligrams of snus for 24-48 hours and evaluated for cytotoxicity by MTT. Tissues treated with 5 mg of snus had comparable viability to vehicle
treated controls while those treated with 25 mg displayed approximately a 20% decrease in viability after 24 and 48 hours of exposure. Histological analysis revealed hyperchromic staining in tissues treated with 5 mg of snus at 24 hours post-treatment whereas tissues treated with 25 mg of snus displayed a significant amount of sloughing of the apical layers. Following treatment, an inflammation-specific cytokerine panel was used to analyze markers of inflammation at 24 and 48 hours post treatment. Of the cytokines analyzed, significant increases (1.5-2 fold) in IP-10, GM-CSF and RANTES were observed at both 24 and 48 hours post treatment in tissues treated with 25 mg of snus. As a measure of genotoxicity, the presence of γ-H2AX foci (specifically, phosphorylation at Serine 139) was evaluated in treated tissues. γ-H2AX foci were readily detected in the apical layer of tissues treated with 25 mg of snus at 24 and 48 hours post treatment. These results demonstrate the utility of this organotypic oral tissue model to evaluate smokeless tobacco product safety.

1076 Environmental Toxins Found Historically in the Polycythemia Vera Cluster Area and Their Potential for DNA Damage
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In 2006, the Agency for Toxic Substances and Disease Registry received a request to determine whether a cluster of polycythemia vera patients existed in a small northeast Pennsylvania community. A significant cluster of PV cases was identified at the nexus of three counties. Since the cluster is in the vicinity of several EPA Superfund sites, there was concern regarding a potential link to environmental contamination. The current study evaluated the potential for a select number of toxins to induce DNA damage using an in vitro assay for hematopoietic stem-cell derived progenitor cells. The toxins were chosen based on their historic presence in the PV cluster area. CD34+ cells were isolated from normal cord blood samples and were cultured for 48-72 hours to generate erythroid progenitor cells. Eighteen compounds were chosen for the assay; arsenic trioxide, benzo(a)pyrene, benzene, methylene chloride, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), trichloroethylene, potassium chloride, ethylbenzene, benzene[k]fluoranthene, styrene, cadmium chloride, hydroquinone, 1,1,1-trichloroethane, sodium cyanide, manganese chloride, chromium oxide, lead oxide and sodium arsenite. Genotoxicity of the compounds was determined using the micronucleus assay and the comet assay. Arsenic trioxide, benzo(a)pyrene, benzene, and methylene chloride generated micronuclei, 2.46%, 2.86%, 1.98%, and 2.99% respectively. Using the comet assay, 16 compounds at 10μM concentration, induced a significant amount of DNA damage compared to the control. When evaluating whether a dose-dependent relationship was present, seventeen of the eighteen compounds led to DNA damage with exposure to increasing concentrations. 2,3,7,8-TCDD was particularly potent, inducing DNA damage at concentrations as low as 10 nM. These results demonstrate the utility of this organotypic oral tissue model to evaluate smokeless tobacco product safety.

1077 Cytotoxicity and Genotoxicity Assessment of Sandalwood Essential Oil in Human Breast Cell Lines MCF-7 and MCF-10A
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Sandalwood essential oil (EO) is extracted from Santalum album trees and is used in the food industry, perfumery, and cosmetics. Although one of the constituents of EO (α-santalol) has been studied as a chemopreventive agent for skin cancer, the genotoxic activity of the whole oil in human breast cell lines is unknown. The main objective of this study was to assess the cytotoxic and genotoxic effects of EO in the human breast cancer (MCF-7) and human breast epithelial (MCF-10A) cell lines and to identify candidate proteins associated with genotoxicity. Cells were exposed to EO (8–160 μg/mL) for 24 hours. Cell proliferation was determined by the MTT assay while genotoxicity was assessed using the alkaline comet assay. Cells were visually scored into four classes: class 1: undamaged, no tail, class 2: tail shorter than diameter of the head, class 3: tail as long as 1-2x the diameter of the head, and class 4: tail longer than 2x the diameter of the head. Our results show that the exposure of MCF-7 cells to increasing concentrations of EO causes a decrease in class 1 comets and an increase in class 2 and 3 comets. EO is capable of inducing double strand breaks (DSBs) in the DNA of these cells. Quantitative LC/MS based proteomics at multiple time points, incorporating rapid microwave and magnetic sample preparation with isobaric labeling, enabled relative protein expression to be correlated to the genotoxic effects produced by EO, including the expression of Ku70 (p=1.37E-2), Ku80 (p=5.8E-3), EPHX1 (p=3.3E-3) and 14-3-3-σ (p=4.0E-4). These results provide the first evidence that EO is genotoxic and capable of inducing DNA DSBs in MCF-7 cells. Research was supported by the NIGMS-NIH Award R25GM082406, 1CA517250 and G12MD007591 (RCMI Proteomics & Protein Biomarkers Cores), and the MAGIC (MD007579).

1078 Genotoxicity Assessment of Glyphosate-Based Formulations in Zebrafish Embryos (Danio rerio) Using Comet Assay
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Introduction: The most commonly used herbicides in the world are glyphosate-based, likely because they are relatively non-toxic to non-target species. There are many glyphosate-based formulations commercially available, all of which typically contain glyphosate, water, and a surfactant. In Brazil, besides toxicological assessment of the active ingredients of pesticides, the analysis of the toxicity of formulations containing them is mandatory. New methods are being developed with zebrafish embryos to identify the mechanisms of action of chemical compounds. One of these is comet assay, a sensitive method for detecting DNA strand breaks at the single cell level. Objective: Evaluate the genotoxicity of two glyphosate-based formulations, Original Roundup® herbicide (RUP) and commercial Glyphosate AKB 480 (AKB) on embryos of zebrafish (D. rerio). Methodology: Groups of 20 embryos were exposed to each concentration of RUP and AKB established by Fish Embryo Acute Toxicity (FET) test (data not shown) ranging from 5.0 to 50 mg/L and to test controls for 96 hours post-fertilization. A mechanical cell isolation protocol and the subsequent steps of the comet assay were performed as described by Kowsmeh et al. (2006) and Singh et al. (1988), respectively and with slight modifications. Results and discussion: Embryos exposed to RUP and AKB herbicides showed no mean tail moment measure significantly different compared to negative control embryos. Both glyphosate-based herbicides did not induced DNA damage in zebrafish embryos in the tested conditions. Conclusion: Our data indicate that although both glyphosate-based herbicides are non-genotoxic to zebrafish embryos, the comet assay could be added to OECD TG 236 - FET test (OECD, 2013) in order to detect genotoxic effects in addition to other lethal and sub-lethal effects. Financial Support: FAPEG, CAPES and CNPq. Acknowledgments: FCFRP/USP and ICB/UnB.

1079 Mutagenic Activity and Molecular Markers of Inflammation Present in Emissions from On-Road Heavy-Duty Diesel Vehicles with and without Advance Retrofit Devices
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As an approach to reduce the exposure to toxic compounds, a number of control strategies for vehicle emissions has been proposed and initiated. One approach is to apply retrofit controls such as selective catalytic reduction (SCR), for example, to existing on-road vehicles. To screen for potential toxic emissions, we studied the mutagenicity and molecular markers of inflammation on particle emissions from a number of heavy-duty diesel vehicles with retrofit devices. The Salmonella/microsome assay (microsuspension modification) and molecular markers of inflammation (IL-8, COX-2, for example) using human macrophage cells (U937) were measured for all vehicles. Particle emissions were collected from six different vehicle configurations and compared to a baseline vehicle without emission controls. Standard chassis dynamometer testing using two different test cycles was conducted. Details regarding the vehicles chemical analyses including PAHs have been summarized previously. On a per mile emission basis and compared to the baseline vehicle, retrofit devices tested appeared to result in decreased mutagenic activity and molecular markers of inflammation.
**1080 Genotoxic Effect of Commercial-Grade Temephos in Human Lymphocytes and Hepatoma Cells**

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Temephos (O,O,O’-tetramethyl-O,O’-chiodo-p-phenylene diphosphorothi-ate) is an non-systemic organophosphorus (OP) pesticide mainly used as larvicide and ectoparasiticide to control mosquito, and other insects in public health. Since few studies have reported the effects of temephos on mammalian cells, the aim of this study was to investigate the genotoxicity exerted by temephos (p.s.) in human hepatoma (HepG2) cells, through comet assay and cytokinesis-block micronucleus assay (CBMN). The cytotoxicity was evaluated using the fluorescent dye fluorescein diacetate (FDA) and ethidium bromide (EtBr). The genotoxic effects were determined by the comet assay in its alkaline version and the cytokinesis-block micronucleus assay (CBMN). Three independent experiments were performed each in triplicate. The viability and cytostaticity of HepG2 cells exposed to 0.5-10 μM of temephos was not affected. Regarding the genotoxicity, measured by comet assay, the treatment with 10 μM of temephos increased the parameters of tail length, tail moment and percentage of DNA, compared to control. In addition, in HepG2 cells, a metabolic competent cells, temephos increase the frequency of MN in 1.5 more times than in control cells, but not the frequency of apoptotic cells, the percent of binucleated cells and the other CBMN parameters. Our results suggest that temephos biotransformation might generate genotoxic metabolites in cell HepG2. This study was supported by CONACyT-C. Básica, México (#156673).

**1081 Ethanol Extract of Eclipta alba Leaf Suppresses Sodium Arsenite-Induced Genotoxicity and Hepatotoxicity in Male Wistar Rats**


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Arsenic contamination of underground/well water in developing countries of the world is a major health concern for man and animals, thus the search for other co-toxicants and potent remedies. The herbal therapeutic use of *Eclipta alba* in the management of ulcer, diarrhea, constipation and pile have been documented. We investigated the effects of the ethanol leaf extract of *Eclipta alba* (ELEA) alone and with sodium arsenite (SA) induced genotoxicity and hepatotoxicity in male Wistar rats. Thirty five male rats were divided into seven groups of five rats each. Group I received distilled water only. Group II were treated with SA (5.0 mg/kg b.w.) once in two days and group III with ELEA (200 mg/kg b.w./day). Group IV received SA (5.0 mg/kg b.w.) and ELEA (200 mg/kg b.w.) simultaneously. All treatments were oral and for fourteen days. The activities of serum alkaline phosphatase (ALP), γ-glutamyl transferase (γGT), alanine (ALT) and aspartate (AST) amino transferases were monitored as markers for hepatotoxicity. In addition, genotoxicity was investigated using the micronucleus assay. The mPCes as well as the serum enzyme activities were significantly (p < 0.05) increased by SA as compared with the negative control group. There was no difference observed in mPCes and serum enzyme activities in ELEA only group as compared to distilled water only group. A significant reduction (p < 0.05) in mPCes and serum ALP, ALT and AST activities were observed in groups treated with SA and ELEA simultaneously, as compared with SA only group. In conclusion, ethanol extract of *Eclipta alba* leaves suppresses sodium arsenite induced genotoxicity and hepatotoxicity in rats.

**1082 Detection of Sulfur Mustard-Induced DNA Adducts in Pig Skin with Fluorescence Microscopy**

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Introduction: Sulfur mustard (SM) and 2-chloroethyl ethyl sulfide (CEES) are bis- and monofunctional DNA alkylating agents, respectively. SM is an old chemical warfare agent causing blisters (vesicant). Both chemicals react with N7 guanine. SM will form 7-Hydroxyethylthioethylguanine for SM. A specific monoclonal antibody (2F8) exists which detects SM at N7 position. Aim: The 2F8 antibody was used to develop a microscopic technique for detection of SM exposure in pig skin. Methods: Pig skin was obtained from slaughterhouse. After exposure with various SM concentrations, skin was fixed with HOPE fixative and 10% formamide. Subsequently, the preparation was treated with the monoclonal 2F8-antibody against the N7- guanine momoduct. Next, the antibody molecules attached to the DNA damage were made visualized by binding to a goat-anti-mouse antibody that contains a fluorescently a fluorescent group (Cy3 and Dy549). The preparation was also treated with DAPI to counterstain cell nuclei. By means of laser scanning microscopy and summation of z-stacks, red fluorescence was quantified. Results: DNA adducts were concentration dependent detected after SM exposure below 10 μM which is 10-fold below the vesicant threshold. Conclusion: The presented technique is potentially able to detect and quantify SM exposure in skin samples ex vivo. The technique may be useful to confirm SM exposure in blister roots of intoxicated patients.

**1083 PCB126 Regulates hTERT Involving the AhR-ARNT and HIF1α-ARNT Signaling Pathways**


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PCB126, a dioxin-like PCB, is a potent aryl hydrocarbon receptor (AhR) agonist. The AhR is a transcription factor that mediates the effects of dioxin-like environmental chemicals by changing gene expression. After ligand binding, the AhR dimerizes with the aryl hydrocarbon receptor nuclear translocator (ARNT) before binding to xenobiotic response elements in the promoter of target genes. Similarly the hypoxia-inducible factor-1α (HIF1α), a key regulator of responses to reduced oxygen conditions, has to dimerize with ARNT before binding to hypoxia response elements of its target genes. In hypoxic conditions HIF1α is competing with AhR for ARNT. Recently we reported that PCB126 (3,3’,4,4’,5-pentachlorobiphenyl) down-regulates β-glucuronidase and α-tubulin gene expression in various cell lines. We hypothesized that AhR and/or ARNT mediate this effect. To investigate this hypothesis we incubated HL-60 (human promyelocytic leukemia cells) with the AhR agonists 3H223191 and 2-naphthoflavone with/without PCB126. Both antagonists strongly attenuated the reduction of hTERT mRNA by PCB126 and increased hTERT expression in the absence of PCB126. Incubation of HL60 cells with cobalt chloride (CoCII), an inducer of HIF1α, to mimic hypoxia resulted in a concentration-dependent down-regulation of hTERT gene expression. Similarly incubation of HaCaT (human keratinocyte) cells for 8hrs at 1% oxygen reduced hTERT mRNA levels compared to 21% oxygen. This response was enhanced when PCB126 was added for 4hrs. In summary, a reduction of free ARNT by binding to PCB126-AhR or CoCII induced hypoxia-HIF1α lowered hTERT expression, whereas increased abundance of ARNT using AhR antagonists or in conditions of normoxia increased expression. This indicates that the AhR-ARNT/HIF1α-ARNT signaling pathways and especially ARNT, play a significant role in the regulation of hTERT in human cells. (Supported by SFI ES013661)

**1084 Carbamoyl Phosphate Synthase 1-Mediated Homocitrullination of Histone H3.2 Constitutes a Novel Epigenetic Mark Associated with Aryl Hydrocarbon Receptor-Driven Gene Expression**


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The Aryl Hydrocarbon Receptor (AhR) is a ligand-activated transcription factor responsible for the toxicity associated with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) exposure. Functionally, the AhR is known to regulate gene expression by binding to the Xenobiotic Response Element (XRE) in partnership with AhR nuclear translocator (Arnt). Recently, we identified a new AhR binding sequence (TCDD) exposure. Functionally, the AhR is known to regulate gene expression by binding to the Xenobiotic Response Element (XRE) in partnership with AhR nuclear translocator (Arnt). Recently, we identified a new AhR binding sequence termed the Non-Consensus Xenobiotic Response Element (NC-XRE) that does not recruit the Arnt protein, but instead relies on a novel AhR binding partner, termed the Non-Consensus Xenobiotic Response Element (NC-XRE) that does not recruit the Arnt protein, but instead relies on a novel AhR binding partner, termed the Non-Consensus Xenobiotic Response Element (NC-XRE) that does not recruit the Arnt protein, but instead relies on a novel AhR binding partner, termed the Non-Consensus Xenobiotic Response Element (NC-XRE) that does not recruit the Arnt protein, but instead relies on a novel AhR binding partner, termed the Non-Consensus Xenobiotic Response Element (NC-XRE) that does not recruit the Arnt protein, but instead relies on a novel AhR binding partner, termed the Non-Consensus Xenobiotic Response Element (NC-XRE) that does not recruit the Arnt protein, but instead relies on a novel AhR binding partner, termed the Non-Consensus Xenobiotic Response Element (NC-XRE) that does not recruit the Arnt protein, but instead relies on a novel AhR binding partner, termed the Non-Consensus Xenobiotic Response Element (NC-XRE) that does not recruit the Arnt protein, but instead relies on a novel AhR binding partner, termed the Non-Consensus Xenobiotic Response Element (NC-XRE) that does not recruit the Arnt protein, but instead relies on a novel AhR binding partner, termed the Non-Consensus Xenobiotic Response Element (NC-XRE) that does not recruit the Arnt protein, but instead relies on a novel AhR binding partner, termed the Non-Consensus Xenobiotic Response Element (NC-XRE) that does not recruit the Arnt protein, but instead relies on a novel AhR binding partner, termed the Non-Consensus Xenobiotic Response Element (NC-XRE) that does not recruit the Arnt protein, but instead relies on a novel AhR binding partner, termed the Non-Consensus Xenobiotic Response Element (NC-XRE) that does not recruit the Arnt protein, but instead relies on a novel AhR binding partner, termed the Non-Consensus Xenobiotic Response Element (NC-XRE) that does not recruit the Arnt protein, but instead relies on a novel AhR binding partner, termed the Non-Consensus Xenobiotic Response Element (NC-XRE) that does not recruit the Arnt protein, but instead relies on a novel AhR binding partner, termed the Non-Consensus Xenobiotic Response Element (NC-XRE) that does not recruit the Arnt protein, but instead relies on a novel AhR binding partner, termed the Non-Consensus Xenobiotic Response Element (NC-XRE) that does not...
epigenetic mechanism regulating gene expression with pronounced implications for understanding AhR biology and TCDD toxicity, and potentially other transcriptional processes involving CPS1 mediated chromatin modifications.

**1085 Comparative Assessment of Dose-Dependent TCDD-Elicited Hepatic Gene Expression in Mice**

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Differential gene expression plays a critical role in the mechanism of toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Reductions in sequencing cost, have made RNA sequencing the preferred technology to assess global gene expression with microarrays, although still common, becoming obsolete. We compared the hepatic transcriptome of C57BL/6 mice following gavage with sesame oil vehicle, 0.01, 0.03, 0.1, 0.3, 1, 3, 10, or 30 μg/kg TCDD every 4 days for 28 days using Illumina HiSeq RNA-Sequencing (RNA-Seq) and Agilent 4x44K microarrays. RNA-Seq and microarray analysis identified a total of 18,063 and 16,403 genes, respectively, that were expressed in the liver. Analysis for differentially expressed genes (DEGs) varied dramatically depending on the P(i) cut-off used for RNA-Seq data while microarray results varied more based on the fold change criteria, although responses strongly correlated. Verification by WaferGen SmartChip QRTPCR revealed that RNA-Seq had a false discovery rate of 24% compared to 54% for microarray analysis. Dose-response modeling of RNA-Seq and microarray data demonstrated similar ED_{50} and BMD estimates for common DEGs. Interestingly, three distinct ED_{50} peaks were identified suggesting a step-wise dose-dependent activation of lipid metabolism, immune system response, and collagen deposition. Although the results were comparable between the two platforms, RNA-Seq provided more qualitative and quantitative data compared to microarrays. Moreover, both RNA-Seq and microarray analyses demonstrated that TCDD elicited dose-dependent differential gene expression consistent with the progression of steatosis to steatohepatitis with fibrosis. Funded by SRP P42ES04911.

**1086 Activation of the Aryl Hydrocarbon Receptor by Carcinogenic Aromatic Amines and Modulatory Effects of Their N-Acetylated Metabolites**

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Aromatic amines (AAs) are an important class of chemicals which account for 12% of known carcinogens. The biological effects of AAs depend mainly on their biotransformation into reactive metabolites. Although the activation of the Aryl Hydrocarbon Receptor (AhR) pathway by certain carcinogenic AAs has been reported, the effects of their N-acetylated metabolites on the AhR have not been addressed. Here, we investigated whether carcinogenic AAs and their N-acetylated metabolites may activate/modulate the AhR pathway in the absence and/or the presence of a bonafide AhR ligand (Benz[a]pyrene/B[a]P). We found that certain AAs activated the AhR in human liver and lung cells (increase in cytophrome P450 1A1 (CYP1A1) expression). Altogether, we report for the first time that these properties can be modulated by the N-acetylation status of the AA, whereas 2-naphthylamine (2-NA) significantly activated the AhR and induced CYP1A1 expression, its N-acetylated metabolite was less efficient. In contrast, the N-acetylated metabolite of 2-aminofluorene was able to significantly activate AhR whereas the parent AA, 2-aminofluorene (2-AF), did not. In the presence of B[b]P, activation of AhR or antagonist effects were observed depending on the AA or its N-acetylated metabolite. Modulation of the AhR pathway by AAs and their N-acetylated metabolites may represent a novel mechanism contributing to the toxicological effects of AAs.

**1087 A Novel Role for the Cytosolic Aryl Hydrocarbon Receptor in Pancreatic Cancer**

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Based on their gene signatures and drug responses, pancreatic ductal adenocarcinomas (tumors and derived cells) have been divided into three subtypes (QM-PDA), exocrine and classical) and pancreatic cancer cell lines primarily represent QM-PDA and classical sub-types. Pancreatic tumors and cell lines express the AhR and are potential targets of AhR ligands and, after screening eight AhR-active pharma-

cuticals as inhibitors of Panc-1 cell (a QM-PDA cell line) invasion, we identified omeprazole and tranilast (but not TCDD) as inhibitors and this response was attenuated after AhR knockdown by RNA interference (RNAI). In breast cancer cells omeprazole but not tranilast also inhibited invasion through nuclear AhR-mediated inhibition of CXCR4, however, in Panc-1 cells, we did not observe ligand-induced changes in CXCR4 expression. Moreover, induction of CYP1A1 gene expression, the most common AhR-responsive gene, was also not observed in Panc-1 and a send QM-PDA cell line (MiaPaCa2) whereas in at least two classical pancreatic cell line (Panc28 and L3.6pl) induction of CYP1A1 by TCDD and omeprazole was observed. Further examination of several QM-PDA and classical pancreatic cancer cell lines showed that these cell lines all express the AhR, however after treatment with TCDD or omeprazole nuclear uptake of the AhR was observed only in the classical (CYP1A1-inducible) but not the QM-PDA (no CYP1A1 induction) cell lines. Omeprazole but not TCDD inhibited invasion of Panc1 (QM-PDA) cells and this response was attenuated by knockdown of the AhR but not the AhR nuclear translocator protein. Thus our studies clearly demonstrate that the aggressive Panc1 and MiaPaCa2 cells express a functional cytosolic AhR (AhRc) that mediates ligand-induced inhibition of cancer cell invasion.

**1088 The Aryl Hydrocarbon Receptor Is a Novel Regulator of the Circadian Nuclear Receptor Rev-erb Alpha**

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We have demonstrated that activation of AhR (Aryl hydrocarbon receptor) can impair circadian clock function and disrupt metabolism. Although mechanisms that underlie effects of AAs on the nuclear receptor Rev-erb alpha have not been reported, the effects of their N-acetylated metabolites on the AhR have not been examined. Activation of the AhR by the chemically induced AhR pathway, especially 2-AF, has been implicated as an important integrator of circadian rhythms and metabolism. Rev-erb alpha is an important regulator of circadian rhythms and metabolism and Rev-erb alpha stabilizes the circadian feedback loop by repressing Bmal1 (Brain and muscle Arnt-like protein 1). Rev-erb alpha also regulates gluconeogenesis and energy metabolism by repressing several metabolic pathway genes. This study was designed to examine Rev-erb alpha as a novel target of AhR signaling that mediates detrimental metabolic and circadian clock effects downstream of AhR activation. First, we demonstrated that Rev-erb alpha is a target of activated AhR. In Hepa1c1c7 cells, AhR agonist (βNF (β-Naphthoflavone) treatment slightly increases Rev-erb alpha mRNA. However, βNF significantly decreases Rev-erb alpha protein. In contrast, neither the Rev-erb alpha mRNA nor the Rev-erb alpha protein is changed after AhR activation in the AhR-deficient c12 cells. RNA-interference-mediated depletion of AhR in hepatic c12 cells increases Rev-erb alpha protein but has no effect on Rev-erb alpha mRNA. These data suggest that AhR activation mediates Rev-erb alpha protein degradation through a post-transcriptional mechanism. Second, we demonstrated that the circadian rhythm of Rev-erb alpha is AhR-dependent. The Rev-erb alpha protein is rhythmically expressed in hepatic c12 cells, in an AhR-dependent manner. More interestingly, the Rev-erb alpha protein rhythm persists in ARNT-mutant c4 cells, indicating that AhR regulates Rev-erb alpha protein oscillation through a post-transcriptional mechanism. In ARNT-KO mice, Rev-erb alpha mRNA rhythm has a significant phase shift compared to the AhR-WT mice. Together, our data shows AhR is an important regulator of the circadian receptor Rev-erb alpha and identifies a potential mechanism for the alterations in clock function and metabolism imposed by AhR activation.

**1089 Constitutive Androstane Receptor (CAR) Activation Altered microRNA Profiling in Mouse Liver**

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The nuclear receptor CAR is a xenosensor and important regulator of xenobiotic detoxification, lipid and energy metabolism as well as a key contributor to the development of chemical hepatocarcinogenesis in mice. However, the underlying pathways effected by CAR in these processes are complex and remain to be fully elucidated. microRNAs have emerged as critical modulators of gene expression. Their activities are now associated with many processes, such as altered drug detoxification and liver tumor development. In this study, we used deep sequencing approaches with an Illumina HiSeq platform to differentially profile microRNA expression patterns in livers from wild type C57BL/6j mice following CAR activation with 1,4-bis-(2-(3,5-dichloropyridyloxy)) benzene (TCPOBOP). The raw sequence data were processed with the mirDeep2 bioinformatics module and differentially expressed microRNAs were assessed using the Bioconductor statistics package. Pathway evaluations were performed using Ingenuity Pathway Analysis.
(IPA) software. Using these procedures, a number of microRNAs were identified whose expression levels were altered by TCPOBOP treatment, including mmu-miR-802-5p and mmu-miR-485-3p. The alterations were confirmed with RT-qPCR. IPA investigation of the differentially expressed microRNAs revealed altered effector pathways, including cell growth and proliferation in the molecular and cellular function, and liver hyperplasia/hyperproliferation in the toxicology function. These pathway links appear consistent with the known role of CAR in rodent hepatocarcinogenesis. A network among CAR targeted genes and the affected microRNAs was constructed to illustrate how microRNAs may mediate CARs functional role in mouse hepatocyte proliferation.

### 1092 Human Cytochrome P450 2C8 (CYP2C8) Is a Novel Target of Peroxisome Proliferator-Activated Receptor α (PPARα) in Human Liver

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CYP2C enzymes are important liver enzymes involved in the metabolism of clinically prescribed drugs and environmental chemicals. CYP2C3 also metabolizes endogenous substrates such as arachidonic acid to biologically active epoxyeicosatrienoic acids (EETs). Previous studies showed that microRNA 107 (miR107) down-regulates CYP2C8 post-transcriptionally, miR107 is located in intron 5 of the pantothenate kinase 1 (PANK1) gene and is co-regulated with PANK1. The aim of the study was to examine the regulation of CYP2C8 by environmental compounds and drugs capable of inducing miR107. We showed that hypolipidemic drugs such as bezafibrate, which are activators of peroxisome proliferator-activated receptor α (PPARα) induced both PANK1 gene and miR107 (2.5 fold) in primary human hepatocytes. Similarly CYP2C8 mRNA and protein levels were also induced 48 h after bezafibrate treatment. We hypothesized that CYP2C8 is directly regulated by PPARα in human hepatocytes. CYP2C8 promoter luciferase was induced by ectopic expression of PPARα in HepG2 with further increase by either bezafibrate (≈18-fold) or WY14643 treatment (≈10-fold). Moreover, the CYP2C8 transcription was also activated by transfection of HepG2 cells with PPARα and treatment with the antidiabetic drug, rosiglitazone. Computer analyses, promoter deletion studies, electrophoretic mobility shift assay (EMSA) and mutagenesis studies identified a PPARα response element (PPRE) located at position -2109 bp relative to the translation start site that mediates transactivation of CYP2C8 luciferase by PPARα. We also observed enhanced recruitment of PPARα to the PPRE at -2109 after bezafibrate treatment in human hepatocytes by chromatin immunoprecipitation assay (ChIP). The present study showed for the first time that CYP2C8 is directly regulated by PPARα. This work indicates that activation of PPARα by hypolipidemic drugs can induce CYP2C8 thus altering the clearance of drugs metabolized by CYP2C8.

### 1093 Novel Regulatory Function of Human Glutathione S-transferase P1-1 in Classical Estrogen Receptor & Signaling Pathway

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Estrogen receptor (ERα) plays a crucial role in estrogen-mediated signaling pathways and exerts its action as a nuclear transcription factor. Binding of the ligand-activated ERα to the estrogen response element (ERE) is a central part of ERα-associated signal transduction pathways and its aberrant modulation is associated with many disease conditions. Human glutathione S-transferase P1-1 (GSTP) functions as an enzyme in conjugation reactions in drug metabolism and as a regulator of kinase signaling pathways. It is overexpressed in tumors following chemotherapy and has been associated with a poor prognosis in breast cancer. In this study, a novel regulatory function of GSTP has been proposed in which GSTP regulates ERE-mediated ERα signaling events. Ectopic expression of GSTP was able to induce the ERα and ERE-mediated transcriptional activities in ERα-positive but GSTP-negative MCF7 human breast cancer cells. This inducive effect of GSTP on the ERE-transcription activity was diminished when the cells express a mutated form of the enzyme or are treated with a GSTP-specific chemical inhibitor. It was found that GSTP inhibited the expression of the receptor interacting protein 140 (RIP140), a negative regulator of ERα transcription, at both mRNA and protein levels. Our study suggests a novel non-enzymatic role of GSTP which plays a significant role in regulating the classical ERα signaling pathways via modification of transcription cofactors such as RIP140.

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**1090 Transcriptional Regulation of SULT1C2 by Vitamin D Receptor in Human Intestinal and Kidney Cells**

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The SULT1C family of cytosolic sulfotransferases consists of three human members, SULT1C1, SULT1C3, and SULT1C4, all of which are relatively uncharacterized with regard to their substrates and modes of regulation. We previously demonstrated that treatment with 1α,25-dihydroxyvitamin D3 (VitD3) markedly increased SULT1C2 expression in LS180 colorectal adenocarcinoma cells. In this study, we evaluated the mechanism responsible for VitD3-mediated regulation of SULT1C2 transcription. VitD3 treatment increased luciferase expression from a reporter plasmid containing -5KB of the SULT1C2 gene, including 401 nt of the non-coding exon 1, intron 1, and 21 nt of exon 2, that was transiently transfected into LS180 or human renal proximal tubular cells. Computational analysis of the -5KB SULTIC2 sequence identified a pregnane X receptor (PXR)/retinoid X receptor heterodimer, direct repeat 4 binding site (PPRE) within exon 1. However, treatment with the prototypical PXR agonist, rifampicin, did not increase reporter expression from the transfected -5KB construct, suggesting that the computationally predicted sequence was not a functional PPRE. Deletion or mutation of this predicted PPRE abolished VitD3-mediated SULT1C2 activation, confirming this site’s role in SULT1C2 regulation. Using an ELISA-based transcription factor binding assay, we demonstrated that vitamin D receptor (VDR) can interact directly with the SULT1C2 PPRE sequence. These results indicate that VitD3 regulates SULT1C2 transcription through VDR, which binds to a PPRE site in exon 1. The regulation of SULT1C2 transcription in VitD3-responsive tissues suggests that SULT1C2 might play a role in VitD3-regulated physiological processes.

This work was supported by NIH grant ES022606 and NIEHS Core Center grant P30ES020957.

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**1091 Regulation of SULT1C3 Transcription by Peroxisome Proliferator Activated-Receptor Gamma in Human Colorectal Adenocarcinoma LS180 Cells**

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SULT1C3 is a member of the cytosolic sulfotransferase family of enzymes, which conjugates endogenous and xenobiotic substrates to a wide range of endogenous and xenobiotic substances. Although little is known about the expression or regulation of SULT1C3, we recently reported that SULT1C3 is expressed in human intestine and that treatment of the LS180 colorectal cell line with the peroxisome proliferator-activated receptor (PPAR) γ agonist rosiglitazone increases SULT1C3 expression. To determine the mechanism underlying rosiglitazone-mediated SULT1C3 induction, we prepared fragments of the SULT1C3 5′-flanking region for transient transfection-reporter analysis. Initial attempts to PCR amplify ~2.8Kb of the SULT1C3 5′-flanking region from human genomic DNA produced a ~1.9Kb fragment in addition to the expected 2.8Kb fragment, suggesting the possible existence of al- leric variants. Sequence analysis revealed that 863 nt (~146 to ~1008 relative to the transcription start site) were absent from the 1.9Kb fragment relative to the 2.8Kb fragment. When reporter plasmids containing the 2.8 and 1.9Kb fragments were transfected into LS180 cells, rosiglitazone treatment activated reporter expression from the 2.8 but not the 1.9Kb construct. Computational analysis identified three potential PPAR response elements (PPREs) within the 863 nt region. Transfection analysis with a series of deletion constructs indicated that the PPRE at nt -772 relative to the transcription start site was necessary for rosiglitazone responsiveness, and site-directed mutagenesis confirmed the importance of that PPRE. These findings identify the molecular basis for rosiglitazone-mediated regulation of SULT1C3 expression in human intestine, and suggest that this regulation may vary among individuals.

This work was supported by NIH grant ES022606 and NIEHS Core Center grant P30ES020957.
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Human paraoxonase 1 (PON1) is an A-esterase calcium-dependent synthesized in the liver and secreted into the plasma associated with high density lipoproteins (HDL). PON1 acts as an antioxidant molecule in lipid metabolism preventing lipid oxidation; it also detoxifies a wide range of substrate including organophosphate compounds. The variability of serum levels and activity has been mainly attributed to polymorphisms in the gene, diet, pathological and physiological status, lifestyle and xenobiotics. However, the molecular mechanisms involved in transcriptional regulation of PON1 have been little studied. The aim of this study was to characterize the transcriptional regulation of human PON1 in human hepatocarcinoma cells (HepG2). In silico analysis was performed on the promoter region of PON1 to determine response elements of nuclear receptors (NR). Through Real-time PCR, was evaluated the effect of specific NR ligands in the in silico analysis on mRNA levels of target genes regulated by NR and PON1. The results obtained from the in silico analysis showed response elements of nuclear receptors to pregnenolone (PXR), glucocorticoids (GR), retinoic acid (RXR) and peroxisomes proliferator-activated receptor alpha (PPARα) with 95% homology. Treatments with desacetyl-ase (GR ligand), rifampicin (PXR ligand) and TCDD (AhR ligand) increased significantly mRNA levels of PON1 at 24 and 48 hours. In conclusion, PON1 is regulated positively by a mechanism that involves activation of the PXR, GR and AhR nuclear receptors.

Cytochrome P450 3A (CYP3A) is a family of drug metabolizing enzymes (DMEs) that metabolize up to 50% of clinically available therapeutics in the market. CYP3As are down-regulated in many diseases such as cancer, infectious diseases, cardiovascular disorders and hepatitis. These enzymes are induced upon activation of nuclear receptors such as pregnane X receptor (PXR), constitutive androstane receptor (CAR). Changes in CYP3A enzyme levels cause drug-drug interactions and adverse reactions in patients. However, the mechanism of CYP3A enzyme modula-
tion is poorly understood. To enhance the therapeutic outcome in patients, we determined the modulation of CYP3A in vivo by treating C57BL/6 mice with preg-
genolone 16-β carbonate (PCN) (mouse PXR activator) and Lipopolysaccharide (LPS). We found that PCN induced Cyp3a11 gene expression by approximately 16±1.6 folds, while LPS significantly attenuated the effect of PCN. Since LPS is known to down-regulate CYP enzymes via pro-inflammatory cytokine-dependent mechanism, we also treated HepG2 cells with TNF-α and IL-1β. Treatment of HepG2 cells co-transfected with CYP3A4-luciferase reporter and PXR plasmid with TNF-α/ IL-1β followed by rifampicin (human PXR agonist) treatment led to attenuation of rifampicin-mediated CYP3A4 activation. Recent studies have shown that mitogen activated protein kinases (MAPKs) are involved in regulation of DMEs. MAPKs comprise of JNK, ERK and p38-MAPKs. Interestingly, we found that treatment of HepG2 cells with the JNK inhibitor (SP600125) found that treatment of HepG2 cells with the JNK inhibitor (SP600125) reduced HO-1 and superoxide dismutase 2 but up-regulated catalase expression in liver, which may adversely affect the detoxification of chemicals. Our findings are complimentary to the literature report on obesity and drug metabolism in ob/ob mice (Cheng et al., 2008), and together provide important information for optimization of dosing strategies in obese patients with concomitant diseases.

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Environmental chemicals can be deposited in adipose tissue (AT) and have the potential to elicit adverse effects via an induction of oxidative stress (OS). OS in AT results in metabolic dysfunction of fatty acid transport, dysregulation of AT expandability and abnormal production of adipokines. The effects of environmen-
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hanced sensitivity of cancer cells to oxidative stress. These results demonstrate that metformin induces miR-34a to suppress sirt expression and increase sensitivity of cancer cells to oxidative.

1099 **Reactive Sulfur Species-Mediated Activation of the Keap1-Nrf2 Pathway by 1,2-Naphthoquinone through Sulfcenic Acids Formation under Oxidative Stress**

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Sulfhydration by a hydrogen sulfide anion and electrophile thiolation by reactive sulfur species (RSS) such as persulfides/poly sulfides (e.g., R-S-SH/R-S-Sn-H(R)) are identified as unique reactions in electrophilic signaling. Using 1,2-dihydroxypropanethione-4-thioacetaote (1,2-NQH2-SAc) as a precursor to 1,2-dihydroxypropanethione-4-thiol (1,2-NQ-SH) and a generator of reactive oxygen species (ROS), we demonstrate that protein thiols can be modified by a reactive sulfenic acid to form disulfide adducts that undergo rapid cleavage in the presence of glutathione (GSH). As expected, 1,2-NQH2-SAc is rapidly hydrolyzed and partially oxidized to yield 1,2-NQ-SH, resulting in a redox cycling reaction to produce ROS through chemical disproportionation reaction. The further oxidized form of 1,2-NQ-SH, 1,2-NQ-SOH, was detected by derivatization experiments with dimedone. This 1,2-NQOH-SOH modified Keap1 at Cys171 to produce a Keap1-S-S-1,2-NQH adduct. Subsequent exposure of A431 cells to 1,2-NQ or 1,2-NQH2-SAc caused an extensive chemical modification of cellular proteins in both cases. Protein adduction by 1,2-NQ through a thio ether (C-S-C) bond was slowly dechlorinated through GSH-dependent S-transarylation reaction, whereas that originating from 1,2-NQ-SH-SAc through a disulfide (C-S-S-C) bond was rapidly restored to the free protein thiol in the cells. Under these conditions, 1,2-NQH2-SAc activated Nrf2 and up-regulated its target genes that were enhanced by pretreatment with buthionine sulfoximine (BSO) to deplete cellular GSH. These results suggest that the RSS-mediated reversible electrophilic signaling through sulfenic acid formation under oxidative stress.

1100 **HGF Confers a Survival Response Against Ethanol and Aldehyde-Induced Toxicity in a Pancreatic Cell Line by a Mechanism Dependent on ERK Activation**

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1Universidad Nacional Autonoma de Mexico, Mexico City, Mexico, 2Faculty of Medicine, University of Tsukuba, Tsukuba, Japan and 3Medicine, Johannes Gutenberg University, Mainz, Germany.

Alcohol and its metabolite acetaldehyde, target practically all cell types in the pancreas inducing inflammation, cell damage and, eventually fibrosis. HGF has been lately positioned as a master regulator of the oxidative stress and cell redox status by activating the canonical signaling pathways such as Erk, Akt 1/2, or stat3. The aim of this work was to address the effect of HGF in the protection of RINm5F cells against ethanol (EtOH)- and acetaldehyde (Ac)-induced toxicity. RINm5F cells were treated with 100 mM EtOH or 200 mM Ac at different times of incubation in the presence or not of a pretreatment (12h) with 50 ng/ml HGF. Cell viability by CCK8 kit was measured and the expression of key cellular damage and survival markers were addressed by Western blot. NADPH oxidase activity and subunits expression were determined. Both toxics significantly decreased cell viability starting in the presence or not of a pretreatment (12h) with 50 ng/ml HGF. Cell viability was enhanced by HGF through GSH-dependent S-transarylation reaction, whereas that originating from 1,2-NQ-SH-SAc through a disulfide (C-S-S-C) bond was rapidly restored to the free protein thiol in the cells. Under these conditions, 1,2-NQH2-SAc activated Nrf2 and up-regulated its target genes that were enhanced by pretreatment with buthionine sulfoximine (BSO) to deplete cellular GSH. These results suggest that there is an RSS-mediated reversible electrophilic signaling through sulfenic acid formation under oxidative stress.

1101 **Nrf2 Response to Whole Smoke in Three-Dimensional (3D) Airway Cultures**


Lung diseases are frequently accompanied by molecular changes, including those associated with the Nrf2 signaling pathway. Cigarette smoke has been shown to activate this pathway in lung tissue. The goals of this study were to assess the effect of cigarette smoke on the Nrf2 promoter and on genes associated with oxidative stress, inflammation and metabolism in human 3D EpiAirway™ tissue models (MatTek, Inc.). Whole smoke exposures (8 – 64 minutes; 8 minutes/cigarette) with Kentucky Reference 3RF cigarettes were conducted under ISO conditions using the VITROCELL VC10 smoke exposure system (VC1). Viability and tissue integrity were assessed with the lactase dehydrogenase and transethelial electrical resistance (TEER) assays, respectively. Nrf2 promoter activation was determined by a luciferase assay, while gene expression changes were assessed via QRT/PCR at 6, 12, 18, or 24 hours post-exposure. Dose-dependent decreases in viability and tissue integrity were observed. Cell viability was > 90% for exposures up to 32 minutes (4 cigarettes); while a maximum response of >80% viability and diminished TEER were observed for the 64 minute (8 cigarettes) treatment group. Therefore, luciferase and gene expression studies were conducted between 8 and 32 minutes. Time- and dose-related increases in Nrf2 promoter activation were observed with levels exceeding 200-fold at the 12 and 18 hours post-exposure time-points. Statistically significant increases (p<0.05) ranging from 2 to >100-fold were observed across the time-course for genes associated with oxidative stress, inflammation and metabolism. Collectively, the data indicate that putative biomarkers of effect in the Nrf2 signaling pathway were responsive to cigarette smoke exposures in human 3D EpiAirway™ tissue models. These models may be useful in evaluating tobacco and aerosol exposure and may further understanding of the biological relevance of the smoke.
purpose of this investigation was to study the in vitro transport of simvastatin and pravastatin by OATP4C1, OATP2B1, OCTN2, P-gp, and BCRP in human kidney and heart. Human proximal tubule epithelial cells (hPTCs) and human cardiomyocytes (hCMs) were exposed to simvastatin (1, 25, and 50 μM), pravastatin (1, 25, and 50 μM), or control (DMSO) for 24 h in a collagen matrix (2D and CO2). Cells were harvested, mRNA and protein isolated, RT-PCR, immunoblotting, and immunofluorescence performed to determine expression and cellular localization of OATP4C1/SLC22A4, OATP2B1/SLC22A5, OCTN2/SLC22A5, P-gp/ABC1B, and BCRP/ABC1G. Statistical analysis was performed by one-way ANOVA and images processed by ImageJ. In hPTCs, SLC04A1 (uptake transporter) and SLC02B1, ABCB1, ABCG2, SLC22A5 (efflux transporters) mRNA was expressed. Transporter expression decreased with increasing doses of simvastatin. In contrast, SLC04A1, SLC02B1, and SLC22A5 mRNA expression increased with dosage of pravastatin. The expression of SLC02B1 in hPTCs was a novel finding. The mRNA expression of ABCG2 was down-regulated with increasing doses of pravastatin. Preliminary results showed variable ABCG2 expression and absence of other targeted transporters in hCMs. mRNA results were confirmed by immunoblotting and immunofluorescence. The results from the current study report the expression of statin transporters in human kidney and demonstrate the effects of simvastatin and pravastatin on regulating SLC04A1, SLC02B1, ABCB1 and ABCG2 expression. Ongoing studies will fully elucidate the effect of chronic kidney disease on statin transporter expression, function and risk of toxicity in kidney and heart.

1104 A Cost-Effective Targeted Sequencing Method for Monitoring Gene Expression

J. M. Yeakley1, N. Abdo2, G. Chappell2,3, P. Shepard1, I. Rusyn3 and J. M. Yeakley1

Monitoring expression of hundreds of genes in high throughput has been hampered by the cost of assays such as RNA-Seq or material limits for parallel qPCRs. We evaluated a highly multiplexed targeted sequencing assay, Templated Oligo Sequencing (TO-Seq), which uses ligation of detector oligos hybridized to RNA targets, sample barcoding during amplification, and pooling for next-generation sequencing. By limiting the assay to known targets and pooling samples, sequencing costs per sample are minimized and data analysis is simplified, enabling high sample throughput experiments. Cell lysates can be used without RNA purification or cDNA synthesis, and the assay exhibits good performance (log2 R2 value over 0.98 and –4 logs of dynamic range) while combining up to 384 samples per lane of an Illumina sequencer. In this study, TO-Seq was used to monitor 120 genes in human lymphoblastoid cell lines from 20 individuals. Cells were treated with 4 concentrations each of 9 compounds, including known toxins. Including controls, this amounted to 768 samples, which took 10 minutes to convert from FASTQ files to a table of reads on a standard PC without requiring sophisticated bioinformatics tools. The results showed good correlations within the same cell line. Responses to compounds varied across chemicals and concentrations, with examples of gene expression induction/repression, and patterns consistent with toxicity. In some cases, a treatment affected different sets of genes at different EC50s, indicating multiple mechanisms of action. In addition, CdCl2 induced MT2A expression by >12 fold, consistent with the literature, and showing that the assay yields biologically relevant results. We conclude that simultaneously monitoring expression of hundreds of genes using TO-Seq presents an effective method for investigating pathways underlying cellular responses to various compounds, and may prove to be a powerful, high plexity tool for high throughput applications that would otherwise be restricted by cost.

1105 Regulation of Cyclin D1 by Arsenic and miRNA Inhibits Adipogenesis

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Low-dose chronic exposure to trivalent arsenic (AsIII) in drinking water represents a global public health concern with established links to metabolic and cardiovascular disease, as well as cancer. While the link between arsenic and disease is strongly established, further understanding of the molecular mechanisms of its pathogenicity is required. Previous reports have demonstrated the ability of arsenic to interfere with adipogenesis, which may mediate its effects on metabolic disease pathogenesis. We hypothesized that microRNA are important regulators of most if not all mesenchymal stem cell processes which are dysregulated by arsenic exposure to impair lipogenesis. Arsenic increased the expression of miR-29b in a time and concentration dependent manner. OATP4C1 regulation or cDNA synthesis, and the assay exhibits good performance (log2 R2 value over 0.98 and –4 logs of dynamic range) while combining up to 384 samples per lane of an Illumina sequencer. In this study, TO-Seq was used to monitor 120 genes in human lymphoblastoid cell lines from 20 individuals. Cells were treated with 4 concentrations each of 9 compounds, including known toxins. Including controls, this amounted to 768 samples, which took 10 minutes to convert from FASTQ files to a table of reads on a standard PC without requiring sophisticated bioinformatics tools. The results showed good correlations within the same cell line. Responses to compounds varied across chemicals and concentrations, with examples of gene expression induction/repression, and patterns consistent with toxicity. In some cases, a treatment affected different sets of genes at different EC50s, indicating multiple mechanisms of action. In addition, CdCl2 induced MT2A expression by >12 fold, consistent with the literature, and showing that the assay yields biologically relevant results. We conclude that simultaneously monitoring expression of hundreds of genes using TO-Seq presents an effective method for investigating pathways underlying cellular responses to various compounds, and may prove to be a powerful, high plexity tool for high throughput applications that would otherwise be restricted by cost.

1106 Early Activation of Aryl Hydrocarbon Receptor Disrupted the Expression of Genes in WNT, BMP2/4, and TGFβ Signal Pathways and Inhibits Binding of Key Cardiac Transcription Factors to Target Genes

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The aryl hydrocarbon receptor (AHR) is a critical mediator of gene-environment interactions. The prototypical ligand for the receptor is the environmental pollutant TCDD. Genome-wide gene expression profiling suggests that activation of AHR by TCDD during embryonic stem cell (ESC) differentiation inhibited the upstream transcriptional regulators including TGF-β, BMP2/4 and WNT3a, and disrupted the expression of homeobox transcription factors (TFs). To further characterize how AHR involves in cardiomyocyte (CM) differentiation, we isolated Ahr+/+, Ahr+-, and Ahr−/− ESC from C57BL6 mice. TCDD treatment significantly suppressed the formation of contractile cardiomyocytes nodes during Ahr+/+ and Ahr+-/− ESC differentiation while Ahr−/− ESC were resistant to TCDD-induced cardiotoxicity. The most critical time window of TCDD toxicity for Ahr+/+ and Ahr+-/− ESC CM differentiation was during the early differentiation time, between day 0 and day 3. Supplementing the medium with anti-TGF-β antibodies, BMP4 or WNT3a during the first three days of differentiation successfully counteracted TCDD cardiotoxicity. Global expression analysis of differentiating cells from day 1 to day 5 showed that activation of AHR disrupts the concerted expression of genes involved in TGFβ, BMP2/4 and WNT signaling pathways. Using chromatin immunoprecipitation with antibodies to key cardiac TFs including NKX2-5, GATA4, TBX5, SHOX2, TBX20 and SMAD, we found that activation of AHR by TCDD significantly inhibits the binding of these key cardiac TFs to their downstream targets. These data suggest that AHR activation by TCDD induces the repression of genes involved in CM differentiation through inhibiting the binding of key cardiac TFs to their targets. One important function of the AHR during development appears to be the coordination of a complex regulatory network responsible for attainment and maintenance of cardiovascular homeostasis. Supported by NIEHS SR01 ES06273.
we highlight several novel genes that are specifically activated in response to the combined stress and may contribute to cancer promoting mechanisms associated with the tumor microenvironment.

1108 Using Authentic Institutional Data to Understand STEM Persistence and Identify Curricular Areas for Targeted Reform

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Increasing persistence of UG students in STEM fields is particularly relevant to toxicology because BS-level toxicology programs are limited and matriculation to graduate level toxicology programs draw from many different STEM majors. To address STEM persistence it is essential to understand conditions unique to each institution and develop processes to integrate needed changes into the culture. This study presents a model process to gather and analyze authentic student data that can inform university stakeholders and help identify curricular areas that may be targeted. In this study >8,000 incoming 1st yr. students (entering 2006-07) at a large urban research university were evaluated across multiple disciplines to assess their movement between majors, graduation rates, academic progression, credit hours earned and other key parameters using a cohort approach. Interestingly, attrition was not specific to STEM as <40% of students in STEM and non-STEM majors earned a degree in their original major choice. ~50% of students who remained in their original major earned a USF degree while >70% of all students who changed from their original major earned a USF degree. Detailed academic analytics of 553 biology majors who left the major but earned a non-STEM degree revealed that 168 (30%) took >7 STEM courses, left the major after 5.2 semesters and had GPA’s over 3.20 when they changed. This population was also much less likely to have participated in high-impact activities such as mentor-led research and only 55% enrolled in intro biology before changing. At graduation, their academic credentials were equal or exceeded students who earned the biology degree. These results show that a large number of qualified students are actively choosing to leave STEM and suggest that direct immersion of students into “majors” courses at matriculation, revising intro courses using inquiry-based pedagogies and engagement of the students early in high-impact activities such as research could inspire students to stay in STEM. Portions of this study were supported by institutional grant 52008123 from HHMI.

1109 “Lotions Are Not Potions” But Are a Good Medium to Bring Together Toxicology Mentors and Students: Results of an SOT K–12 Education Outreach Workshop


Dozens of high school students and science teachers participated in a half-day “Lotions Are Not Potions” workshop during the 2014 Annual Meeting of the Society of Toxicology in Phoenix, AZ. Sponsored by the SOT K-12 Subcommittee and Education Committee, the workshop focused on introducing students to the science and profession of toxicology. The objectives were achieved through interactive hands-on product safety activities, keynote presentations by prominent toxicologists, and dilutions, pH and environment, and ADME. At completion of the experiments, the students answered workbook questions related to the lessons. NorCal toxicologists were present to address the student and parent questions. One metric of program success was the participation of students from the previous year’s as teaching students this year. Their primary role was demonstrating the experiments to their peers. These teaching students also performed a Toxicology Skit written by ToxSA Students. These outreach activities had the following outcomes: introduction of toxicological concepts to K-12 students, instruction of undergraduate students in teaching toxicology, and increased awareness of the role of toxicology in the participant’s daily lives. We plan to expand our outreach in 2015 by organizing a Toxicology Summer Camp to students to increase overall awareness and knowledge of toxicology for area students.

1110 NorCal SOT K–12 Student Outreach: The Key Is to Have FUN FUN FUN!

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Students of all grades are taught science but the field and applications of toxicology are not included. Making science relevant and fun is critical to engaging young students and can be accomplished with hands-on toxicology experiments. The Northern California Society of Toxicology (NorCal SOT) sponsored K-12 education outreach events this year. All events were held at local schools. A K-5 event occurred in March 2014 and a K-12 event was held during a summer camp with 20-30 students actively participating. Students were introduced to Paracelsus, concept of “the dose makes the poison”, dose-response, differentiation between exposure and dose, factors that can affect dose, and different routes of exposure. They performed a hands-on experiment using night crawler worms and caffeine. The 6-8th grade activity took place in April 2014 in conjunction with Peninsula Girl Scouts and Berkeley Toxicology Student Association (ToxSA). Nearly 40 students attended. The girls performed simple hands-on experiments which were organized in 4 different modules covering concepts such as dose-response, concepts and dilutions, pH and environment, and ADME. At completion of the experiments, the students answered workbook questions related to the lessons. NorCal toxicologists were present to address the student and parent questions. One metric of program success was the participation of students from the previous year’s as teaching students this year. Their primary role was demonstrating the experiments to their peers. These teaching students also performed a Toxicology Skit written by ToxSA Students. These outreach activities had the following outcomes: introduction of toxicological concepts to K-12 students, instruction of undergraduate students in teaching toxicology, and increased awareness of the role of toxicology in the participant’s daily lives. We plan to expand our outreach in 2015 by organizing a Toxicology Summer Camp to students to increase overall awareness and knowledge of toxicology for area students.

1111 Development of Environmental Health Animated Tutorials for K–12 Students and Teachers


The U.S. National Library of Medicine (NLM) is the largest biomedical library in the world. It provides credible health information resources for scientists, health professionals, and the lay public. The NLM Division of Specialized Information Services (SIS) provides resources in toxicology, environmental health, chemistry, HIV/AIDS, as well as resources for specific populations, including K-12 students and their teachers. Focus group data and informal feedback during meetings and conferences indicated that science teachers were interested in utilizing multimedia online environmental health and toxicology resources which include animations. To address this need, in 2014 SIS implemented an animation production program to enhance the Environmental Health Student Portal (EHSP) with animations on the portal’s topics: water and air pollution as well as potentially hazardous chemicals in everyday environments. To date, SIS has produced animations educating middle school students about a range of topics, such as potential health effects of mercury, pesticides, lead, ozone, and particulate matter. The animations supplement the existing EHSP resources, including videos, games, activities, and lesson plans for middle school. The animations were produced with GoAnimate, a low-cost online tool. Production includes five steps: research, outline, storyboard, animation, and evaluation. This method allows for a high degree of content customization. The animations are reviewed by a subject matter expert for content accuracy prior to release. To view the animations, visit the NLMI/NIH YouTube Channel (https://www.youtube.com/user/NLMNIH), or the NLM Environmental Health Student Portal (http://kidsenvirohealth.nlm.nih.gov/).

1112 Using the Life Science Teaching Resource Community’s Digital Library to Enhance Toxicology Teaching

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The Life Science Teaching Resource Community digital resource library is a repository of more than 6,700 peer-reviewed teaching resources and 600 registered users. Enabling in the Archive Scholarly Vision and Change Program was a useful way to critically evaluate teaching methods and how they aligned with the National Science Foundation’s Core Concepts for Biological Literacy and Core Competencies. By the nature of toxicology, three of the six of the NSF core com-
Design and Assessment of Classroom and Laboratory Activities during a One-Week Toxicology High School Program

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The core concepts and practical applications of Toxicology are not included in the standard high school science curriculum. The Toxicology, Health and Environmental Disease (THED) Summer Program exposes high school students to the biomedical sciences, and introduces the fundamental principles of Toxicology through lecture, discussion and lab-based activities. Pre- and post-surveys assessed student motivation for participation and understanding of course topics. In the first 2 years of the program, 96 students entering 10th-12th grade participated. Students identified the three most important motivations for joining the program to be 1) gaining hands-on experience, 2) exploring their interest in science and 3) clarifying a college major. Female participants indicated a greater interest in clarifying their college major than males. Self-assessment of research abilities for all students revealed improved confidence in DNA isolation (39%), analysis of patient findings (27%), cell work and stains (26%) and oral presentation (17%). Further analysis by grade level showed rising sophomores had greater improvement in team work (19%), as compared to an overall 7% improvement. A True/False quiz tested student understanding of fundamental scientific concepts. The average score increased from 68% in the pre-survey to 81% in the post-survey. Most significant improvement occurred in the areas of histopathology and dose-response relationships. Interestingly, while upperclassmen reported greater satisfaction with most lab-based activities, sophomores tended to be more interested in social benefits, such as meeting other students. In conclusion, the THED Summer Program successfully introduced the principles of Toxicology to students, and enhanced laboratory-based skills. In addition, interaction with graduate program faculty and students gave participants insight into future training opportunities in the biomedical sciences.

The Science of Terrorism: A Model Interdisciplinary Undergraduate Capstone Course


The Science of Terrorism is an interdisciplinary, upper-level undergraduate elective taught at the U.S. Coast Guard Academy. Interdisciplinary courses are often capstone courses, drawing on concepts learned in disparate courses, encouraging effective thinking, developing multiple perspectives, motivating students to learn, and constructing meaning in the classroom (Lattuca 2004). As future officers in the U.S. Coast Guard, our students must be prepared to address complex scenarios requiring diverse skills such as oil spill response, humanitarian crises, border defense, and biological or chemical terrorism. To address this need, “The Science of Terrorism” was created as an elective capstone course drawing on faculty from across the campus. The course is composed of several modules: bioterrorism, which discusses likely bioterror agents such as smallpox, plague, and viral hemorrhagic fevers; chemical/toxicological terrorism, which covers toxicology basics up to mechanisms of action for common chemical weapons; nuclear and radiological terrorism, discussing radiation poisoning and dirty bombs; medical countermeasures, covering drug and vaccine development for agents of terror; cyberterrorism, discussing attacks on networked computer systems; and epidemiology and geographic information science (GIS), discussing the use of computer modeling and information to describe and respond to a terror event. Guest lecturers from outside of the science department discuss other complex issues, such as the mechanism by which science is used to inform policy and the ethics of the use of biological and chemical weapons. The course culminates in a multi-day table top exercise similar to those routinely used in the government and response communities. Student and faculty assessments will be discussed. Course materials, learning objectives, and other materials will be made available for interested faculty through digital libraries to facilitate adoption of parts of the course at their own institutions.

The Undergraduate Student’s Perspective on Toxicology Education

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Over the past few years, the SOT Undergraduate Education Subcommittee has been focused on enhancing undergraduate instruction in toxicology by gathering toxicology web resource materials for instructor use. During 2015-2016 academic year, the committee was interested in understanding the needs of undergraduate students in order to better tailor the resources available for both instructors and students. The Subcommittee in conjunction with the Education Committee circulated a survey to undergraduates and received 76 responses. These responses ranged from students at research institutions to small liberal arts colleges. From these responses it was found that 68.1% want to pursue a graduate degree and of these 66.7% are interested in specifically research in a toxicological field suggesting that exposing students to toxicology at the undergraduate level is especially important. This poster will also highlight other responses from this survey which include both educational resources the students would like to see implemented in the classroom but also activities and resources they would like to have available at the Regional Chapter and Annual Meetings. The poster will not only present the survey results but it will make recommendations for future undergraduate resources. Most importantly, this survey indicates that students are very interested in the field of toxicology but that their interests may be enhanced if additional resources and opportunities are made available.

“CREATTE” an Ecotax Research Program on a Shoestring

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Obtaining funding for a new research program can be difficult for non-tenure track faculty. Absent department or college support, researchers often self-finance scaled down projects to gather pilot data. The Undergraduate Office of Research (OUR) at the University of South Florida (USF) has created seed grants dubbed ‘CREATTE’ for professors who offer an authentic research experience to undergraduate students within a structured course. The funding covers equipment, supplies and graduate student support for project management. Careful planning can ensure a sustainable project within several terms. Each autumn, undergraduate students who enroll in Ecotox are given the opportunity to work on a CREATTE project. There are two project options: Option 1: Using Daphnia or Duckweed bioassays, students evaluate the toxicity of different concentrations of pesticides/herbicides commonly used in Florida. They establish an LC50 and then work back to a subacute/subchronic toxicity. Option 2: Students evaluate inter- and intraspecific variations in the toxicity of different concentrations of pesticides/herbicides commonly used in Florida by conducting bioassays on both Daphnia and duckweed then comparing the results. The Ecotox CREATTE project allows students to work from a defined research question, apply methods of inquiry relevant to the project to produce original findings and present the findings to peers and professionals in formal and informal venues such as the USF Undergraduate Research Colloquium and the regional SOT chapter and national chapter meetings. Their work also becomes part of a larger graduate student research project examining the effects of mixed pesticide exposures on common toxicological models. Programs such as this train students in the art of research and collaboration while simultaneously increasing opportunities for skill development among those considering research careers.

C. elegans Dose-Response Lab Using Avoidance Indices

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Introducing the dose-response concept using a simple animal model quickly engages undergraduate students. The C. elegans animal model lends itself to this demonstration since it is inexpensive, easy to maintain, and easy for students to handle. In this lab, students used the dry drop test to calculate an avoidance index and duration of response of C. elegans to a compound. Students were given stock solutions of each compound, then calculated and performed serial dilutions of their assigned compound. Worms were exposed to each concentration by placing a small drop of the compound in their path of forward movement. Working in pairs so that students were blinded, each student determined if the worm responded with avoidance and, if so, how long the worm engaged in backward movement (n=60).
per student). Students calculated the avoidance index and an average duration of response for each concentration of their compound. Data for each endpoint were calculated by averaging scores across students. The results revealed student perceptions of the challenges and successes of designing and implementing authentic learning activities. Overall, this approach engaged students in using appropriate model systems and presented their findings in formal scientific papers. Students received feedback throughout the process via formative writing assignments, rubrics, peer review, and large group discussions, which made the project manageable for the students and instructor. The ongoing low-stakes feedback revealed student perceptions of the challenges and successes of designing and interpreting original experiments and provided opportunities to relate their experiences to those faced by all researchers. Overall, this approach engaged students in the creative and analytical aspects of scientific inquiry while introducing them to several model systems and central toxicology concepts.

**1118 Risky Business: Incorporating Risk Assessment and Risk Management into an Undergraduate Environmental Toxicology Course**

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Environmental Toxicology is an upper division course at Northern Kentucky University which is part of the Environmental Science major and also serves students in Biological Sciences. Since most of the students in the course have not completed biochemistry or basic ecology, it is a challenge to introduce many principles of toxicology and the effects of toxicants on ecosystems. Further complicating curriculum development is the lack of any fundamental toxicology courses in the department. To overcome these obstacles, the course relies on evidence-based pedagogical techniques including team-based learning and case studies to reinforce the principles of environmental toxicology from Silent Spring to hydraulic fracturing and mountaintop mining. The culminating project includes elements of both risk assessment and risk management as students identify critical issues in environmental toxicology and propose cost-effective, socially acceptable solutions to complex problems. The project includes a written report and class presentation where students must defend their plans before their peers.

**1119 Molecular Biology Lab Class As a Vehicle for Teaching Environmental Toxicology**

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Relatively few undergraduate life sciences programs offer classes in toxicology. In the molecular biology lab course at Kenyon College, I compensate by drawing from my research expertise in molecular toxicology to construct a course with significant toxicology content. The semester is organized around a two-phase lab project. In phase I, students use RT-PCR with degenerate primers to amplify a partial CYP1A cDNA encoding CYP1A, the best-characterized gene induced by aryl hydrocarbon receptor (AHR) agonists, from a fish. Specimens are collected from local waters or obtained from bait shops and biological specimen suppliers. Fish are treated with a sub-lethal dose of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) or PCB mixture Aroclor 1254. The PCR product is cloned into a plasmid vector and sequenced. Sequences are subjected to bioinformatic and phylogenetic analysis to determine whether each is indeed CYP1A, the number of different CYP1A paralogs represented, and their orthology to known sequences. Importantly, the six species examined thus far have no prior CYP1A sequence in GenBank, so the class isolates these sequences to those faced by all researchers. Overall, this approach engaged students in using appropriate model systems and presented their findings in formal scientific papers. Students received feedback throughout the process via formative writing assignments, rubrics, peer review, and large group discussions, which made the project manageable for the students and instructor. The ongoing low-stakes feedback revealed student perceptions of the challenges and successes of designing and interpreting original experiments and provided opportunities to relate their experiences to those faced by all researchers. Overall, this approach engaged students in the creative and analytical aspects of scientific inquiry while introducing them to several model systems and central toxicology concepts.

**1120 Use of Multiple Model Systems to Develop Student-Designed Research Projects in an Undergraduate Toxicology Course**

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Recent reports on best practices in science education have highlighted the effectiveness of developing scientific competencies through student-directed inquiry and engagement in the scientific process (Discipline-Based Education Research, NRC, 2012; Vision and Change, AAAS, 2009). The upper level undergraduate toxicology course described here used original, student-designed research projects to introduce a breadth of toxicology techniques and concepts while engaging students in all aspects of the scientific process. During initial lab sessions, students in the course were introduced to commonly used model systems including Daphnia magna, Saccharomyces cerevisiae, Caenorhabditis elegans, and Raphanus sativus (radish). These labs addressed water and soil quality, mutagens, and oxidants as students collected and analyzed dose-response data, seed germination, simple behavioral analyses, and qualitative assessments of fluorescence. After these initial labs, small groups of students developed original hypotheses based on observations from the first labs, personal interests, and primary literature. They tested their hypotheses using appropriate model systems and presented their findings in formal scientific papers. Students received feedback throughout the process via formative writing assignments, rubrics, peer review, and large group discussions, which made the project manageable for the students and instructor. The ongoing low-stakes feedback revealed student perceptions of the challenges and successes of designing and interpreting original experiments and provided opportunities to relate their experiences to those faced by all researchers. Overall, this approach engaged students in the creative and analytical aspects of scientific inquiry while introducing them to several model systems and central toxicology concepts.

**1121 Student-Centered Learning: From Student, by Student, and for Student**


Student-centered learning pedagogy has shown to improve student’s academic performance. Student-centered learning instructional strategies include a wide range of activities with the common component of: involving students in doing things and thinking about the things they are doing. They must read, write, discuss, or be engaged in solving problems with higher-order thinking tasks as analysis, synthesis, and evaluation. The present report describes an activity in which student-centered learning was implemented as part of an undergraduate toxicology course: Analytical Toxicology (TOX416). The course is the required core course for undergraduate students who study Forensic Science with concentration on Forensic Toxicology at John Jay College of Criminal Justice. In this activity, teams of up to 2 students were charged to develop a presentation based on scientific journal articles concerning the analytical techniques and the related issues in forensic toxicology. Student presenters provided one question which they think the student audience should learn from their presentation. Student audiences were asked to write the answer during the presentation. Student audiences also were requested to ask the presenter(s) questions at the end of presentation. Strengths and limitations of the activity were noted. Student test score was improved after implementing this activity as compared to the test score before the activity. The major strengths of the activity were student became an active learner, student developed the habit of reading scientific journal articles, student obtained the capability of presenting scientific papers, and students engaged in learning by discussing and asking questions. A limitation of the activity is finding the appropriate assessment tool to assess this activity.

**1122 Implementing Authentic Science Learning through Multitiered Collaboration**


An authentic science learning model through collaborative learning suitable for large urban public colleges with limited resources was proposed and implemented in the course of Instrumental Analysis (science major), Environmental Science (non-science major), and Undergraduate Research at John Jay College, the City University of New York. The goal of this project is to enhance student learning of science concepts and skills by introducing them to real world problems and solutions. Using sustainability-chemistry as an example, this project allowed students at different academic levels to work collaboratively on field-based environmental projects to investigate pollutants in Hudson River and other EPA Superfund sites in New York City, establish close communication among participants and mentors, and develop positive attitudes towards science learning, and cultivate social awareness. This research is funded by The National Science Foundation NSF TUES DUE-1245314. Dec. 1, 2013.
Regional chapters have traditionally used K12 outreach to educate their communities about toxicology and risk assessment. SOT provides funds for a variety of regional chapter activities; including speakers at regional meetings, student travel to regional meetings, webinar development and strategic activities such as outreach events. Proposals are submitted to the SOT Council Subcommittee for review and approval quarterly. The application can be found on the SOT website. The application process is not onerous but does require some advance planning as chapters are limited to a single annual funding application. The support for strategic activities such as outreach requires a brief explanation of how the chapter’s activities further the SOT vision of creating a safer and healthier world by advancing the science of toxicology. Chapters should consult the SOT strategic plan to shape their answer. Metrics for measuring success are an important part of any application. The Southeastern Regional Chapter of SOT (SESOI) received strategic funding for outreach at Yeal of America hosts FAIR, a community event for persons living with disabilities. Historically, FAIR has experienced difficulty getting volunteers from scientific groups for outreach/education activities other than basic ‘living with disability’ programming. SESOI volunteers included the K12 Outreach representative and graduate and undergraduate members. Members of the US Toxification Student Association (TSA) were also present. Approximately 40 disabled children and adults rotated through hands-on activities that included elephant toothpaste, lava lamps and compostable toilet roll seedling planters. The TSA also ran a version of ToxLand – a walk through toxicology awareness game for children. This event increased discipline awareness, helped build for the future of toxicology through community outreach and allowed us to develop metrics on outreach impact.

### 1124 Tutorial Video Series: Using Stakeholder Outreach to Increase Usage of ToxCast Data


The limited amount of toxicity data on thousands of chemicals found in consumer products has led to the development of research endeavors such as the U.S. EPA’s Toxicity Forecaster (ToxCast). ToxCast uses high-throughput screening technology to evaluate thousands of chemicals for potential toxicity. At the end of 2013, U.S. EPA released ToxCast chemical data on almost 2,000 chemicals through the interactive Chemical Safety for Sustainability (iCSS) Dashboard. The iCSS Dashboard provides public access to the high-throughput screening data that can be used to inform the evaluation of the safety of chemicals. U.S. EPA recognized early in the development of ToxCast that stakeholder outreach was needed in order to translate the complex toxicological information featured in the iCSS Dashboard and data, with the goal of educating the diverse user community through targeted efforts to increase data usage and analysis. Through survey feedback and the request of stakeholders, a series of tutorial videos to demonstrate how to access and use the data has been planned, and the first video of the series has been released to guide data usage. This presentation will describe the video tutorial strategy including an overview of: 1) Stakeholder outreach goals and approach; 2) Planning, production, and dissemination of tutorial videos; 3) Overview of Survey Feedback; 4) Overview of tutorial video usage statistics and usage of the ToxCast data. This stakeholder-outreach approach is an ongoing effort that has improved public access, understanding, and usability of the iCSS Dashboard and ToxCast data. This abstract does not necessarily reflect U.S. EPA policy.

### 1125 Improving 3R Application in Regulatory Testing

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Despite all efforts made by the scientific community to use in vitro models as much as possible, biological, medical, and pharmaceutical research still relies heavily on animal testing. Today the progress in veterinary science has allowed us to better understand all factors influencing animal welfare and the impact it has on the quality of experimental results. Therefore, there is considerably changed the conditions for using animals for experimental purposes. This shift took place through the gradual establishment of ethics committees in all research institutes and with the application of the 3Rs. On the principle of the 3Rs to Replace animals, to Reduce the number of animals used and to improve the experimental conditions (Refinement), a real willingness to work together exists between researchers, technicians and ethics committee, realizing that ethics is not only a regulatory requirement but that each team member has something to gain in a collaborative approach that contributes to its success. Twelve years after the creation of the first ethical committee at our site, the ethical approach is supported by many members. Proposals with regard to the 3Rs are received from several sources, including scientists, operational but also from Pharmaceutical companies that have their own Ethical Policy, asking CROs to comply with. Several initiatives in relationship with 3Rs were launched either at a local level on site, either at a global level within Covance like sensitization/irritation in vitro alternative methods development, microsampling techniques, group housing solutions, caging improvement, food reward, implementation of rescue protocol to minimize the risk to loose animals due to an adverse effect already well known. All these initiatives considerably changed the approach of animal use within the Institution. In addition, Companies like Covance implement 3Rs recognition and award programs to promote, recognize and reward internal contribution towards the 3Rs. These programs give the opportunity to make visible to the whole Company all the efforts made by animal user to improve animal well being.

### 1126 Is Open Discussion on Animal Research Beneficial to Science and the Industry?

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The life sciences industry in the UK has been the focus of long running campaigns against animal testing from certain animal rights organisations which has led to companies closing their doors to the general public. Consequently, the public perception of the work carried out in the sector has been driven by one side of the argument with little explanation of the actual work performed in animal facilities and the reasons behind it. Public opinion has often been influenced by one sided and out of date information and photographs with no perspective. In the past few years there has been an initiative towards openness to expand the knowledge base of the general public and increase their understanding of why, when and how we use in animals in research. In the UK, companies involved in animal research were asked to sign up to a Concordat on Openness developed by the organisation “Understanding Animal Research”. It was launched in May 2014 with 72 signatories and is still growing. This initiative has also encouraged companies to work together which has resulted in opportunities to reduce the numbers, refine procedures and, where appropriate, replace the use of animals in research. This review examines some of the ways this initiative has been implemented and developed by a signatory company performing regulatory toxicology studies. It encapsulates some of the real gains which have been made in the 3Rs, gaining public understanding of the work and also attracting new talented scientists into the industry.

### 1127 Risk Communication and Perceptions for Highly Beneficial Medical Devices and Pharmaceuticals with Limited Scientific Evidence for Adverse Consequences


Negative news media reports regarding potential health hazards posed by implanted medical devices and pharmaceuticals can lead to a ‘reverse halo effect’, an overgeneralization of adverse events attributed to category products based on limited information. This ‘reverse halo effect’ can significantly impact rates of use and exploration of devices even with tenuous scientific support. We analyzed the occurrence of such a ‘reverse halo effect’ on four historical case studies: 1) intrauterine devices for contraception (IUDs); 2) silicone gel-filled breast implants (SGBI); 3) metal-on-metal hip implants (MoM); and 4) Tyabar, a pharmaceutical treatment for multiple sclerosis. We found that, although these products were removed from the market for different reasons, common factors altered public risk perceptions and patterns of continued use. First, negative reports on one product rapidly spread to affect the patterns of use and rates of exploration for dissimilar products or medical devices in the same category. Second, litigation-related media reports led to a sustained stigma on an entire category of drugs or medical devices regardless of the scientific findings pertaining to safety. Third, recovery of product safety reputation and prevalent use may take decades in the US as a result of litigation-driven stigma, while the same products may exhibit prevalent use and good safety records in other countries. And fourth, stigma may affect reported rates of product failure and/or exploration rates for extended periods and may be driven predominantly by patient fears rather than adverse clinical outcomes. We conclude that the ‘reverse halo effect’ associated with a stigma rather than an objective risk-benefit assessment of medical products can have important negative outcomes and we suggest a framework for thinking about risk communication that minimizes adverse clinical outcomes, reduces unintended social impacts, and reinforces safe and effective treatments.
Regulatory bodies within the USA and other countries use scientific data from various sources to assess the human safety of chemicals and drugs. We have conducted a survey to assess whether researchers in academic institutions are aware of how their published data can be used by regulatory bodies to make decisions regarding human health and safety. Researchers from academic institutions were randomly selected to receive an email request to complete a survey generated and analyzed using the web-based program SurveyMonkey. The survey contained ten questions designed to assess the researcher’s involvement in academic research and knowledge of the regulatory process. Fifty-three researchers completed the survey out of 500 who received the survey between September-October 2014. Of those who responded, 82% were PhD level scientists, 8% Master’s level scientists, and 10% Bachelor’s level scientists. Of the respondents, 94% were actively engaged in research. Greater than 90% of the respondents publish up to ten articles per year in peer reviewed journals and greater than 90% publish up to two articles per year in non-peer reviewed journals. The majority of respondents, 81%, indicated they are aware that their published data can be used in the regulatory process but 70% responded that they are only somewhat knowledgeable about regulatory processes, while 11% indicate no knowledge of regulatory processes. 65% of the respondents indicated that they have not received training about how the regulatory process uses scientific data. In conclusion, researchers actively engaged in academic research publish in peer reviewed journals and non-peer reviewed journals. While the majority of researchers are aware that their data can be used in the regulatory process, they indicate being only somewhat knowledgeable about how the data is used in the regulatory process and have not received training about the regulatory process. The results of this survey indicate a training opportunity in academic institutions on how published scientific data can impact the regulatory process and regulatory decision making.

We have developed a multidisciplinary one health approach for increasing environmental knowledge and building global health capacity for sustainable livelihood among youths and adults community members in Koinadugu District, Sierra Leone. The 10 year post war recovery and progress in Sierra Leone has been eroded by an unprecedented Ebola outbreak. The sustainability innovation program focused on real time knowledge exchange and building workable models of community and people based solutions to health problems that use locally available resources for addressing community needs. Our three week program involving Project 1808, Inc, University of Wisconsin Madison and University of Sierra Leone students, staff and faculty engaged and connect students, teachers, community members, university faculty, and various stakeholders through small pointed projects, this program increase health awareness among 400 students on addressing community health issues such as Ebola. It also enhanced global thinking and local action through knowledge transfer, building creativity and innovation culture for development. Overall, we observed increase engagement, ownership, leadership, greater sense of optimism, hope as well as civic responsibility regarding community health needs.

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Exposure to the environmental contaminant 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) adversely affects B cell activation and the differentiation into antibody-secreting plasma cells; however, the molecular mechanisms underlying this phenotype are still unclear. We hypothesized that TCDD-mediated suppression of B cell activation is mediated, in part, by SHP-1, a protein tyrosine phosphatase inhibiting signaling downstream of the B cell receptor. SHP-1 was initially identified as a putative target of the aryl hydrocarbon receptor (AHR) through a genome-wide CHIP-on-chip and gene expression analysis. SHP-1 mRNA and protein levels were upregulated in a TCDD-mediated concentration dependent manner in CD40

able period of exposure (CDC, 2014). Pregnant women are, about 20 times more likely to get listeriosis than the general population; with resultant negative maternal-infant outcomes possible, such as miscarriage, stillbirth, sepsis, and meningitis (WHO, 2014). Notable outbreaks of listeriosis in the United States have resulted in over 100 deaths since 1985 (CDC, 2014); and have been attributed to contaminated food sources, such as soft cheeses, processed meats, unpasteurized dairy products, fruits, and salad products. Public health agency function in the history of toxicologic outbreaks of listeriosis was investigated, including those of the Centers for Disease Control and Prevention (CDC), the Food & Drug Administration (FDA), and the U.S. Department of Agriculture (USDA). The “Listeria Initiative” from the CDC was piloted in the United States in 2004, as part of a national case-based surveillance system of laboratory-confirmed cases of human listeriosis to identify outbreaks rapidly, and decrease time to public health interventions. The FDA, the CDC and state and local public health professionals have investigated reports of outbreaks collaboratively across multiple states, when necessary. The USDA and the five regional Food Safety Information centers (The Food Safe) guide and participate in a Fighting BAC® public health campaign. Regional poison control centers can play a role in toxicovigilance (or real-time exposures), descriptive statistics provided by the National Poison Data System, and have the capacity for poison prevention information, in order to educate the public. The cooperation of multiple public health agencies during outbreaks, coupled with community health education of toxicologic principles and food safety can mitigate the risk of contamination with proper vigilance, especially of vulnerable populations.

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Food poisoning and pregnancy: Vigilance and Vulnerability

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Listeriosis is a public health concern, mainly associated with contamination of foods by the bacterium, Listeria monocytogenes. It is the third leading cause of death from food poisoning/food-borne illness. Pregnancy is a particularly vulner-
Taken together, this study for the first time, suggests impairment of early human B fold less than VH) and PAX5 (9.5 fold less than VH), during B cell development. Additionally, TCDD reduced the total number of lineage-committed B cells that were suppressed mRNA expression of two critical transcription factors, EBF1 (12.5 fold less than VH). Likewise, TCDD-treatment significantly (P<0.05) downregulated the expression of Igα and 3' RR induced immunosuppression. RNA sequencing (RNASeq) was used to identify human will trigger a conserved, B cell-specific mechanism involved in TCDD-induced suppression of primary B cell activation in a population of human donors. We hypothesize that incorporation of population variability will not linearize the dose-response curve as suggested by the NRC. This hypothesis will be tested via well-established TCDD-elicited suppression of immunoglobulin (IgM) secretion from CD40L-activated human B-cells. Primary human B-cells will be isolated from 50 unique human donors and exposed to increasing levels of TCDD (0.0001 - 30 nM). The number of IgM secreting B-cells and amount of IgM secreted will be assessed following TCDD exposure. Preliminary results indicate that TCDD has a significant suppressive effect on the number of IgM secreting B cells at the top TCDD concentrations (1, 10, and 30 nM) (p<0.05). Moreover, preliminary results suggest the dose-response better fits a non-linear, sigmoidal statistical model as opposed to a linear model based on Akaike Index Criteria (AIC) model comparison. Results also support that reactive oxygen scavengers can modulate TCDD-induced changes in IgM secretion. Taken together, the preliminary results support our hypothesis and further analysis of unique donors will better assess the effects of genetic variability on the dose-response curve.

1135 The Effects of Genetic Variability on the Shape of a Dose-Response Curve: 2, 3, 7, 8 Tetrachlorodibenzo-p-dioxin (TCDD)-Induced Suppression of CD40L-Activated Human Primary B Cells

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Recently, the National Research Council (NRC) hypothesized that the traditional nonlinear dose-response curve for non-cancer endpoints will become linear when the genetic variability of a population is taken into consideration. This hypothesis has not been adequately tested and, if correct, will have broad implications for risk assessment. The purpose of this project is to generate preliminary data to close this knowledge gap by determining the shape of the dose response curve of TCDD-induced suppression of primary B cell activation in a population of human donors. We hypothesize that incorporation of population variability will not linearize the dose-response curve as suggested by the NRC. This hypothesis will be tested via well-established TCDD-elicited suppression of immunoglobulin (IgM) secretion from CD40L-activated human B-cells. Primary human B-cells will be isolated from 50 unique human donors and exposed to increasing levels of TCDD (0.0001 - 30 nM). The number of IgM secreting B-cells and amount of IgM secreted will be assessed following TCDD exposure. Preliminary results indicate that TCDD has a significant suppressive effect on the number of IgM secreting B cells at the top TCDD concentrations (1, 10, and 30 nM) (p<0.05). Moreover, preliminary results suggest the dose-response better fits a non-linear, sigmoidal statistical model as opposed to a linear model based on Akaike Index Criteria (AIC) model comparison. Results also support that reactive oxygen scavengers can modulate TCDD-induced changes in IgM secretion. Taken together, the preliminary results support our hypothesis and further analysis of unique donors will better assess the effects of genetic variability on the dose-response curve.

1136 Differential Modulation of the Human 3' IgH Regulatory Region Enhancers by TCDD and B Cell Stimulation in Mouse and Human B Cells

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The persistent environmental pollutant 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is a potent inhibitor of immunoglobulin (Ig) expression in animal models. We have identified the IgH 3' regulatory region (3'RR) as a sensitive target of TCDD, which may explain TCDD's inhibitory effect on Ig expression. The 3' RR contains three enhancers and is duplicated in the human gene, whereas only one 3'RR exists in mice but it contains four enhancers. The human hs1,2 enhancer contains a polymorphic region, which does not exist in mice, and is associated with various immunological disorders. The polymorphic region contains a core binding motif of a dioxin response element (DRE) that is thought to be the binding site of the AhR, for which TCDD is a high affinity ligand. The objective of this study was to understand how the unique structural qualities of the human 3' RR affect transcriptional activity. Utilizing a luciferase reporter plasmid, we found the human hs1,2 enhancer exhibited a strong activating response to TCDD treatment in mouse and human cells, which appears to be mediated by an NF-1 binding site rather than the DRE. Additionally, B-cell stimulation increases human hs1,2 enhancer activity in mouse B cells, but decreases activity in human B cells. It is unknown if these responses are an artifact of removing the hs1,2 enhancer from the context of the other 3'RR enhancers. Therefore, we also constructed a new luciferase reporter with three enhancers as well as the intervening sequence between the hs3 and hs1,2 enhancers. An in silico analysis indicates this additional sequence may have functional properties due to the presence of DRE-like and AP1 binding sites, which have never been evaluated in this context. Studies with this plasmid are ongoing and should provide further insight into the function of the 3' IgH RR and its individual enhancers, thereby shedding new light on the etiology of specific human diseases and the immunotoxicity of AhR ligands. (Supported by NIH R01ES014676, WSU Emerging Sciences Grant)
2,3,7,8 Tetrachlorodibenzop dioxin (TCDD) is a potent and persistent environmental toxin that is known to inhibit immunoglobulin (Ig) gene expression in various animal studies. TCDD is thought to mediate its effects through the aryl hydrocarbon receptor (AhR) and thereby a subsequent alteration in gene expression. The mouse 3'Igh regulatory region (3'IghRR) is a sensitive transcriptional target of TCDD that may mediate the inhibitory effect of TCDD on Ig expression. Suppression of antibody production in response to TCDD in animal models suggests that human B cells could also be a sensitive target of TCDD. The primary goal of this study is to determine the effect of TCDD on human Ig expression and class switch recombination (CSR) utilizing a human Burkitt lymphoma cell line (CL-01) model that can be activated to secrete IgM and undergo CSR to IgG, IgE antibody isotypes. Ig transcripts were evaluated using RT-PCR and subsequent agarose gel electrophoresis revealed the presence of IgM, IgG1 and IgA1 transcripts when treated with Toll-like receptor ligands or LPS-4 and CD40L. Sandwich ELISA demonstrated an inhibitory effect on IgM and IgG antibody secretion with TCDD treatment. An AhR antagonist reversed the effect of TCDD on IgG, supporting a functional role of AhR in human Ig expression. However, current results may suggest the cells have already undergone a spontaneous class switch rather than being induced to CSR by the current stimuli. Resultantly, ongoing efforts are focused on trying to establish the appropriate stimuli responsible for inducing CSR and evaluating the effect of TCDD and AhR antagonism on the ability to induce CSR versus effects on Ig expression in pre-switched cells. As the human 3'IghRR is involved in many autoimmune disorders and also in the regulation of Ig expression, current studies could help elucidate not only the role of the AhR in Ig gene expression but also the relevance of AhR ligands and the 3'IghRR in autoimmune disorders with a significant antibody component. (Supported by NEIHS R01ES014676 and WSU Emerging Science Grant)

The aryl hydrocarbon receptor (AhR) is a sensor of small chemicals and orchestrates responses as diverse as xenobiotic catabolism and immune responses. We and others have shown that the inducible expression of the immunosuppressive enzyme indoleamine-2,3-dioxygenase (IDO) is absent in AhR-deficient Langerhans cells and BM-derived dendritic cells (DC). IDO generates kynurenine from tryptophan, DC thus can curb availability of the essential amino acid tryptophan and thereby create an immunosuppressive micro-milieu. In addition kynurenin, as an AhR ligand, can ensure continuing AhR activity. IDO expression can be triggered by LPS via the toll-like receptors and IFN-γ/STAT1. The failure of AhR-deficient DC to induce IDO is not due to a defect in the respective signalling cascades, as toll-like receptors were detectable, and LPS or IFNγ could induce TNFα, IL6, CXCL10 and IRF1 or the IDO downstream enzyme kynureninase. We therefore asked whether prostaglandin E2 (PGE2) signalling, which is known to induce IDO mRNA, cooperates with AhR-signalling in IDO mRNA induction. Treatment of wild-type bone-marrow derived DC with LPS and PGE2 enhanced IDO in a synergistic fashion, while PGE2 treatment alone induced IDO only moderately. Abrogation of IDO induction in AhR-deficient mice could be overcome by stimulation with LPS and PGE2. Inhibition of PGE2 with acetylsalicylic acid led to a decreased IDO induction of wild-type BM-DCs and no detectable expression at all in AhR-deficient DCs. Thus, PGE2 contributes to shifting DC towards IDO production and thus ultimately down-modulation of inflammation. Moreover, PGE2 has a role in by-passing the need for AhR-signaling in IDO induction by DC. Our results reveal a novel cross-talk of AhR signalling with other inflammatory pathways in dendritic cells.

The aryl hydrocarbon receptor (AhR) is a ligand-activated cytosolic transcription factor that regulates xenobiotic-metabolizing enzymes. It mediates the toxicity of various environmental chemicals, such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). TCDD inhibits the differentiation of B-cells into antibody-secreting cells and inhibits immunoglobulin (Ig) expression in various animal models. We have previously determined that TCDD-induced inhibition of the mouse Ig heavy chain gene is AhR-dependent. This inhibition may be mediated by binding of the AhR to dioxin response elements (DREs) within the 3'Igh regulatory region (3'IghRR) and inhibition of 3'IghRR activity, a significant transcriptional regulator of Ig expression. However, there are structural differences between the mouse and human 3'IghRR. The mouse contains four enhancers (hs3A; hs1,2; hs3B; and hs4), whereas the human contains three (hs3; hs1,2; and hs4). In the human hs1,2 is known to be highly polymorphic and has been associated with several autoimmune diseases. The current study focuses on elucidating the role of the AhR in human Ig expression. Using two different shRNA constructs (shAhR11

The aryl hydrocarbon receptor (AhR) is known to be a key regulator of immune function, particularly in the context of immune suppression and induction of regulatory T cells (Tregs). Our study aimed to investigate the role of AhR in the immune response to TCDD, a potent environmental contaminant known to induce immunosuppression. We found that TCDD treatment of AhR-positive mice results in a reduction in splenic CD4+ Foxp3+ regulatory T cells, indicating a role for AhR in promoting Treg development.

Furthermore, we observed a decrease in IFN-γ production in TCDD-treated mice, suggesting a shift towards a more regulatory immune response. This aligns with previous studies demonstrating the importance of AhR in immune regulation, particularly in the context of immunotoxicity.

Our findings not only highlight the role of AhR in immune suppression but also provide insights into the mechanisms by which TCDD affects the immune system. This understanding could have implications for the development of strategies to mitigate the immunotoxic effects of environmental pollutants.
& shAhR12) and the chemical AhR antagonist (CH-223191), we disrupted the AhR signaling pathway in a human B-cell line (CL-01). Interestingly, decreased AhR protein levels and function minimally affected TCDD-induced inhibition of IgM secretion but reversed the inhibitory effect of TCDD on IgG secretion. Furthermore, disruption of AhR antagonist induced elevated FICZ secretion in stimulated B cells, which was not replicated by the AhR knockdown suggesting a mechanistic difference between the chemical antagonist and AhR knockdown. With the growing number of human immune-related disorders correlating with the polymorphic hI2 enhancer, understanding the role of the AhR in IgH activation and Ig expression could provide insight into potential therapeutic interventions. (NIEHS RO1ES104676): SAGM

1142 Aryl Hydrocarbon Receptor Ligand-Specific Effects on Cytokines in TLR-Activated Dendritic Cells
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The AhR has been described as a critical ligand-binding transcription factor modulating the development, differentiation and function of dendritic cells (DC) and T cells. Different ligands of the AhR have been reported to have anti-inflammatory or immunostimulatory effects on the development of pathological immune responses and endpoints. The effects of the specific ligand and the downstream activated AhR pathway on immune regulated endpoints in vivo and in vitro is rather complex and may depend on the presence and participation of the specific cell types and their environment. In the current study we investigated the effects of various ligands of the AhR such as TCDD, 3HCC, Kyn and FICZ on the expression of cytokines in TLR activated and non-activated DC. Results show, that TCDD and FICZ, but not Kyn or 3HCC, significantly induced the expression of TGFα1, TGFα1B and AhR in human DC. The cytokines interleukin (IL)-β and IL-8 were also significantly induced by the treatment with TCDD and FICZ alone. However, in TLR activated DC we found antagonistic and agonistic effects of AhR ligands depending on the TLR ligand and the target gene. The TLR-mediated induction of IL-6 and IL-12 for instance was significantly suppressed by TCDD and FICZ whereas IL-1β and IL-8 were synergistically induced by AhR ligands in TLR activated DC. Chemokines such as CXCL2 and CXCL3 were significantly increased by the TLR8 ligand CL075, but only moderately elevated by ligands of TLR4 (LPS) and TLR3 (poly(C)). On the other hand, CXCL10 was significantly induced by LPS and poly(C) only and antagonized by TCDD, FICZ as well as 3HCC and Kyn. The results indicate that the specific TLR ligands may direct the outcome of AhR signaling with the NFκB pathway and the regulatory or immunostimulatory response in DC.

1143 Low-Level Arsenic Exposure Affects the Innate Immune Response in Embryonic Zebrafish
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Inorganic arsenic (AsIII, AsV) is a naturally occurring pollutant and known human carcinogen. Over 100 million people worldwide, including 15 million in the U.S., are chronically exposed to As-polluted water at levels higher than the U.S. Environmental Protection Agency maximum of 10 ppb. Exposed individuals are at higher risk of numerous diseases, and form an alarming global public health problem. Although arsenic is a suspected immunosuppressant, there has been little progress identifying the molecular mechanism. Our lab found that low-level arsenic exposure alters transcriptional pathways regulating the innate immune system. To better understand how arsenic alters innate immunity, transgenic zebrafish embryos, in which neutrophils (NP) express green fluorescent protein, were exposed to 10 and 100 ppb As at 5 hours post fertilization for 96 hours. Afterwards, Edwaardiliana tarda (E. tarda) bacteria were microinjected into the larval otic vesicle and NP migration was measured. Consistent with previous studies, iAs exposure abolished NP migration. Next, we grew E. tarda in 10 and 100 ppb iAs, injected it into the otic vesicle of unexposed larvae, and observed a robust immune response, suggesting that iAs affects innate immune function rather than masking the pathogen. We then analyzed NP population number by flow cytometry and found no difference between naive and exposed zebrafish, further supporting a defect in immune function, possibly related to cell-cell signaling. Analysis of the transcriptome and proteome of NPs isolated from naive and exposed zebrafish is underway, as are a number of functional assays to determine the level at which NP function is adversely affected by exposure to iAs. Our results support the hypothesis that iAs functions as an immunosuppressant, thus increasing susceptibility to disease and death, and demonstrate the versatility of zebrafish for immunotoxicology studies. Similar studies are planned for macrophages.

1144 Effects of Single Source versus Multisource Copper Indium Disulfide Nanoparticles on Mouse Macrophage Gene Expression
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Due to a wide array of applications for nanoparticles, the industry is currently growing rapidly. However, the industry is growing faster than the toxicological research to compliment it. There are numerous types, formations, and production methods for nanoparticles. This study examined the effects of all of copper indium disulfide nanoparticles, produced in two different ways, on mouse macrophages. Single source methods of production are typically more expensive but have higher purity and yields, while multi source methods are typically easier and cheaper. However, the two production methods could result in particles with completely different toxicological effects. Using MoSeq cDNA sequencing we identified a profile of genes differentially expressed in macrophages exposed to the different nanoparticles. Multisource copper indium disulfide nanoparticles resulted in 26 genes differentially expressed compared to the untreated control, where 16 of these genes are indicative of immune and inflammatory response. Single source copper indium disulfide nanoparticles resulted in 12 genes up regulated, but not related to immune or inflammatory response. These results suggest the single source method of production for copper indium disulfide nanoparticles yields a less immunotoxic particle than the multisource method.

1145 Cadmium-Associated Dysregulation of Proinflammatory Cytokines in Human Placenta
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OBJECTIVE: To identify the associations between cadmium (Cd), zinc (Zn), and pro-inflammatory cytokine levels in human placenta.EXPERIMENTAL PROCEEDURES: Human placental tissue samples were collected from 20 normotensive and 20 preeclamptic women. Cd and Zn metals levels were quantified by inductively coupled plasma mass spectrometry. Cytokine levels were measured using enzyme-linked immunosorbant assay and relationships between Cd and Zn metals, and cytokine levels were assessed via multivariable regression model analyses. Additionally, human JEG-3 trophoblasts were dosed for 48 hours with 2 μM Cd followed by extractions of total RNA. Extracted RNA were used in nanostring technologies immune panels to assess gene expression profiles in Cd dosed JEG-3 cells. RESULTS: In human placenta, interactive effects were observed between Cd, Zn, and cytokine expression levels in preeclampsia that were not seen in normotensive women. In relationship to placental Cd levels, preeclampsias displayed increased levels of the pro-inflammatory cytokine IL-17 and decreased levels of the anti-inflammatory cytokine IL-10. In JEG-3 cells 19 immune related genes were significantly altered in response to Cd, five are known to be associated with preeclampsia including CCL2, CDKN1A, CD64, SELE and TNFSF10. CONCLUSIONS: Identification of Cd and Zn associated differential expression of pro-inflammatory cytokine levels may have implications for immune function in the placenta. These results identify a novel relationship between placental Cd, Zn, and cytokine protein levels in preeclamptic women.

1146 Inorganic Arsenite Suppresses Phosphorylation of the Kinases Syk and PI3K in Mast Cells
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Exposure to arsenic (As) causes many types of human diseases worldwide. Mast cells are ubiquitous in the human body and play crucial roles in numerous physiological processes and diseases. Upon antigen or Ca2+ ionophore stimulation, mast cells secrete myriad effectors, such as histamine and β-hexosaminidase, from their granules. Using rat mast cells (RBL-2H3), we previously demonstrated that inorganic arsenite inhibits antigen-mediated mast cell degranulation. Here we show that arsenate causes similar effects. We previously found that As affects neither stimulated F-actin ruffling nor degranulation stimulated by the G-protein activator compound 48/80. Also previously, we found no As effect on degranulation stim-
ualted by either A23187 Ca2+ ionophore or thapsigargin, both of which bypass early mast cell signaling events. Taken together, all these findings suggest that arsenic’s target in mast cells lies upstream of Ca2+ influx. Indeed, we found that As inhibits antigen-stimulated Ca2+ influx. Thus, we present new ELISA data on As effects on the early signaling molecules Syk kinase, phospholipase C-γ (PLC-γ), and phosphoinoside 3-kinase (PI3K). We found that As interferes with phosphorylation of Syk. Therefore, we investigated downstream substrates of Syk and found the antigen-stimulated phosphorylation level of the p-85 subunit of PI3K is decreased by 750ppb As exposure, whereas the phosphorylation level of PLC-γ is unaffected by As. These data provide a mechanism underlying As inhibition of mast cell degranulation and suggest that the important kinase Syk and PI3K may be affected by As in numerous other cell types.

1449 Disruption of Lipid Raft Membrane Integrity by Benzo(α)pyrene Exposure Increases Macrophage Susceptibility to HIV-1 Infection
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Macrophages, cells of the innate immune system, perform surveillance and police their surroundings to clear pathogens from the host. Previous studies suggest that macrophage membranes are an important target for viruses and particulate matter. Previous research also suggests that benzo(a)pyrene, [B(a)P], an environmental toxicant, modulates membrane cholesterol and may impact viruses like HIV-1, due to a requirement for membrane cholesterol and intact lipid rafts allowing for entry into host cells. CD4+ monocytes were cultured in the presence of M-CSF and B(a)P (1, 5 or 10μM) for 6 days. Macrophage lipid rafts were isolated by sucrose density gradient centrifugation and 1 μl fractions were collected. The raft fractions were identified by CD59, a raft protein, and were fractioned for cholesterol and B(a)P metabolites. Cholesterol oxidase assays after HPLC indicated that the largest quantities of B(a)P metabolites and cholesterol were found within the lipid raft fractions. Positive infection of MDMs with M-tropic, BAL strain HIV virus was measured by luciferase assay and macrophages exposed to 10μg B(a)P were found to be infected with HIV at a rate 3-fold higher as compared to control. Internal p24, a unique HIV capsid protein indicative of infection within the cells, was quantified by FACS analysis. MDM’s exposed to 5 and 10μM B(a)P were found to have an average increase of internal p24 protein of at least 30% as compared to control. Taken together, these results suggest that B(a)P exposure confers an enhanced susceptibility phenotype on macrophage membranes to HIV-1 infection.

1450 Inhibition of Acute Phase Response after Bacterial Infection in Zebrafish Developmentally Exposed to Benzo[a]pyrene
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Benzo[a]pyrene (BaP) is a potent environmental contaminant and known mammalian immunotoxicant. A number of studies have indicated a role of chronic BaP exposure on immune modulation in aquatic organisms. A relatively unexplored area for aquatic toxicology, involves the assessment of innate immune response potential postdevelopmental exposure to BaP. The aim of this study was to identify biomarkers for BaP mediated innate immune modulation during development using zebrafish as a model organism. Six hours post fertilization, zebrafish embryos were acutely exposed to three different concentrations of BaP (0, 5, 500 ng/L) prepared in embryo media with 0.1% DMSO as vehicle. At 48 hpf, BaP was removed and larvae were immersed challenged with 108 cfu/ml of Edwardsiella tarda, an opportunistic bacterial pathogen of fish for four hours. Twenty-four hours post infection, larvae were sacrificed for the isolation of RNA. RNA was used for quantitative RT-PCR expression profiling of genes that are vital for host innate immune function (TNFα, HAMP1, SAA & IL1β). Our results indicated significant increase in expression of TNFα, HAMP1 and SAA after infection with E. tarda in vehicle exposed larvae as compared to the uninfected larvae (p<0.01). Exposure of BaP (both concentration) however significantly inhibited the induction of acute phase gene, SAA by 28-30%. Role of other genes in BaP mediated innate immunity suppression is also investigated. These results indicate a significant suppression of the acute phase genes due to developmental exposure to BaP that can be used as a biomarker for future studies.
1151 Influence of ELF-MF on the Juvenile Immune System of CD-1 Mice
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In the context of the association between childhood leukemia and extremely low frequency magnetic fields (ELF-MF) exposure, the European project ARIMMORA aims to gain mechanistic insights on the impact of ELF-MF exposure in in vitro and in vivo models. In our studies the biological effects of ELF-MF exposure especially on changes in the juvenile immune system in C57CD1(ICR) mice were investigated. The mice were exposed (20 h/d, 7 d/week) to continuous linear polarized sine-shaped 50 Hz electromagnetic fields of 10 μT, 1 mT, and 10 mT, starting with pregnant dams on day 10 p.c. and continuing to F1 offspring up to 90 days of age. At different time points, samples of spleen and peripheral blood were taken to evaluate potential changes in the number and activation status of T-cell, B-cell, and monocyte/macrophage compartments ex vivo by means of flow cytometry and measurement of cytokine secretion by ELISA. On day 28, a reduced number of CD8+ cytotoxic T-lymphocytes was seen in peripheral blood at all exposure levels compared to sham treatment. No alterations of this cell compartment were observed in spleen of all treatment groups. The effect was moderate but significant. Whether the observed reduction of T-lymphocytes has a functional effect cannot be definitely concluded from these studies. In addition, none of the tested doses of the ELF-MF signal mediated a significant induction of microneurites in the peripheral blood erythrocyte fraction. The absence of an effect speaks against a direct DNA-damaging potential of ELF-MF exposure. The research leading to these results has received funding from the European Union’s Seventh Framework Programme (FP7/2007-2013) under grant agreement no. 282891.

1152 Exposure to Produced Water from “Fracking” Induces Immunotoxicity in Adult Male Mice
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1114 Embryonic Thymocyte Responsiveness to HPTE Is Dependent upon Concurrent TCR Signaling, but Not Estrogen Receptor Signaling
The immune system and the thymus in particular are sensitive to the toxic effects of environmental chemicals, such as the organochlorine pesticide methoxychlor. Though largely banned from use, these chemicals and their breakdown products can still be found in the environment. Inducer of thymus toxicity is known to be a risk for the thymus responds to exposures with atrophy and death of thymocytes. Loss of thymocytes or alteration of their maturation pathway can cause long-term immune dysfunction and immune-related disease. An important issue that remains unresolved is the mechanism whereby organochlorines such as methoxychlor and its primary metabolite HPTE induce cell death. Using an in vitro model of thymocyte differentiation that mimics in vivo events during development, we examined the effects of HPTE on embryonic thymocyte survival and differentiation in C57BL/6 mice. To address the mechanism, we also determined whether apoptosis was induced and whether classical or nonclassical estrogen receptors were involved. Lastly, we probed the relationship between signaling through the T cell receptor (TCR) and susceptibility to those effects. We found that although apoptosis was observed in thymocytes, the death that occurred appeared to be only partly due to apoptosis. A small percentage of dead and dying cells showed the classical signs of apoptosis, including annexin V staining and caspase-3 activation. In addition, in experiments in which the classical receptor ER alpha, or the nonclassical receptor GPR 30 was inhibited, rescue from death was incomplete. Finally, when signaling through the TCR occurred concurrently with HPTE exposure, significant death occurred. Whereas, when TCR signaling occurred prior to exposure, death was less pronounced. Taken together these data suggest that HPTE may not be mediating its effects through estrogen receptor signaling, but may depend in part upon T cell receptor signaling to induce death.

1153 Protein Phosphorylation Profiling Identifies Potential Mechanisms for Direct Immunotoxicity
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Signalling networks are essential elements that are involved in diverse cellular processes. One group of fundamental components in various signalling pathways concerns protein tyrosine kinases (PTKs). Various toxicants have been demonstrated to exert their toxicity via modulation of tyrosine kinase activity. The present study aimed to identify common cellular signaling pathways that are involved in chemical-induced direct immunotoxicity. To this end, an antibody array-based profiling approach was applied in order to assess the effects of five immunotoxicants (lin-dane, dicofol, A. TBT, TBTQ, TBTQ, and DON), two immunomodulatory drugs (rapamycin and mycophenolic acid), and two non-immunotoxic control chemicals (urethane and mnnitol) on the phosphorylation of 28 receptor tyrosine kinases and 11 crucial signalling nodes in Jurkat T cells. The phosphorylation of ribosomal protein S6 (RPS6), the kinases Akt, Src, and p44/42 was affected by at least three of the immunotoxicants and/or immunosuppressive drugs, with the largest effect observed for RPS6. Flow cytometry and Western blotting were used to further examine the effect of the immunotoxicant TBTQ on the components of the mTOR/p70S6K-RPS6 pathway. This revealed that both TBTQ and the mTOR inhibitor rapamycin inactive RPS6 but via different mechanisms. Finally, analysis of the protein phosphorylation and previously obtained transcriptome data suggest that TBTQ exerted its effect on cell proliferation through a mechanism different from the effect of TBTQ was further confirmed by in vitro Trans-well based chemotaxis assay.

1154 Enhanced Histopathology in an Immunotoxicity Tiered Testing Strategy
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Enhanced histopathology (EH) of the immune system is a systematic method for examining potential chemical-induced changes in cellularity or architecture in individual compartments of lymphoid organs using semiquantitative descriptive terminology. EH was incorporated into the immunotoxicity tiered testing approach for nine chemicals to obtain greater insight about the putative target cell populations, the critical site(s) within affected immune organs, and the correlation between histopathology and functional measures in toxicity screening. For each chemical, a board-certified toxicological pathologist conducted the EH assessment. Pathology findings were then peer-reviewed by a National Toxicology Program (NTP) pathology working group. Immunotoxicity studies included evaluation of innate, humoral, and cell-mediated immunity. Treatment-related histopathological alterations were detected in four NTP studies. Dibenzanthracene treatment resulted in microscopic abnormalities in the T-cell compartments (4 of 8 mice, 2500 pg/kg) of the thymus, spleen and lymph nodes, loss of mature lymphocytes, increased numbers of tingible-body and pigmented macrophages in spleen and lymph nodes, and decreased numbers of follicle germinal centers in splenic white pulp, with concomitant decreases in spleen weight, all spleen cell populations, T-dependent antibody responses, cell-mediated immunity, and resistance to Plasmodium yoelii. EH identified lesions in two studies with minimal adverse immunological effects. microscopic abnormalities in the T-cell compartments of the thymus, spleen, and
lymph nodes were diagnosed in nano-resveratrol treated mice, but no effects were observed on immune function. There were no treatment-related lesions identified in three studies; those chemicals induced some limited immunological effects. There were no cases of chemicals that induced clear immunotoxicity without histological lesions. Using the compartmentalized, histologic evaluation of EH can contribute to the screening of potentially immunomodulatory compounds and to the identification of specific cellular targets and effects.

**1156 Significant Differences in Specific Immune Cell Populations, in Particular CD8+ T Cell Subsets, in Chinese-Origin vs. Mauritius-Origin Cynomolgus Monkeys**

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The unique ability of flow cytometry to simultaneously examine the intricate details of multiple, specialized immune cell subsets allows for documentation of species variation. The objective of this study was to compare the frequency of 20 immune cell subsets between 2 different strains of cynomolgus monkeys (Chinese-origin vs Mauritius-origin) using single platform flow cytometry methods. Ten (5/sex) peripheral blood samples from Chinese-origin and Mauritius-origin animals were analyzed for the following cell subsets: total T cells, helper T cells, cytotoxic T cells, B cells, Natural Killer cells, central memory CD8+ T cells, naïve CD8+ T cells, effector memory CD8+ T cells, central memory CD4+ T cells, naïve CD4+ T cells, effector memory CD4+ T cells, naïve B cells, memory B cells, germinal center B cells, regulatory T cells, total monocytes, classical monocytes, non-classical monocytes, neutrophils, and basophils. Both the relative percentages and absolute counts of each subset were compared between monkey origins. A pairwise t-test was used to compare differences (p < 0.05) between individual populations followed by Bonferroni’s method to correct for multiple hypothesis testing. Results demonstrate there were no significant differences in the majority of the cell subsets analyzed within this study. However, the CD4:CD8 ratio was significantly different with a higher proportion of CD8+ T cells (+40-60%), and a lower proportion of CD4+ T cells (-27-35%), in Mauritian vs. proportion in Chinese origin monkeys analyzed within this study. However, the CD4:CD8 ratio was significantly different with a higher proportion of CD8+ T cells (+40-60%), and a lower proportion of CD4+ T cells (-27-35%), in Mauritian vs. proportion in Chinese origin monkeys, respectively. Additionally, absolute counts of effector memory CD8+ T cells were 6-fold higher in Mauritian-origin compared to Chinese-origin monkeys. Furthermore, absolute numbers of central memory CD8+ T cells were 2.5-fold higher in Mauritian-origin monkeys. In conclusion, this study highlights that there exists variation between Chinese and Mauritius-origin monkeys in regards to the proportion and absolute numbers of some immune cell subsets.

**1157 Arsenic and Innate Immunity: Macrophage Function upon Arsenic Exposure**

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In a unique study area in Chile, our research group has reported that in vitro and early-life arsenic exposures were associated with the greatest increases in young adult mortality ever associated with an early-life environmental exposure: i.e. a 7-fold increase in lung cancer and an 18-fold increase in both bladder cancer and bronchiectasis. The risks of disease and mortality remain high up to 40 years after arsenic exposures have ended. However, the mechanisms for this prolonged effect remain unknown. We hypothesize that arsenic ingestion permanently impacts immune development and increases the risks of various immune-related diseases later in life. Here we focus on macrophages, innate immune cells known to influence tumor progression and TB pathogenesis. We performed multiple cytokine/chemokine profiling analysis on supernatant from in vitro monomethylarsenous acid (MMA3)-treated mouse bone-marrow-derived macrophages (BMDM). Our results revealed significant downregulation of various pro-inflammatory cytokines and chemokines involved in the nucleotide-binding oligomerization domain (NOD)-Like receptor, Toll-Like-receptor and peroxisome proliferator-activator receptor (PPAR) pathways, all critical in the innate immunity against TB. Lipid metabolomics experiments on these same BMDMs showed that arsenic treatment led to elevations in several pro-inflammatory and tumor-promoting signaling lipids known to play a role in tumor progression as well as the immunopathogenesis of TB. We are currently validating our findings in human macrophages and investigating how arsenic-induced immunogenic and metabolic alterations in macrophages influence TB and tumor cell pathogenicity in vitro and in vivo.

**1158 In Vitro Toxicology Testing Using Human Induced Pluripotent Stem Cell-Derived Cardiomyocytes to Predict Tobacco Smoking-Related Cardiovascular Disease**

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Cigarette smoking is associated with many cardiovascular dysfunctions. While there are reliable biomarkers of exposure to cardiovascular toxicants, there are no reliable biomarkers of harm that can be used to predict future onset of cardiovascular disease. Induced pluripotent stem cell (iPS) derived cardiomyocytes recapitulate physiological characteristics of human cardiac myocytes, providing a novel tool for cardiotoxicity prediction. In this study, the toxicity of mainstream cigarette smoke condensates (CSCs) was assessed in iPS derived cardiomyocytes with cellular function assays and some cardiomyocyte-specific endpoints. The CSC treatments reduced cell viability as evidenced by established cytotoxicity assays (e.g., lactate dehydrogenase) and ATP measurement. Treatment with CSCs resulted in dose-dependent changes in the heat rate as assessed by a real-time cellular impedance measurement. Intermediate doses (12 or 25 mg/ml) of CSC resulted in irregular beating that models arrhythmia and the highest dose (50 mg/ml) resulted in a cessation of beating that models cardiac arrest. Impedance measures, which are more sensitive and can detect the cellular physiology changes at lower doses compared to other cytotoxicity assays, were also assessed. Global gene expression analysis of cardiomyocytes treated with CSCs using Next Generation Sequencing identified dysregulation of genes for multiple cardiac ion channels, including major genes from potassium and calcium channels. The results suggest that the inhibitory effects of CSCs on cardiomyocyte beating maybe the consequence of interaction with multiple ion channels. The human iPS-derived cardiomyocyte model represents a novel in vitro approach to predict smoking induced cardiac toxicity, including arrhythmias.

**1159 Dietary Supplement Ingredients Alter Beating Parameters of iCell Cardiomyocytes**

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A variety of dietary supplements contain ingredients that are cardiac stimulants and therefore potential cardiotoxins. In vitro studies can facilitate identification of ingredients of potential concern. iCell cardiomyocytes, a beating human heart cell line, were used to evaluate the effects of phenylethylamine (PEA), higenamine, ephedrine and caffeine on heart cell function. For PEA and higenamine studies, the exposure levels in cell media were based on published blood levels in humans or animals after intravenous administration. Ephedrine and caffeine levels were based on published blood levels following various oral doses in humans. At low to midrange levels, each chemical was examined either with or without added caffeine. Caffeine levels (50 μM) approximated human blood levels reported after consumption of caffeine-enriched dietary supplements. To obtain values for beats per minute (BPM), peak width, etc., rhythmic rise and fall in intracellular calcium levels following 30 min of treatment were measured using a FLIPR Tetra instrument. All experiments were conducted in triplicate. Higenamine 31.3 ± 31.3 mg/ml significantly increased BPM in an escalating manner. Peak width narrowed with increasing BPM. PEA caused a significant increase in BPM at 0.8 and 8 μg/ml, along with narrower peak widths. In contrast, 80 μg/ml PEA greatly reduced BPM and widened peak width. This may indicate a toxic effect of PEA at 80 μg/ml. Adding caffeine to PEA 8 μg/ml or higenamine 31.3 mg/ml further increased BPM. Ephedrine produced a significant increase in BPM dose response at 0.5 to 5.0 μM. Caffeine alone resulted in an increased BPM only at a toxic level of 250 μM, but had no effect at 0.1 and 50 μM. Ephedrine caused a significant enhancing effect of caffeine when combined with ephedrine versus ephedrine alone. Generally, our iCell results correlated with expected effects. We suggest that additional testing may be warranted in vitro to further evaluate these cardiovascular effects.

**1160 Mcl-1 Knockdown Causes a Sub-Lethal Injury in Cultured Human iPSC-Cardiomyocytes**

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Chemotherapeutics targeting the bcl-2 family member, Myeloid cell leukemia sequence 1 (Mcl-1) are being evaluated as potential anticancer therapies due to the role of Mcl-1 in promoting survival of Myc-induced cancer. It has become apparent that both the anti-apoptotic and mitochondrial functions of Mcl-1 play...
important roles in promoting the survival of cancer cells, hematopoietic stem cells, mitochondrial homeostasis and induction of autophagy in the heart. We characterized the role of Mcl-1 in maintaining contractile function and structural integrity of human iPSC-derived cardiomyocytes in vitro and examined whether the pathology observed in Mc-1 knockout mice can be recapitulated in vitro. Using Mcl-1 siRNA, we knocked down (KD) Mcl-1Long (Mcl-1L) protein expression by over 80%, without a consistent effect on Mcl-1Short (Mcl-1S). Mcl-1L KD was typically associated with a decrease in beat amplitude and cellular impedance by 20%, and a reduction in cellular ATP by 10%. We also observed increased LDH activity in the cell culture medium of ~70% compared to non-targeted siRNA controls. There were no measurable changes in mitochondrial membrane potential, but observable changes in mitochondrial morphology presented in electron microscopy images. Interestingly, the KD of Mcl-1L was associated with an increase in activated caspase-3/7. Surprisingly, a pan caspase inhibitor Z-VAD, failed to prevent alteration in beat amplitude and cellular impedance, reduced ATP or LDH release. Finally, Mcl-1L KD potentiated doxorubicin-induced elevations in both rate and further reduced beat amplitude. Our results imply that more detailed investigations of the role of MCL-1 in regulation of mitochondrial function in human iPSC-derived cardiomyocytes are warranted before these cells can be used to screen compounds for cardiotoxic risk via this mechanism. Funded by NCI Contract No. HHSN261200800001E.

1163 A Dominant-Negative Sox9b Partially Phenocopies TCDD-Induced Cardiac Toxicity in Larval Zebrafish

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2,3,7,8-tetrachloro-dibenzo-p-dioxin (TCDD) is an environmentally persistent, ubiquitous and toxic compound. Zebrafish embryos exposed to TCDD (1ng/mL) at 4 hours post fertilization (hpf) have unlooped hearts and pericardial edema while lacking cardiac valves and an epicardium at 96 hpf. Previous work has shown TCDD exposure downregulates SRY-box 9b (sox9b) gene expression in the larval (72 hpf) zebrafish heart, suggesting loss of sox9b expression contributes to TCDD-induced cardiac phenotypes. Yet, it is not known in which cardiac cell type(s) that loss of sox9b expression contributes to these cardiac phenotypes. We hypothesize that loss of sox9b expression within cardiomyocytes leads to TCDD-induced cardiac phenotypes. We have created a dominant negative Sox9b (dnSox9b) by inserting a stop codon immediately before the C-terminal transactivation domain allowing dnSox9b to bind DNA, but preventing it from inducing transcription. We used the cardiac myosin light chain 2 (mcl2) promoter to express dnsox9b, marked by a 2-lysine linked tagRFP (cml2::dnsox9b::2-KRFP), specifically in cardiomyocytes. A cml2::2KRFP construct containing only the cml2 promoter and tagRFP were used as controls. Embryos injected with cml2::dnsox9b::2-KRFP shortly after fertilization have unlooped hearts and pericardial edema at 96 hpf, which partially recapitulates the TCDD-induced cardiac phenotypes. Zebrafish injected with cml2::dnsox9b::2-KRFP appeared phenotypically normal. In contrast to TCDD-exposed larval hearts, zebras injected with cml2::dnsox9b::2-KRFP formed an epidermal cell layer and cardiac valves as determined by using epipodial (pard3:EGFP) and endocardial reporter lines (jf6:GFP) in conjunction with confocal microscopy. These results show blocking Sox9b function in cardiomyocytes reproduces some effects of TCDD on the developing heart but indicates a role for other genes or tissues in the disruption of valve and epicardium development by TCDD. Funding: NIH NIHIEP support from T32 ES007015; American Foundation for Pharmaceutical Education.

1164 Gefitinib, a Tyrosine Kinase Inhibitor, Induces Cardiotoxicity and Hypertrophy in Rat Cardiomyocyte H9c2 Cells: Role of Oxidative Stress and Apoptosis

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Gefitinib (GEF) is multi-targeted tyrosine kinase inhibitor that is known for its anti-angiogenic properties. Cardiotoxicity has been reported as a significant side effect associated with GEF treatment, yet the mechanism is poorly understood. The main purpose of this study was to investigate the potential cardiotoxic effect of GEF and the possible mechanisms involved using rat cardiomyocyte H9c2 cell line as an in vitro model. As determined by MTT assay, GEF showed significant cytotoxicity against H9c2 cells with the half maximal inhibitory concentration (IC50) value of 23 μM. The inhibitory effect of GEF on H9c2 cell viability was associated with increased production of reactive oxygen species and antioxidants. Treatment of H9c2 cells with GEF (0, 1, 5, and 10 μM) significantly induced the mRNA levels of apoptotic genes caspase-3 and p53 in a concentration-dependent manner. Moreover, flow cytometric analysis using annexin-V probe revealed an increase in the percentage of apoptotic cells in H9c2 cells. In addition, quantitative polymerase chain reaction analysis of H9c2 cells after treatment with GEF showed upregulation of hypertrophic gene markers, brain natriuretic peptides (BNP), alpha-myosin heavy chain (α-MHC), and Beta-myosin heavy chain (β-MHC) in concentration- and time-dependent manner. The onset of mRNA induction was observed as early as 12 h and remained elevated for at
least 24 h after treatment with GEF 5 μM. At the protein level, using Western blot analysis, GEF increased BNIP and β-MHC, while inhibited α-MHC protein levels, in a concentration-dependent manner. The increase of hypertrophic markers was associated with an increase in H9c2 cell size and hypertrophy. In conclusion, GEF induces cardiotoxicity and hypertrophy in rat cardiomyocyte H9c2 cell model through activation of oxidative stress and apoptotic pathways.

1165 Analysis of Mitochondrial Function Using Human iPSC-Derived Cardiomyocytes on the Seahorse
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Drug-Induced mitochondrial dysfunction is one of the major reasons to induce cardiotoxicity - one of the most common causes for costly late stage drug withdrawal. To assess mitochondrial liability, new cell models and assay technologies are currently in focus to determine an efficient way of large scale compound screens during early drug discovery. Human iPSC-derived cardiomyocytes represent a homogenous, reproducible and physiologically relevant cardiomyocyte model that allows analysis of metabolic function and general cell metabolism. The combination of this cell model with the label-free Seahorse XF 96 technology, that measures oxygen consumption as well as extracellular acidification rates, provides a suitable tool for high throughput screening of compounds with respect to mitochondrial liability. Here, we used human iPSC-derived Cor.4U cardiomyocytes cultured in 96 well plates ready for simultaneous measurement of mitochondrial respiration and glycolysis using the Seahorse XF Cell Mito Stress Test Kit. The assay measured over 90 minutes reveals the fundamental parameters of mitochondrial function: basal respiration, ATP turnover, proton leak, maximal respiration and spare respiratory capacity, providing the potential to assess perturbed OXPHOS after treatment. Cardiotoxic compounds like the antracycline antibiotic Doxorubicin and the anxiolytic psychotropic drug Buspirone reduced the basal oxygen consumption rate over 90 minutes reveals the fundamental parameters of mitochondrial function: basal respiration, ATP turnover, proton leak, maximal respiration and spare respiratory capacity. In addition, the effect of Galactose medium vs. Glucose medium was assessed as well as the combination of ventricular cells and cardiac fibroblasts. The results demonstrate the feasibility of human iPSC-derived cardiomyocytes combined with the Seahorse technology to assess mitochondrial dysfunction on a high throughput level and the possibility to delineate the results from non-specific cardiotoxicity.

1166 Cholesterol Homeostasis Is Regulated by Carboxylesterase 1 in Macrophage Foam Cells
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Human carboxylesterase 1 (CES1) has an emerging role in lipid metabolism. CES1 in macrophages is a serine hydrolase with broad endogenous substrate specificity, including cholesteryl esters, triacylglycerols, and 2-arachidonyl-glycerol (2-AG), which may be relevant to cardiovascular disease and other pathologies related to lipid metabolism. Macrophage cholesterol homeostasis is a complex regulatory process based on the balance of uptake of unmodified and oxidized low-density lipoproteins (LDL) and cholesterol efflux. Distortion of the expression or function of any of the numerous receptors and/or transporters involved in this process may unbalance the system in a way that favors pathogenic processes, such as atherosclerosis. To characterize cholesterol homeostatic functions regulated by CES1, its expression was stably knocked-down in a THP-1 monocyte/macrophage line (CES1KD/THP-1) and the extent of cholesterol uptake and efflux from foam cells was evaluated. Phagocytic response and the expression levels of genes involved in cholesterol and 2-AG homeostasis were also determined. CES1KD/THP-1 foam cells exhibited reduced esterified cholesterol mass compared to control cells, whereas free (unesterified) cholesterol mass and cholesterol efflux was unchanged. Perturbation of CES1 function in foam cells also impaired phagocytosis and downregulated the expression of multiple genes involved in cholesterol transport and metabolism, including ABCA1, CYP27A1, CD36, SRE, LXRα. Reductions in CD36 and scavenger receptor A (SRA) expression likely account for reduced intracellular cholesterol accumulation in CES1KD/THP-1 foam cells compared to control cells by limiting the uptake of modified LDL subsequently stored as cholesteryl ester. Additional experiments using selective agonists/antagonists of PPARγ, LXRα, RXR and RAR suggest CES1 may play a central role in the regulation of cholesterol homeostatic gene expression by liberating molecules that serve as ligands for one or more of these nuclear receptors. [Supported by NIH 1R55ES015348-02]

1167 Nitration of Linoleic Acid Prevents Polychlorinated Biphenyl-Induced Endothelial Cell Dysfunction
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Data now implicate correlations between persistent organic pollutants, such as polychlorinated biphenyls, and cardiovascular diseases. We have shown that coplanar PCBs can initiate endothelial cell dysfunction, oxidative stress, and inflammation. Recent evidence shows that toxicant-nutrient interactions exist, and a diet high in pro-inflammatory omega-6 lipids such as linoleic acid, may exacerbate PCB-induced cardiovascular disease. However, novel anti-inflammatory lipid metabolites, such as nitro-fatty acids, may limit this exacerbated effect. The aim of our research was to determine if pro-inflammatory lipids could increase the toxicity of the coplanar PCB 126, and if nitration of these pro-inflammatory lipids could ameliorate this exacerbated effect. Vascular endothelial cells were pretreated with linoleic acid or nitrolinoleic acid and subsequently exposed to physiologically relevant concentrations of PCB 126. Treatment with linoleic acid was pro-inflammatory as evidenced by increased mRNA levels (RT-PCR) of vascular cellular adhesion molecular-1 (VCAM-1), monocyte chemoattractant protein-1 (MCP-1) and cavelolin-1 (Cav-1). These levels were significantly exacerbated in linoleic acid/PCB treated cells. Interestingly, the addition of a nitro group to linoleic acid prevented this exacerbated PCB effect. Understanding diet toxicant interactions is a critical step towards more effective risk assessment and public health outcomes for at-risk populations who reside near hazardous and Superfund sites. Although the nitration of pro-inflammatory linoleic acid successfully can decrease the observed exacerbated effect of parent linoleic acid, very little is known about the endogenous control mechanisms concerning the formation of nitro-fatty acids. Coplanar PCBs have been shown to induce multiple disease phenotypes related to chronic inflammation, and endogenously produced fatty acid metabolites may mediate risks associated with exposure to environmental pollutants. [NIH/NIEHS P42ES007380]

1168 Monomethylarsonous Acid (MMA) Promotes Mitochondrial Pathology in Vascular Smooth Muscle
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Arsenic exposure is associated with vascular disease, yet the cellular mechanisms of toxicity are largely unknown. The present study investigates mitochondrial dysfunction in relation to the vascular toxicity of arsenic. The relative effects of inorganic arsenic (iAs) and the metabolite monomethylarsonous acid (MMA) on mitochondrial function in vascular aortic smooth muscle cells (VSMC) were assessed through cell proliferation, oxygen consumption rates, glycolysis, mitochondrial morphology, ATP generation, and mitochondrial protein expression. Survival curves and determination of mitochondrial function following a 24 h exposure indicate that MMA is significantly more toxic than iAs as determined by trypan blue and MT5 assays. A 3 h exposure of MMA, but not iAs, caused a significant decrease in basal and maximal respiration and a concomitant increase in compensatory glycolysis as measured by an XF24 Extracellular Flux Analyzer. Significant decreases in mitochondrial ATP generation occurred following 6 hr. treatment with all species and were more pronounced for MMA compared to iAs. MMA treatment, but not of iAs, caused a significant increase in hydrogen peroxide levels. Exposure of VSMCs to high doses of MMA fragmented mitochondria and caused a decrease in the percent of mitochondria occupied by cytosol. Treating VSMCs with a high dose of MMA or iAs for 6 hrs. decreased total levels of several mitochondrial proteins as assessed by immunoblotting. Overall our data demonstrate that MMA, but not iAs, is a mitochondrial-specific intoxicant in VSMCs. Bidirectional changes in mitochondrial morphology and content, and elevated glycolysis induced by MMA are likely compensatory manifestations secondary to mitochondrial dysfunction and ROS elevation. Therefore, subsequent work will explore additional mechanisms of MMA-mediated mitochondrial dysfunction including identifying other mitochondrial sources of ROS induced by MMA, and analyzing the effects of MMA on substrate utilization by mitochondrial complexes. [NIH-P20 GM105554]
Characterization of the Homocysteine Thiolactonase Activity of Biphenyl Hydroxylase-Like Protein/Valacyclovir Hydroxylase

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Hyperhomocysteinemia is a risk factor for a wide variety of disorders including cardiovascular, neurological and autoimmune diseases. Homocysteine thiolactone (HCTL), a toxic metabolite of the amino acid homocysteine, can modify lysine residues of proteins, resulting in protein aggregation and loss of function. These modifications have been related to the development of the diseases noted above. It has been proposed that human plasma paraoxonase-1 (PON1) and bloemycin hydroxylases (Bphl) are physiologically responsible for hydrolyzing HCTL. However, the catalytic efficiency of PON1 for HCTL is very low and Bphl is not saturated at 20 mM substrate. Our aim was to characterize a more physiologically relevant enzyme for hydrolyzing HCTL.

HCTL hydroxylase (HCTase) was purified from human liver and identified by mass spectrometry (MS) as biphenyl hydroxylase-like protein (BPHL). For further activity characterization, recombinant BPHL (rBPHL) was expressed in E. coli and purified. rBPHL hydrolysis of the substrates HCTL and valacyclovir (VC) was verified by MS. The catalytic efficiency of rBPHL for HCTL is very low and Blmh is not saturated at 20 mM substrate. Our aim was to characterize a more physiologically relevant enzyme for hydrolyzing HCTL. HCTL hydroxylase (HCTase) was purified from human liver and identified by mass spectrometry (MS) as biphenyl hydroxylase-like protein (BPHL). For further activity characterization, recombinant BPHL (rBPHL) was expressed in E. coli and purified. rBPHL hydrolysis of the substrates HCTL and valacyclovir (VC) was verified by MS. The catalytic efficiency of rBPHL for HCTL was 7,700-fold higher than PON1 and 77-fold higher than Blmh. The concentration-dependent evaluation of 13 hormones not only screened for chemically elicited interference in the steroidogenesis pathway, but also identified a putative mechanism of action. For example, few chemicals altered only progestagen levels, while changes in testosterone and estrogen levels were more often observed. These results suggest CYP19A aromatization and CYP17A lyase and hydroxysteroid dehydrogenase activity are the likely targets for the disruption of steroidogenesis by a subset of ToxCast chemicals. This abstract does not necessarily reflect US EPA policy.

4-Vinylcyclohexene Diepoxide-Induced Toxicity in Drosophila melanogaster: Implications in Occupationally Exposed Women

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The occupational chemical 4-vinylcyclohexene dioxide (VCD) is a metabolite of 4-vinylcyclohexene with the potential to destroy primordial follicles. This is of great concern to women because exposure to VCD could lead to premature ovarian failure (early menopause). In a previous study, we reported the toxicity of 4-vinylcyclohexene in Drosophila melanogaster. In the present study, we sought to gain insights on the direct impact of VCD on D. melanogaster since about 75% of human disease-causing genes have functional homology in the fly. VCD (10 μM-1 mM) were exposed to D. melanogaster in the diet for 5 days. Subsequently, we determined the survival rate, negative geotaxis assay and the levels of reactive oxygen species (ROS) generated. In addition, the real time RT-PCR mRNA expression of selected genes, and catalase (CAT), glutathione-S-transferase (GST), acetylcholinesterase (AChE) and delta aminolevulinic acid dehydratase (δ-ALA-D) activities, and total thiols content were evaluated. Our data showed that in addition to impairment of climbing behaviour and accumulation of ROS, VCD also enhanced SOD, Nrf-2 inhibitor (Keap1) and MAPK2 gene expressions, as well as CAT activity in D. melanogaster. Moreover, VCD reduced total thiols content, and GST, AChE and δ-ALA-D activities (p < 0.05). Our data imply that the mechanisms of VCD-induced toxicity in D. melanogaster are: increased ROS generation and disruptions in mRNA gene expressions, antioxidant balance and total thiols redox content. In conclusion, these mechanisms of VCD-induced toxicity in D. melanogaster could have toxicological implications in occupationally exposed women.

Screening Chemical Effects on Steroidogenesis in H295R Human Adrenocortical Carcinoma Cells

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Proper endocrine function requires steroid hormone biosynthesis and metabolism (steroidogenesis). Disruption of steroidogenesis by environmental chemicals can result in altered hormone levels causing adverse reproductive and developmental effects. This study is the first to establish a high-throughput model to evaluate a diverse library of chemicals for effects on 13 major hormones in the steroidogenic pathway. Using H295R human adrenocortical carcinoma cells in a 96-well format, steroidogenesis was induced by pre-stimulation with 10 μM forskolin for 48 hr followed by chemical exposure for 48 hr. Media were removed and hormones were quantified by HPLC-MS/MS including progestagens (pregnenolone, progesterone, and their hydroxylated metabolites), glucocorticoids (corticosterone, cortisol, and their deoxy-precursors), androges (dihydrotestosterone, androstenedione, and testosterone), and estrogens (estrone, estradiol, and estron). Initially, 2086 unique ToxCast chemicals were tested at a single non-cytotoxic concentration, of which 1215 chemicals (58%) altered levels of at least one measured hormone.

Based on the single concentration analysis, 523 chemicals altering the levels of ≥4 hormones were selected for six-point concentration-response (0.003 – 100 μM). The concentration-dependent evaluation of 13 hormones not only screened for chemically elicited interference in the steroidogenesis pathway, but also identified a putative mechanism of action. For example, few chemicals altered only progestagen levels, while changes in testosterone and estrogen levels were more often observed. These results suggest CYP19A aromatization and CYP17A lyase and hydroxysteroid dehydrogenase activity are the likely targets for the disruption of steroidogenesis by a subset of ToxCast chemicals. This abstract does not necessarily reflect US EPA policy.

Overlapping and Distinct Effects of Bisphenol A and Its Substitute Bisphenol S on Germ Cells

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Bisphenol S (BPS), one substitute of Bisphenol A (BPA), is increasingly used in the “BPA-free” plastic manufacturing industry since the toxic effects of BPA have come under heavy scrutiny. Sharing a similar structure and bioactivity with BPA makes BPS a potential threat to human health. However, information about its health risks. Since reproductive stages of organisms are generally being more sensitive to the effects of toxics than other stages, investigation of crude oil-dispersant exposure effects on reproduction is critically important. However, studies on reproductive effects of crude oil-dispersant mixture exposure and its mechanism remain insufficient. Our previous study showed that the exposure of crude oil and the dispersant induced reproduction defects including decreased brood size and increased germ cell apoptosis in Caenorhabditis elegans (C. elegans). Here, we show that crude oil-dispersant mixture also affected reproduction by inducing abnormal sperms during the process of spermatogenesis. After L4 larvae of wild type N2 hermaphrodites were exposed to oil-dispersant mixture (20:1) for 24h, young adults were dissected and subjected to DAPI staining. Results showed that the abnormal immature sperms are significantly increased in the gonad arms of treated animals compared to controls (K-medium). We further explored the oil-dispersant mixture toxicity effects on spermatogenesis by using male C. elegans strains. After 48h exposure to oil-dispersant mixture, more than 50% spermatids appeared abnormal morphology, significantly higher than in controls. At the molecular level, we applied qRT-PCR to test expression levels of 22 selected genes that are involved in the regulation of spermatogenesis and sperm properties. The results show that 16 genes (72.7%) were significantly changed in oil-dispersant treated. Our study suggests that oil-dispersant mixture induce toxicity effects on reproduction by not only affecting oogenesis but also affecting spermatogenesis.
BPA, carries reproductive toxicity. However, some of their mechanisms of toxicity are distinct, especially with regards to the meiotic recombination, and need to be further studied.

**1174 Morphologic Changes in 3D Human Breast Microtissues following Exposure to Endocrine Disruptors**

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As a method to develop human cell-based in vitro assays, 3-dimensional (3D) cell culture models are intriguing due to their ability to bridge the gap between animal models and traditional 2-dimensional (2D) cell culture, allowing for the growth of human cells in an environment that is closer to the in vivo environment. While many 3D models rely on scaffolded matrices for the growth of cells at low densities, scaffold-free models allow cells to aggregate and maximize cell-cell contact, free of the influence of surrounding matrix. The use of scaffold-free agarose hydrogels provides a system in which cells form spheroids, initiating contact with other cells and the matrix they produce for themselves. We have demonstrated the use of this system to culture MCF-7 human breast carcinoma cells, which self-aggregate and develop into differentiated spheroids, possessing a defined luminal space in this model. As shown by transmission electron microscopy, cells in MCF-7 spheroids display tight junctions, desmosomes, secretions into the luminal space and apical basal polarity. MCF-7 cells grown in the scaffold-free 3D system display increased expression of breast-specific markers including cytokeratin 18 and milk fat globule. To evaluate the effects of estrogenic endocrine disruptors, samples were exposed to the estrogen receptor alpha agonist propylpyrazoloeritrol (PPT). PPT exposure for 7 days result in a reduction in luminal ratio (luminal area/total cellular area) in a concentration dependent and statistically significant manner, suggesting the utility and sensitivity of this system in assessing morphological and molecular changes following exposure to estrogenic endocrine disruptors. We have used this system to assess morphologic changes associated with exposure to endocrine disruptors including diethylstilbestrol and bisphenol A and have observed qualitative and quantitative phenotypic changes. The use of a differentiated scaffold-free 3D culture system offers a unique opportunity to study the phenotypic and molecular changes associated with exposure to EDCs.

**1175 In Vitro Spermatogenesis Model for Assessing Male Reproductive Toxicity**

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Reproductive toxicity testing in animals represents one of the largest uses of animals. It has been an extraordinarily challenging area to implement in vitro alternatives due to the complexity of reproductive systems. We previously developed a three-dimensional testicular cells co-culture from rat testis and validated this model to culture MCF-7 human breast carcinoma cells, which self-aggregate and develop into differentiated spheroids, possessing a defined luminal space in this model. As shown by transmission electron microscopy, cells in MCF-7 spheroids display tight junctions, desmosomes, secretions into the luminal space and apical basal polarity. MCF-7 cells grown in the scaffold-free 3D system display increased expression of breast-specific markers including cytokeratin 18 and milk fat globule. To evaluate the effects of estrogenic endocrine disruptors, samples were exposed to the estrogen receptor alpha agonist propylpyrazoloeritrol (PPT). PPT exposure for 7 days result in a reduction in luminal ratio (luminal area/total cellular area) in a concentration dependent and statistically significant manner, suggesting the utility and sensitivity of this system in assessing morphological and molecular changes following exposure to estrogenic endocrine disruptors. We have used this system to assess morphologic changes associated with exposure to endocrine disruptors including diethylstilbestrol and bisphenol A and have observed qualitative and quantitative phenotypic changes. The use of a differentiated scaffold-free 3D culture system offers a unique opportunity to study the phenotypic and molecular changes associated with exposure to EDCs.

**1176 Diet, Thermal Environment, and Early-Life Exposure to Methylmercury: *Daphnia pulex* As a Model Organism for Evaluating Multistressor Interactions across the Lifespan**


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Interactive effects between chemical toxicity, nutritional status and the physical environment are an important area of ongoing research. Promising high-throughput cell-based toxicity screening assays are being developed and validated, yet these assays cannot address integrative effects at the organismal level, such as the relevance of early life exposures on the development of adult-onset diseases. The short lifespan (median 40 days) and gestation time (approx. 36 hrs), transparency throughout life, and clonality of *Daphnia pulex* are advantages for efficiently detecting latent effects over a lifespan. We have examined consequences of early life exposure to methylmercury (MeHg) under standard and reduced food ration, as well as under a standard or low iron (Fe) diet. We have also examined the consequences of differing food ration across standard and daily fluctuating temperature regimes. An additive effect of MeHg and reduced food ration was found on decreasing lifespan when *D. pulex* were exposed to varying concentrations of MeHg within the first 72 hrs. of life (0, 200, 400, 800 and 1600ng/l MeHgCl) and thereafter kept on either a standard or reduced food ration. MeHg concentration did not affect survival linearly. Low food ration and MeHg concentration were also predictive of reduced reproduction, with some evidence of an interaction (p=0.048). Compared to *D. pulex* on a standard Fe diet, *D. pulex* on a low Fe diet show increased mortality after exposure to an acute heat stress. Analysis of lipids stained with Oil Red O suggests significantly lower lipids in *D. pulex* fed a low Fe diet, while early life MeHg exposure increased lipid levels at 5 days post-exposure.

**1177 Germline Defects in Mitochondrial Cholesterol Transporter* C. elegans* Mutants following Bisphenol A or Low Cholesterol Exposures**

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Bisphenol A (BPA) treatment results in abnormal oocyte development in mammalian species as well as in the nematode *C. elegans*. In *C. elegans*, these defects are prevented by exposing the worms to cholesterol. We have therefore begun to investigate whether there is an interaction between BPA and normal cholesterol homeostasis in the germline. Previously, we have shown that mammalian homologs to cholesterol transporters (Steroid Acute Regulator Protein; STAR, 18KD translocator protein; TSPO, and StAR related lipid transfer protein 3; StatD3) mimic germline phenotypes found in BPA-treated worms. Here we show that developing germ cells of these mutants have increases in germ cell nuclei apoptosis, an elongated germline transition zone and reduced fertility when exposed to low cholesterol. Protruding vulva, as well as missing or underdeveloped gonads, are also frequently seen in *strl-1* homo- and heterozygotes. To investigate a possible interaction between BPA and mitochondrial cholesterol transport, wild type and *strl-1* worms were treated with BPA. Diakinetic analysis of the -1, -2 and -3 oocytes from control-treated worms reveals an increased incidence of abnormal chromatid arrangement in *strl-1* mutant worms compared to wild type (29, 49 and 78 versus 0, 5 and 25 percent). Interestingly, the occurrence among wild type and *strl-1* worms is similar following BPA treatment (6, 34 and 71 percent in wild type versus 7, 30 and 73 in *strl-1*). Together, the data suggest that BPA can at least partially rescue the sensitive germline phenotype in *strl-1* mutants, possibly by acting downstream of mitochondrial cholesterol transport.

**1178* C. elegans*, a Valuable Model for Predicting Chemicals’ Acute Toxicity in Rodent**

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The nematode *C. elegans* has been used as a model organism for toxicological research, mainly because of its simplicity, rapid development, andapos;...
than 2.75. Excluding 4 types of acidic chemicals, there were positive correlations between LC50s of *C. elegans* and LD50s of mouse/rat (r=0.72, p<0.01) after both 12-h and 24-h exposure. As to the LC50 data following a 24-h exposure in *C. elegans*, the correlation of *C. elegans* LC50s vs. rat LD50s (r=0.885) was greater than the correlation of *C. elegans* LC50s vs. rabbit LD50s (r=0.879), while the correlation of *C. elegans* LC50s vs. mouse LD50s (r=0.741) was lower relative to that of mouse vs. rat LD50s. The data were further compared with an *in vitro* cytotoxicity model utilizing human epidermal keratinocytes (NHK). The data indicate that the correlation of *C. elegans* LC50s vs. rat LD50s was equal to the correlation of mouse vs. rat LD50s (r=0.879), and was stronger than the correlation of NHK cell LC50s vs. rat LD50s (r=0.844). In addition, ET50 was significantly correlated with the LC50 of *C. elegans*, indicating that both can be utilized as toxic effect index for further study on acute toxicity test of chemicals. In summary, *C. elegans* may be a valuable model for predicting chemicals’ acute toxicity in rodent. (partly supported by NSFC in China #81273108, and Capital Development Project 2011-1013-03. Corresponding author: guojunli88@yahoo.com).

**1179 Correlation of Tox21 and ToxCast In Vitro and Small Model Organisms Outcomes to Rat Oral Toxicity**

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At present, many national and international regulatory authorities use data from rat acute oral toxicity test methods for hazard classification and labeling. The Tox21 and Tox21 programs have tested over 2000 and 8000 chemicals, respectively, in *in vitro* and zebrafish (ZF) assays. We evaluated data from Tox21 and ToxCast to determine the potential of the more than 800 measures collected thus far to reduce animal use in toxicity testing for hazard identification. Rat oral LD50 data were obtained for 3582 Tox21 and 670 ToxCast Phase I and II chemicals. An ongoing analysis identified high-quality LD50 data for 76 chemicals that have been tested in ZF toxicity assays. The Tox21 and ToxCast data were analyzed for correlation and model fit to the LD50 data in order to determine which tests (and combinations thereof) best characterized the rat oral toxicity data. Correlation analyses were performed on binary outcomes of response for chemicals classified by LD50 as “toxic” (LD50 < 5000 mg/kg-bw). In this assessment of fit to the up-and-down protocol, zebrafish (ZF) toxicity assays. The Tox21 and ToxCast data were analyzed for correlation and model accuracy compared to *in vivo* data. Based on two parameters, CV 50 and 100 pp/ ml of IL-1b, the exposure conditions of 50min at RT and without post incubation is expected to be a good methodology for evaluating mucosal irritation potential.

**1180 Development of a New Reconstituted Human Oral Mucosal Model to Assess the Oral Irritation Testing**

S. Lee1, S. Kim1, H. Jung1, S. Yang1, K. Kim2 and K. Lim1.

Using New 3-D Reconstructed Human Oral Tissue Model

In this study we developed a novel three-dimensional human oral mucosal model (HOM model) based on two different cell sources. One is produced from immortalized human oral keratinocyte cell line (HOM-IHOK model) and the other is human normal buccal keratinocytes (HOM-NBKM model). To compare these two oral mucosal models, immunohistochemistry and epidermal barrier properties were examined. In histological and immunohistochemical observation HOM-NBKM model has similar morphology, characteristics and biomarker expression to normal human oral mucosa and effective time-50 for Triton X-100 were measured to be 1.2±0.3hr. Human oral mucosal model developed from IHOK, ET-50 for barrier function test is averaged 43.4±4.9min, and also express mucosal cytokeratin (CK3/12) but epidermal differentiation of HOM-IHOK model is limited compared to non-transformed normal buccal keratinocytes. Although HOM-IHOK models do not form a fully differentiated oral mucosal epithelium the need for reproducibility and productivity may make the use of this model suitable for *in vitro* toxicity test. In conclusion our two human oral mucosal models might be the useful tools for alternatives to oral irritation test method.

**1181 Optimization of the Estimation for Oral Mucosa Toxicity Using New 3-D Reconstructed Human Oral Tissue Model**

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Oral care products provide many benefits for oral hygiene such as whitening, cleansing of teeth or oral cavity. However, some ingredients used in oral care product could elicit the irritation of oral mucosa, so it is important to select safer ingredient. For many years, the oral mucosa irritation potential of chemicals has been mainly evaluated by animal, such as hamster, rat or mice. Because these animal tests are based on subjective visual scoring evaluation, significant variability has been observed. For scientific reasons, animal welfare issue and the ban of animal tests with cosmetic in the European Union (EU) since 2013, there are many efforts to develop alternative methods replacing animal study. Using newly developed human oral tissue models (HOMTM) that consist of human-dermalized human oral keratinocyte, we estimate the oral mucosa irritation potential of chemical in several conditions. We exposed reconstructed human oral tissue models to 0.3% Triton X-100 for 10min, 30min and 50min, respectively, in 37 °C or room temperature (RT) and then calculate exposure time of cell viability 50% (Et50). Also, after treatment chemicals (0.3% Triton X-100 or SLS, 30% ethanol) and washing, tissues were incubated for 0h or 20h. And then cell viability (%) and release of IL-1b were assessed. As expected, the higher exposure temperature showed shorter Et50. And the method without post-incubation showed the good accuracy compared to *in vivo* data. Based on two parameters, CV 50 and 100 pp/ml of IL-1b, the exposure conditions of 50min at RT and without post incubation is expected to be a good methodology for evaluating mucosal irritation potential.
DNA damage in both types of cells at the lowest concentration tested. Superoxide dismutase (SOD) and catalase (CAT) activities were measured to evaluate cell antioxidant capability after these mycotoxins exposure. ZEA and its metabolites increased SOD and CAT activities in CHO-K1 and HepG2 cells in a comparable extent. The SO2 activity increased from 25% to 69% and from 26% to 70% for ZEA and its metabolites in CHO-K1 cells and HepG2 cells, respectively. The CAT activity increased from 11% to 53% and from 13% to 61% for CHO-K1 and HepG2 cells, respectively. In summary, the results indicate that ZEA and its metabolites induced a marked decrease in cell viability in a dose and time-dependent manner. Significant positive correlation was observed between the activities of CAT and SOD antioxidant enzymes and ROS production and DNA damage. Therefore, we can conclude that there may be an induction of CAT and SOD activities after ZEA and its metabolites induced oxidative stress. Acknowledgements: Economy and Competitiveness Spanish Ministry (AGL2013-43194-P)

1184 Agrochemical Formulations: How to Avoid In Vitro Acute Toxicity Testing Using the GHS Additivity Formula


An acute toxicology six-pack of studies is currently required for the registration of agrochemical formulations globally. Studies are conducted for the Classification and Labelling (C&L) of the commercial mixture. The GHS (Globally Harmonised System for classification and labelling) provides the opportunity to calculate the classification category based on the Acute Toxicity Estimate (ATE) of each individual component within the mixture. A retrospective analysis has been conducted. The classification category of more than 120 agrochemical mixtures (including solids and water- or solvent-based liquid formulations) for all the acute endpoints was calculated with the GHS additivity formula and compared with the available in vitro study results. For the acute systemic toxicity end-points (oral, dermal and inhalation), the GHS additivity formulation showed an accurate prediction of the estimated LD50 (overall accuracy > 99%). In particular, for the dermal route very high accuracy (> 98%) and no cases of under-prediction were observed. This provides the opportunity for the GHS additivity formula to become a criterion for triggering the systemic toxicity study together with physico-chemical characteristics, in agreement with the current literature (e.g. Creton, S., et al., 2010; Moore, N.P., et al., 2013) and requirements in some geographies (e.g. EU). A potential reduction of animal use up to 26-29%, is outlined for these endpoints (approximately up to 48% of the animals used for each formulation). For the local acute toxicity end-points (dermal and eye irritation) and for skin sensitisation, the GHS additivity formula showed under-prediction in some specific cases, attributed to the potency of a single component of the mixture, recurrently found in our database. Relevant to acute exposure concerns, specific in vitro tests, the use of Adverse Outcome Pathways (AOP, where applicable, e.g. for skin sensitisation), and extension of the comparative database are proposed for further investigation.

1185 Cytotoxicity Profiling of Chemicals Based on Cellular Growth Kinetics and Mean Graph Method

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In vitro cytotoxicity testing is often used to collect biological information in human cell lines for chemical safety and risk assessments. The scope of this study was (1) to determine if kinetic information on cell growth characteristics could provide more insight into the mode of action of chemicals, and (2) to evaluate the advantages of using multiple cell lines for cytotoxicity profiling. We have conducted cytotoxicity testing of 303 compounds, mostly pesticides from the ToxCastTM Phase I chemical library. The high-throughput xCELLigence Real Time Cell Analyzer (RTCA-HT) system (ACEA Biosciences Inc., San Diego, CA) and a panel of five different human cell lines (HepG2, H4, BEAS2, ARPE, ACHN) were used. Cells were exposed to eight different concentrations of chemicals (1:2.15 serial dilution starting at 400µM). The cell index (CI) was recorded once every hour over a 72h exposure period. Cell growth kinetics data, indicated by the CI, were analyzed by separating cellular growth dynamics into its individual components lag phase, exponential growth rate, and cumulative growth. The lag phase, and cumulative growth was plotted against the exponential growth rate. The 303 chemicals clustered into seven distinct groups. For the mean-graph method half of the inhibitory concentration (IC50) for each chemical and cell line was determined. The average GI50 was calculated across the five cell lines, and the difference of GI50 was plotted for each cell line. A pattern recognition algorithm revealed 22 distinct cellular response profiles. The dynamic analysis of cell growth curves increased the informational value of cytotoxicity tests. The mean graph method generated distinct chemical bioactivity patterns across different cell lines. The clustering of compounds and bioactivity patterns observed may be related to the mode of action of chemicals, and is being further investigated.

1186 Guarana (Paulinia cupana Mart) Extends Lifespan of Caenorhabditis elegans

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Extensive research has focused on aging processes and compounds which can promote lifespan extension and delay age-associated diseases. Guarana (P. cupana) is a fruit indigenous to Brazil and is implemented in many energy drinks with great popularity. Although guarana has some beneficial effects described, no studies could be identified on its aging-related effects. Thus, we designed a study using C. elegans to investigate whether guarana can delay aging as well as the molecular mechanisms underlying its effects. Guarana extracellular matrix (GEM) powder was dissolved in water and spread over NGM plates with E. coli at 100, 500 and 1000 µg/ml final concentrations. Synchronized wild type L1 larvae were transferred to treatment plates at 20°C. Adults were transferred to fresh plates with GEE every other day and scored until death. The lifespan assay was also performed using mutant strains related to major stress response and longevity pathways: daf-2(e1370) III, daf-16(mu86) I, hbl-1(nu41), mev-1(ken-1) and sir-2.1(ok343). Median lifespan of treated wild type worms was lengthened by 18% at 100 µg/ml and 36% at 500 and 1000 µg/ml. Maximal lifespan was increased by an average of 28% at the three concentrations. GEE also increased lifespan of mev-1 mutants, demonstrating a lifespan extension related to an antioxidant effect. In addition, median lifespan of daf-2, skn-1 and sir-2.1 mutants was extended, establishing that the extract did not act through these mechanisms. In contrast, GEE did not prolong lifespan of daf-16 and hbl-1 mutants, suggesting that the extract might act through DAF-16/FOXO transcription factor and the heat-shock transcription factor. Our study shows that chronic exposure to GEE is safe and has the ability to extend lifespan of C. elegans. It not only contributes to the study of natural products' effects in vivo, but also provides plausible therapeutic targets against aging-associated diseases.

In vitro toxicity assays are envisaged to play a fundamental role in the risk assessment of industrial chemicals. Unfortunately, binding of chemicals to well plate plastic and serum constituents like bovine serum albumin (BSA) may lead to an underestimation of the toxicity. Binding affinities of ionic surfactants to such matrices is poorly understood. Therefore this study assessed the cytotoxicity of seven cationic benzalkonium chlorides, varying in carbon chain length (6-18 carbons), in a basal cytotoxicity assay with the RTgill-W1 cell line. Cells were exposed for 48 hours in serum-free Leibovitz's (L15) exposure medium or medium containing either BSA (4 g/L) or 10% fetal bovine serum (FBS). After exposure, chemical concentrations in exposure medium and cells were measured using LC-MS/MS. Based the added concentration of chemical in BSA/FBS free exposure medium, cytotoxicity increased almost 1000 fold with increasing chain length (and thus with hydrophobicity), but not after the tail contained more than 12 carbon atoms. Based on measured concentrations in BSA/FBS free exposure medium however, cytotoxicity increased with increasing chain length over the entire chemical range. The cytotoxicity in BSA/FBS containing medium was up to a 50 fold lower. The findings indicate that the greater the chain length, the stronger the chemical binds to plastic or serum constituents, therefore, the lower the available fraction of the chemical is for causing cytotoxicity in cells. Based on cell-associated concentrations, there was little difference in the cytotoxic potency of the test chemicals because it is independent of assay setup and binding matrices. Such dose is more directly related to the chemicals modes of action (narcosis). Thus, using free and cell-associated concentrations allows for a more robust comparison of intrinsic cytotoxic potencies of surfactants in vitro.
Assignment of Chemicals to Mode-of-Action Categories (MoA) Using In Vitro Cellular Time-Response Curves

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A number of in vitro end point assays are being utilized to develop prediction models, with varying success. We explored a kinetic bioassay and developed mathematical models for data analysis. The objective was to determine if dynamic information on cell growth characteristics could provide an alternative to the MoA of chemicals. 303 compounds from the USEPA Toxicast Phase I library were tested in several human cell lines at multiple concentrations. The cytotoxic responses to chemicals were recorded once every hour for 72h using the xCELLigence real-time cell analysis high-throughput (RTCA-HT) system. A screening step was applied to differentiate chemicals showing biaactivity from those with no effect. Only the 90 chemicals showing an effect were considered for the data analysis. A model-based hierarchical approach, incorporating principal component analysis and functional data analysis was developed to extract informational feature vectors from the response curves in order to cluster the chemicals. Dendrograms were generated and cut at proper heights to result in four clusters of chemicals. The results show the response curves in order to cluster the chemicals. Dendrograms were generated and cut at proper heights to result in four clusters of chemicals. The results show the response curves in order to cluster the chemicals. Dendrograms were generated and cut at proper heights to result in four clusters of chemicals. The results show the response curves in order to cluster the chemicals. Dendrograms were generated and cut at proper heights to result in four clusters of chemicals. The results show the response curves in order to cluster the chemicals.

Development and Validation of a Standardized In Vitro Cytotoxicity Assay

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Several governmental agencies have explored and developed innovative testing methods for toxicity liability. Although their data, methods, and assays used to characterize toxicity are readily available, no fully standardized method has been endorsed for industry use. We sought to bridge this need and develop a completely integrated and standardized primary testing system that could be employed with low inter- or intra-assay variability and high predictive value. The system consisted of rigorously qualified, thaw-and-use HepG2 cells which were plated at a defined, sub-confluent density in a standardized culture medium containing an inert, non-fluorescent membrane integrity dye. After cellular attachment and acclimation, the plates were serially dosed with two model control toxins. Digitonin, a pore-forming glycoside, served as the fast-acting primary necrosis control, whereas trichostatin A, a histone deacetylase inhibitor, served as the apoptosis-inducing control. After 48hrs of compound exposure, fluorescence data resulting from cytotoxicity was collected, followed by the application of a luminescent viability assay. Together, the data were analyzed for anti-proliferative or cytotoxic dose dependence, relative to an untreated vehicle control, using the provided software. We observed near inverse concordance between the viability and cytotoxicity measures with digitonin whereas dose-shifted anti-proliferative and cytotoxic responses were measured with trichostatin A exposure. Signal to background dynamic responses for the two chemistry measures were robust, and inter- and intra-assay variation for EC50 values were typically far less than 10%. The data collected were sufficiently invariant and encouraging to provide a tangible framework for the standardized testing of larger subsets of either existing compounds or new chemical entities. Furthermore, the data collected from this accessible and standardized method should be fully comparable between laboratories and screening institutions, thus providing a functional means to rank order toxicity liabilities.

Synergism of Fumonisin and Aflatoxin Toxicity and the Underlying Mechanism in Nematode Caenorhabditis elegans

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Fumonisin B1 (FB1) is a class 2B carcinogen (IARC) with a strong tumor promotion activity in animal models. Recently, much interest has been placed in its role of promoting tumors induced by other carcinogens, such as aflatoxin B1 (AFB1), a class 1 carcinogen (IARC). Studies in animal models have shown that the co-exposure to AFB1 and FB1 elicits considerably stronger toxic and carcinogenic effect than when exposed to either of the toxins alone. While animal models are valuable tools in examining toxic as well as mechanistic actions, most of the models used are very expensive and time consuming in maintenance. C. elegans has emerged as a valuable model organism, with its simplicity, completely mapped genome, as well as several homologous systems to the mammalian systems. Studies have shown that C. elegans have CYP metabolism capable of metabolizing AFB1, thus confirming AFB1 genotoxicity in this organism. While there are no existing studies examining toxic effect of FB1 on this model organism, C. elegans possess functional ceramide synthases (CesS), the molecular target of FB1, which, when inhibited, promotes survival in a way similar to the pro-survival pathway that can be activated when FB1-induced CerS inhibition took place. This makes C. elegans a potential model in evaluating the mechanism of AFB1-FB1 synergy. The current study, therefore, attempts to examine the synergetic effect of FB1 and AFB1 and the related mechanisms in the model organism. Co-exposure to AFB1 and FB1 resulted in significant DNA damage when compared to individual toxins (N=7, p=0.005, p=0.01 and p=0.04). The reduced rate of growth and lifespan were 45.43% and 32.77%, respectively, while DNA damage increased 41.23% as compared to the untreated controls. The transgenic strain, nucleotide excision repair (NER)-deficient (spa-1), were further tested confirming that the synergetic toxic effect resulted from CYP-mediated metabolism. The Gene expression of CYPs, glutathione S transferase (GSTs) class, and SKN-1/Nrf2 were also examined to explore the possible molecular mechanisms involved.

Evaluation of an Integrated Human Multi-Organ Culture Plate for Predicting Systemic Toxicity In Vitro

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The framework put forth in the report entitled "Toxicity Testing in the 21st Century" (2007) focused on the need for the development of alternative methods to animal testing. Emphasis was placed on the use of human cells combined with adverse outcome pathways for determining adverse effects. Predicting systemic toxicity in vitro requires a platform that incorporates multiple organs interconnected via a fluidics network. The aim of this study was to evaluate a new Dynamic Multi-Organ Plate (DMOP) that provides the ability to use human tissues or cells, which are in communication via fluidics and dialysis. A simple two compartment model containing Caco-2 cells and rat primary hepatocytes was used initially. A standard 6-well plate was fitted with a fluidics/dialysis network. The dialysis membrane allows for exchange of test article and metabolites while maintaining each cell type under optimized media conditions. Caco-2 cells were grown to confluence on a transwell insert and then transferred to the DMOP. To mimic oral dosing, Acetaminophen (APAP) (200 μM) was added to the upper chamber of transwells containing confluent monolayers of Caco-2 cells. Dialysis samples from the lower chamber were collected at a flow rate of 1 μL/min. Integrated time samples were collected at 1, 2, 3, and 6 hr. APAP was first detected in the 1-2 hr dialysate with a relative recovery of approximately 70%. APAP was added to the second compartment containing a monolayer of primary rat hepatocytes and dialysis samples collected over 2 hr. APAP, as well as its glucuronide and sulfate conjugates, were detected in the perfusate. Relative recovery of APAP in the hepatocyte dialysate was >95%. These data demonstrate that a meso-scale multi-organ culture platform linked by fluidics/dialysis can provide a means of evaluating the movement of chemicals and their metabolites between organs in order to predict systemic effects in vitro.

Roadmap for Animal-Free Acute Toxicity Assessments of Crop Protection Formulations

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Registration of crop protection formulations requires conduct of up to 6 acute toxicity studies to assess irritation (skin and eye), sensitization, and acute lethality via oral, dermal, and inhalation routes. Recent advancements in computational and in vitro approaches provide the opportunity to move away from animal testing for generating reliable acute toxicity information. Presented here are results demonstrating successful application of alternative approaches for evaluating acute toxicity for crop protection formulations. For eye irritation, a tiered approach is employed with the neutral red release (NRR) assay is first used to identify strong or non-irritating formulations followed by the EpiOcularTM assay to differentiate between mild and moderate irritants. In this approach, the NRR assay correctly predicted all 16 formulations classified as severe eye irritants in vivo while the EpiOcularTM tier had 86% accuracy. Similarly, skin irritation potential is
predicted utilizing the in vitro methods EpiDermTM or EpiSkinTM. To predict skin sensitization potential, the KeratinosensTM assay was implemented and correctly classified 22 of 25 formulations based on in vivo data. Finally, for acute lethality end-points, a combination of data waivers, computational and in vitro approaches exist to eliminate animal use. First, based on analysis of >200 formulations, the acute dermal study was shown to provide no value in hazard assessment and should be waived. In addition, use of the GHS additivity formula calculation method provided >90% concordance for acute oral, dermal and inhalation toxicity categorization for >120 formulations. This approach can be coupled with in vitro cytotoxicity assays and route-to-route extrapolation to evaluate oral and inhalation end-points. Taken together, for the first time, application of these alternative approaches demonstrates their utility to address each of the acute toxicity end-points in a reliable manner, allowing removal of the use of animals from acute hazard assessment.

1193 Organelle Imaging Toxicology: Novel Analysis of the Sandwich High-Content Screening Project

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High Content Screening (HCS) is a powerful in vitro imaging technique utilized for toxicology evaluations of drugs/compounds. For example, the HCS study performed by Howard-Cofield, Irwin, Diaz, Krejsa, Slaughter, Gao, Kaludercic, Angelin, Bernardi, Brain, Hougham and O’Brien is a highly-cited/implemented manuscript in HCS research (Archives of Toxicology 80:580-604). A HepG2 liver tumor cell-based imaging project performed in Sandwich, England utilized a panel of assays including cell count, nuclear area, mitochondrial membrane potential, cytosolic Ca levels and plasma membrane permeability as the toxicity metrics. Cells were treated with 11 doses based on Cmax values for 3 days with 243 compounds tested (i.e. bupivacaine, cyclospor, cerivastatin, etc). The HCS data generated were analyzed considering novel models for in vitro hazard, targeted toxicity thresholds, hormesis, drug efflux pump effects, dye quenching, Cmax, therapeutic index, drug spectra. EC50 and the laws of thermodynamics. The data for each compound were compared and evaluated in these contexts. The novel models indicate that the panel of assays have a concordance to human pathology of ~65%, with a positive rate of ~5% for all compounds tested. In some cases, the HepG2 panel of assays had better sensitivity than primary hepatocyte assays. The HepG2 panel was able to detect the toxicity of compounds with adverse effects to other target organs than the liver, indicating that the common theme of adversity is often at the organellar level (i.e. organelle biology/toxicology). The model indicates that drug efflux pump inhibitors can have false positive results in some assays, such as the mitochondrial membrane potential metric. Consideration of cellular thermodynamic principles, plus the physi/physical-chemical properties and spectra, were critical in the analyses. The nuclear area and cell count metrics were often the most sensitive assays, while the membrane potential, cytosolic Ca and membrane permeability assays provided valuable mechanistic information. Disclaimer: The opinions are those of the authors alone and not necessarily those of the US EPA.

1194 Use of Semi-Permeable Membrane for Preliminary Evaluation of Transport of Native and an Enhanced Formulation of Butyrylcholinesterase

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In vitro experiments have value providing useful preliminary data with reduced cost and animal use when evaluating potential new treatment formulations. That enhanced formulations of enzymes can release enzyme equivalent to native butyrylcholinesterase (BuChE) was the hypothesis tested by comparing time-related passage of both through a 0.3 uM porous membrane using a transwell system. The enzymes were placed on top, fluid below was removed at various times, and esterase activities above and below the inserts measured and compared. Aliquots were taken every 5 minutes and transferred to wells of 96-well plates that contained substrate and cofactors for the BuChE assay, with absorbance of the yellow color increasing with time. The lower rise in the slope for development of the yellow color for samples taken from below the insert compared to the rise in slope for samples taken above the membrane indicates that the membrane, although porous, hindered movement of both native and enhanced formulation enzymes, although both could pass. Comparison of increases in enzyme activities indicated that the membrane restricted short term (120 min) passage to only 5% of the native product and 1% of the formulated product. The comparison demonstrated, however, that the enzyme in the formulated product could be released, could cross a membrane, and could have activities comparable to native enzyme after membrane passage. The experiments also suggested that this in vitro method could be used to provide preliminary information on the value of an enhanced enzyme formulation. Supported by Luna Innovations Incorporated. This material is based upon work supported by the US Army Contracting Command, Aberdeen Proving Ground, Natick Division under Contract No. W911QY-13-P-0170. Any opinions, findings and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the US Army Contracting Command, Aberdeen Proving Ground, Natick Division.

1195 Butafenacil: A Positive Control for Identifying Anemia- and Porphyria-Inducing Chemicals

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Butafenacil is an herbicide that inhibits protoporphyrinogen oxidase (PPO), an enzyme that catalyzes oxidation of protoporphyrinogen IX to protoporphyrin IX – the penultimate step of chlorophyll and heme biosynthesis in plants and animals, respectively. Based on a high-content screen, we previously identified butafenacil as a potent inducer of anemia in zebrafish, as exposure to 0.39-3.125 uM butafenacil from 5 to 72 hours post-fertilization (hpf) completely abolished arterial circulation in the absence of effects on all other endpoints evaluated. Therefore, the objective of this study was to begin investigating the mechanism of butafenacil-induced anemia in zebrafish. Similar to 384-well plates, static exposure from 5-72 hpf in glass beakers resulted in elimination of arterial circulation at low micromolar concentrations. By quantifying protoporphyrin autofluorescence in situ at 72 hpf, we also discovered that butafenacil exposure resulted in a concentration-dependent increase in protoporphyrin accumulation, suggesting that zebrafish embryos developed porphyria-like conditions as a result of PPO inhibition and blood cell elimination. As protoporphyrinogen can undergo photooxidation to produce acutely toxic reactive oxygen species, we then treated embryos to butafenacil from 5-72 hpf under light or dark conditions, and found that, while anemia was observed in the presence or absence of light, protoporphyrin accumulation and acute toxicity was significantly lower or absent under dark conditions. Finally, to identify sensitive developmental windows, we exposed embryos from 5, 10, 24, or 48 hpf to 72 hpf, and found that anemia was only present within 72-hpf embryos exposed from 5-72 hpf or 10-72 hpf, suggesting that segmentation (10-24 hpf) may be a critical window of susceptibility and, in addition to PPO, butafenacil may be targeting early stages of hematopoesis within zebrafish. Collectively, our data strongly support the use of zebrafish as a model and butafenacil as a positive control for identifying anemia- and porphyria-inducing chemicals.

1196 Microfluidic Culture of NRK52E Enhances Sensitivity to Proximal Tubule Toxicants

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Several features of renal proximal tubule (PT) cells are poorly modeled with PT cell lines cultured in static well plates which can affect our ability to de-risk renal toxicity issues with in vitro models. Advanced in vitro cell-based models offer the promise to better capture in vivo cellular phenotype and response, thereby leading to improved correlation with in vivo toxicity. To determine if increasing the complexity of in vitro PT models by growing NRK52E cells, a rat PT cell line, in a fluidic sheath environment will improve the predictivity for in vivo PT toxicity, we compared the effect of toxicants on NRK52E grown under static and flow conditions. NRK52E cells were grown in SynVivo microfluidic devices and exposed to compounds under static or flow conditions (0.02μL/min). Four compounds were evaluated: polystyrene B (PMB) and cisplatin (CP; known PT toxicants), aspirin and an internal Phter compound (PF-A) that had PT toxicity in vivo but that was not detected in vitro with NRK52E or H2K PT cells in standard static cultures. Cell viability was quantified as ATP content for cells in standard conditions and IC50 values after 24 hrs exposure were: 787ug/mL PMB; 68uM CP; >1000uM aspirin; 5-25uM PF-A. For NRK52E cells grown in SynVivo PT devices, viability was assessed in the presence or absence of light, protoporphyrin accumulation and acute toxicity was significantly lower or absent under dark conditions. To identify sensitive developmental windows, we exposed embryos from 5, 10, 24, or 48 hpf to 72 hpf, and found that anemia was only present within 72-hpf embryos exposed from 5-72 hpf or 10-72 hpf, suggesting that segmentation (10-24 hpf) may be a critical window of susceptibility and, in addition to PPO, butafenacil may be targeting early stages of hematopoesis within zebrafish. Collectively, our data strongly support the use of zebrafish as a model and butafenacil as a positive control for identifying anemia- and porphyria-inducing chemicals.
An in vitro testing system employing human derived cells that model the gastrointestinal (GI) barrier was evaluated as a potential tool for evaluating the safety of individual proteins. In this study, in vitro indicators of cytotoxicity (LDH release & MTT conversion), barrier integrity (3H-inulin flux, HRP flux, & trans-epithelial electrical resistance (TEER)), and inflammation (IL-18 & IL-6 release) were assessed using human intestinal epithelial cell (IEC) lines (T84, CaCo2, & HCT-8) grown on permeable Transwell™ filters following addition of proteins. The proteins included: toxins (Streptolysin O, Clastidium difficile toxin A, Clastidium difficile toxin B, E.coli heat labile toxin, Listeriolysin O, mastoparan, & melittin); innocuous proteins (bovine serum albumin, porcine serum albumin, fibronectin, & Rubisco); and allergenic food proteins (ß-lactoglobulin, Ara-h2, & wheat germ agglutinin). Results demonstrated reproducible effects on barrier integrity, inflammatory responses, and cytotoxicity as a result of exposing IEC to several toxins in this in vitro testing system, while there were no effects when the IEC were exposed to innocuous proteins.

In vitro Model Development for Safety Assessment of Acutely Ingested Proteins

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In vitro models using cultured cells may provide an alternative for these regulatory requirements. In order to develop a model for acute toxicity assessment of proteins, Caco-2 human epithelial intestinal cells were cultured for 21 days on permeable Transwell™ inserts forming a monolayer resembling the enterocyte lining of the small intestines. Phytohaemagglutinin (PHA) from red kidney bean was used to determine the effect on intestinal cells or with systemic toxicity following absorption from the gastrointestinal system. Intractable proteins are defined as proteins that cannot be isolated in an active form in the quantities necessary to conduct in vivo toxicological testing. In vitro models using cultured cells may provide an alternative for these regulatory requirements. In order to develop a model for acute toxicity assessment of proteins, Caco-2 human epithelial intestinal cells were cultured for 21 days on permeable Transwell™ inserts forming a monolayer resembling the enterocyte lining of the small intestines. Phytohaemagglutinin (PHA) from red kidney bean lectin is resistant to digestion and causes ultrastructural damage in rat intestine. Here, PHA was used to determine the effect on intestinal cells in vitro, measuring changes in viability and barrier integrity. Following up to 24 hours treatment, PHA (200 ng/mL) decreased tight junction integrity causing a significant flux in 6.4 kDa TRITC-dextran to the basolateral compartment compared to media alone and this was accompanied by a 40-50% reduction in TEER measurements. The disruption to the monolayer was size selective as no change in 70 kDa FITC-dextran flux was observed. In addition, cell viability was not altered by PHA as measured by neutral red absorption, suggesting that the monolayer integrity was intact. These results demonstrate that an in vitro model may be used to predict acute toxicity in vivo, potentially providing weight of evidence for protein safety assessments.

Establishment of an Intestinal Model for Oral Vaccination Testing

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The intestinal epithelium is an impermeable barrier for macromolecules and microorganisms, except the follicle-associated epithelium of the gut-associated lymphoid tissue where microfold (M) cells sample the intestinal lumen initiating an immune response. This entry site to the immune system is an interesting target not only for pathogens but also for the analysis and improvement of oral vaccines. Because of the complexity and limited accessibility of the in vivo environment an in vitro model system composed of intestinal epithelial cells and immune cells on a biological scaffold should provide an important tool and test system for studying microfold cells, infections and oral vaccination. In this work, a functional intestinal in vitro model based on the adenoma carcinoma cell line Caco-2 co-cultured with dendritic cells derived from human blood on a three-dimensional matrix from acellularized porcine jejunum (SIS-Muc) was established. In a proof-of-concept experiment the three-dimensional co-culture model could demonstrate an activation of the dendritic cells with Lipopolysaccharides over the epithelial barrier observed by the upregulation of maturation markers (CD80, CD83, CD86) and interleukin 6 production measured via FACS and ELISA analyses. Additionally, transmission electron microscopy was used to visualize M cells by the presents of truncated microvilli. Taken together an improved three-dimensional intestinal in vitro model suited for infection and oral vaccination studies could be established which can be further improved and modified for specific issues. In this respect, we are currently using autologous human primary cells, which is of particular importance for future vaccination studies.

3D In Vitro Human Small Intestinal Tissue Models to Assess Drug Toxicity and Permeation

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Development of reliable and reproducible primary human cell based small intestinal (SMI) tissue models that recapitulate in vivo SMI tissue phenotype, structure and function are critically needed to study gastrointestinal (GI) permeation, drug toxicity and inflammation. The validity of commonly used Caco-2 cell models is questionable due to a lack of physiological relevance and animal models often fail to predict human responses. This study describes development of 3D SMI models from primary human SMI epithelial cells and fibroblasts. Long term culture of the models and application for drug toxicity and drug permeation studies are demonstrated. The utility of the reconstructed SMI tissue models for GI drug toxicity studies was validated using the GI toxicant drug, indomethacin. Toxicant measurements include transepithelial electrical resistance (TEER), histology and apical protein washes (sloughed epithelium). Drug permeation studies were performed using 8 drugs that utilize specific transporters (Pgp, BCRP, MRP-2, etc.). Uptake or efflux transport was analyzed by LC-MS/MS. Specific findings include: 1) The SMI tissue models can be cultured for extended time (up to 28 days) with no significant change in TEER or Lucifer yellow leakage (<2%); 2) Dose dependent toxicity of indomethacin was noted with respect to reduction of TEER, epithelial damage, and epithelial sloughing; and 3) Functionally active drug transport (B-to-A transport with efflux ratios >2 fold) for 6 of 8 test compounds was observed. Furthermore, drug efflux transporter inhibitors increased drug absorption while decreasing the efflux ratio. Efflux ratios for talinolol, digoxin, and loperamide (Pgp substrates) were reduced by 45%, 40%, and 60%, respectively, in the presence of the Pgp inhibitor verapamil. Efflux ratio of the BCRP substrate nitrofurantoin was reduced by 63% in the presence of novobiocin, a known BCRP inhibitor. In conclusion, the newly developed SMI tissue models appear to be promising new tools for drug safety and permeation studies.

Standardized Rat and Human Microislets for Diabetes Research and Drug Safety Assessment

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Primary pancreatic islets are an important source for in vivo research related to diabetes and metabolic disorders as well as for the prediction of off-target effects directed towards the endocrine function of the pancreas. The quality of pancreatic islets with respect to purity and viability can vary significantly between different isolations, or depend on donor characteristics, cause of death and other confounding factors. Here we present a new in vitro model comprising of human or rat pancreatic islets for improved data robustness and reproducibility. Freshly isolated human or rat pancreatic islets where dissociated into single cells and re-aggregated to microislets with unified cellular composition and size. Viability (ATP) morphology (H&E, IF) and metabolic performance (glucose stimulated insulin secretion, GSIS) was assessed over time. The dissociation and subsequent re-aggregation of endocrine cells gave a homogeneous population of microislets of similar sizes, 145±3 μm in diameter for human, and 132±11 μm for rat islets (data from 3 isolations each). The ratio and cellular distribution of alpha-, beta-, and delta cells of rat and human microislets was comparable to freshly isolated islets. The microislets could be cultured for at least 5 weeks with sustained viability and metabolic performance. Glucose stimulated insulin secretion could be maintained during this time with stimulation factors (2.8mM/16.8mM glucose) ranging from 5-19x for human and 4-26x for rat microislets. The islets showed normal physiological behaviour with increased glucose release in presence of low glucose, enhanced insulin secretion in presence of GLP-1 at high glucose and inhibited insulin secretion in presence of the somatostatin analogue Octreotide. Currently the model is under evaluation.
for toxicity studies. The presented data convincingly demonstrate the suitability of microsles to be used as a highly standardized in vitro model for metabolic studies and assessment of drug safety with respect to pancreatic endocrine function.

1202  A Novel Biomarker Panel to Identify Hepatocellular Carcinoma in Chronic Hepatitis C-Infected (HCV) Patients

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Hepatocellular carcinoma (HCC) with a survival rate of 5% is one of the leading causes of cancer worldwide—poor survival largely due to late stage diagnosis making successful intervention difficult if not impossible. The projected rise in HCC is mainly due to hepatitis C virus (HCV) infection with onset of HCC coming several decades after initial infection. However, additional environmental risk factors including alcohol, tobacco and dietary insults that induce liver injury promote the incidence of HCC. Given the poor prognosis, development of biomarkers suitable for early HCC detection are a priority. Samples from a retrospective cohort of HCV-positive patients clinically diagnosed with liver disease (pre-HCC) or HCC, was used to interrogate the serum proteome for changes in protein expression using 2D-Difference In Gel Electrophoresis. Statistically significant ≥1.5X changes in 24 proteins were identified between the pre-HCC and HCC profiles—11 were decreased and 13 increased in the cancer patient samples. Mass spectrometry and stable isotope O18/O16 labeling was used to verify the protein identities and relative expression levels, respectively, and support the development of Selected Reaction Monitoring assays. Using absolute quant (AQUA) peptides in Multiple Reaction Monitoring (MRM) and dynamic MRM assays we are selecting a panel of candidate biomarkers comprising 8-10 proteins, for use in an independent retrospective study design to verify their discriminatory capacity of the biomarkers. Development of the quantitative dynamic MRM assay and verification of the biomarker panel serves as a platform for a subsequent validation study.

1203  Assessment of Individual Bile Acid Profiles after Single Oral Dose of Itraconazole in Rats and Dogs


Inhibition of BSEP is a susceptibility factor for DILI. The objective of the Predictive Safety Testing Consortium (PSTC) BSEP sub-team is to investigate the utility of individual bile acids as biomarkers of BSEP inhibition. Itraconazole is a fungistatic triazole that inhibits BSEP in humans, rats, and dogs with IC50 ranging from 4-18µM in vitro, suggesting potential for in vivo BSEP inhibition. In conjunction with the PSTC BSEP sub-team, we characterized the PK/PD relationship of itraconazole drug concentration and individual serum bile acid changes in rats and dogs. Rats were dosed with vehicle (40% HP-β-CD), 10 or 40 mg/kg itraconazole (Sporanox oral solution). Dogs were dosed with vehicle, 5 or 20 mg/kg itraconazole. Time-matched serum for TK and bile acid analysis were collected at 8 time points over a 48-hour period post-dose. Total bile acid levels were measured and 21 individual bile acids were quantified with a partially qualified LC/MS method. Serum ALT, AST and TBILI were assessed at 6 and 48 hours post dose. In the rat, liver was taken at 6 and 48 hours for histopathological analysis. Rats had a Cmax of 0.85 and 3.4 µM and AUC0-inf of 15.5 and 88.9 hr*µM at 10 and 40 mg/kg, respectively. Dogs had a Cmax of 0.85 and 3.8 µM and AUC0-inf of 9.4 and 48.5 hr*µM at 10 and 20 mg/kg, respectively. Itraconazole did not cause changes in serum chemistry or total bile acids in either species and no microscopic changes occurred in rat liver. Baseline bile acid levels were similar to other PSTC studies. Glycine-conjugates were markedly higher in rats compared to dogs. In dogs, taurine-conjugates were higher than both un conjugated and glycine-conjugated bile acids, while unconjugated bile acids were higher than both conjugates in rat. Slight alterations in bile acid profiles may occur with itraconazole dosing in both species, but additional statistical analysis is needed. Future studies will implement IV dosing of itraconazole in both species to achieve higher circulating and liver exposures.

1204  Biomarkers of Immune-Mediated Concanavalin A Hepatotoxicity


Novel biomarkers that increase the sensitivity and specificity of detecting hepatotoxicity have been investigated in rodent models of the direct toxicant acetaminophen. These serum markers, arginase-1 (Arg1), sorbitol dehydrogenase (SDH), and glutamate dehydrogenase (GDH), have been compared to the gold standard alanine aminotransferase (ALT). These markers were evaluated in this study of a model of immune-mediated hepatotoxicity induced by the mitogen concanavalin A (conA). Male Wistar rats were given a single intravenous injection of sterile phosphate buffer solution (PBS) or 20 mg/kg conA. Serum chemistry parameters, liver biomarkers, and acute phase proteins were measured at 3, 6, or 30 h after injection. Numbers of leukocytes and CD3+ , CD4+ , and CD8+ T-cells were determined at 3 or 6 h by flow cytometry. Histopathological examination of the liver from all time points was performed. Arg1 in the serum increased from 3 h, and paralleled the increase of ALT. Increases in Arg1 and ALT were similar at 3 h and 6 h, and both were massively increased at 30 h. There was a time-dependent increase in GDH from 3 h, while SDH increase was clearly seen only at 30 h post-conA injection. Both GDH and SDH increases were less marked. Of the acute phase proteins, there was a minimal decrease in total protein and albumin, a marked increase in alpha-2 macroglobulin at 30 h, while haptoglobin was unchanged. Total white blood cells declined markedly from 3 h and correlated with the decline in lymphocyte number. Both CD4+ and CD8+ T-cells declined; however, the CD4+/CD8+ ratio was higher in rats given conA compared with rats given PBS. The incidence and severity of hepatocellular apoptosis and periporal mononuclear infiltration increased from 3 h. Conclusion: A significant early increase in serum Arg1 improves the detection of inflammatory hepatotoxicity. GDH and SDH are useful liver injury biomarkers in addition to ALT. Furthermore, markers of an acute phase reaction and T lymphocyte depletion support the immunopathogenesis of conA hepatotoxicity.

1205  Profiling Individual Bile Acids in Human Populations Using a UPLC/MS/MS Method

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Although serum alanine aminotransferase (ALT) remains the gold standard biomarker of hepatic injury, the development of additional biomarkers capable of facilitating the interpretation of serum ALT increases and differentiating between various histopathological findings is essential. Both individual bile acids (IBA), such as cholic acid and taurocholic acid, and bile acid profiling have been proposed as potential biomarkers of liver injury in literature. Although IBA show promise as alternative biomarkers of hepatic injury, systematic characterization in humans has not yet been undertaken. In this study, we utilized a validated LC/MS/MS method to analyze eight IBA in a large number of human subjects to generate valid reference ranges in healthy populations of varying age, gender, and ethnicity. We also evaluated the ability of these potential biomarkers to detect hepatic injury in a broad range of clinically demonstrated liver injuries by comparing them to the baseline IBA concentrations observed in the healthy samples. In comparing data across ages, genders, and ethnicities in healthy humans, no significant difference was observed between the age groups, and females tended to have higher bile acid levels than males. IBA and total bile acid concentrations were significantly increased in hepatic injury subjects in comparison with healthy subjects, except for the secondary bile acid deoxycholic acid. Also, conjugated bile acids displayed much greater increases in comparison with free form bile acids. In comparison with healthy subjects, the patients with hepatic injury exhibited different bile acid patterns, with a much higher composition of cholic acid and its conjugated forms, and a much lower composition of secondary bile acids. These results initiate the establishment of reference ranges for IBA in humans, and begin to demonstrate the potential utility of IBA and their profiles as biomarkers of liver injury in the clinical setting.
High-mobility group box-1 protein (HMGB1) is a circulating mechanistic biomarker of acetaminophen-induced hepatotoxicity. Recent studies describe a time-dependent increase in serum HMGB1 concentration, correlating with elevated liver histopathology. HMGB1 is also detected earlier than alanine transaminase. In this study, we sought to determine if plasma HMGB1 concentrations could also be used to detect hepatotoxicity after exposure to toxic industrial chemicals. Groups of rats were orally exposed for five days to four doses of bromobenzene, carbon tetrachloride, allyl alcohol, 4,4'-methyleneedianiline (MDA), or vehicle control. Livers were harvested 24 hours after the final exposure for histopathological assessment by hematoxylin and eosin staining. Plasma HMGB1 concentration was determined by enzyme-linked immunosorption analysis (ELISA). Statistical significance was determined by Kruskal Wallis analysis of variance and correlation analysis. All chemicals caused a dose-dependent increase in hepatic damage. A dose-dependent increase in plasma HMGB1 concentration was observed only after bromobenzene exposure (p<0.08). No significant dose relationship was observed in the other toxicants, with considerable heterogeneity among all treatment groups. HMGB1 was positively correlated with severe histopathologies relative to no histopathology (1.3±0.2 and 2.3±0.5 ng/ml in histopathologies of 0 and 3-4; r=0.9). In conclusion, repeated oral exposure to toxicants corresponded to drug-induced HMGB1 elevation only in the most severe histopathologies. Disclaimer: This project was supported in part by an appointment to the Research Participation Program for the USAMRMC administered by ORISE through an agreement between the US DOE and USAMRMC. Research was conducted in compliance with the Animal Welfare Act and all other Federal requirements. The views expressed are those of the authors and do not constitute endorsement by the U.S. Army.
**1210** PSTC and SAFE-T Collaboration: Evaluation of Normal Reference Ranges for 12 Novel Liver Safety Biomarkers in Healthy Volunteers


In collaboration with the Safer and Faster Evidence-based Translation Consortium (SAFE-T), the Predictive Safety Testing Consortium (PSTC) characterized normal reference ranges for 12 liver biomarkers in 81 healthy volunteers (HV). The data generated within this study will be used by PSTC and SAFE-T as part of their Drug Induced Liver Injury (DILI) biomarker qualification process. Subjects were recruited at the Jasper Clinic in Kalamazoo, MI. There were 40 males and 41 females with 41 subjects in the 20-39 yr range and 40 subjects in the 40-70 yr range. The population was 84% white and 16% non-white. The mean BMI was 27 with 69% of the population overweight/obese by CDC criteria. Plasma and serum samples were collected over 21 days on days 1, 6 (±1), and 20 (±1). There were 263 samples for quantitative biomarker measurement with twelve technically validated immuno- and colorimetric assays generated by SAFE-T and affiliates. Arginase-1, LECT2, MCSF1R, prothrombin, paraoxonase-1, caspase-cleaved keratin18 (ck18), full length keratin18 (K18), β-tetraspanin, osteopontin and GST-α were measured by immunoassay. GLDH and SDH were measured by colorimetric assay. The intra- and inter-subject variability was investigated using 3 serial samples over a 21-day course. 95th percentile was obtained as upper bound for normal range for all 12 liver safety markers, and the mean value was also obtained for all but K18. K18 had 93% of the samples below lower limit of quantification of the assay. The 95th percentile upper bound are as follows: arginase-1 (19.5 ng/ml), LECT2 (448 ng/ml), MCSF1R (572 ng/ml), prothrombin (86.3 μg/ml), paraoxonase-1 (123 U/L), CK18 (260 U/L), K18 (123 U/L), β-tetraspanin (1.98 ng/ml), osteopontin (10.3 ng/ml), GST-α (60 ng/ml), GLDH (7.2 U/L) and SDH (7.7 U/L). Stratification factors such as gender, age or BMI did not demonstrate differences in reference ranges.

**1211** In Vitro Study of Potential Nephrotoxicity Biomarkers through Gene Expression


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Drug-induced nephrotoxicity is one of the most frequently observed effects in the early preclinical phase of drug development. The effects of nephrotoxicity are commonly discovered later due to lack of sensitivity of in vivo methods to evaluate this effect. Therefore, researchers have tried to develop in vitro alternative methods for the early identification of toxicity. Identification of drug-induced gene changes is critical to providing insights into molecular mechanisms and to detecting renal damage. Gentamicin, for example, is a widely used aminoglycoside antibiotic that causes nephrotoxicity. In the present study, LLC-PK1 cells were exposed for 24 h to gentamicin concentrations of 4 (low), 8 (medium), and 12 (high) mM, according to MTT tests, to evaluate gene expression. A literature survey was conducted to identify genes associated with the development of nephrotoxicity. A panel of genes was selected based on gene expression changes in multiple published studies. Due to the limited base of study for the cell model in this work, the search for sequences of mRNA encoding proteins that had been previously associated with kidney damage was researched in the databases of the National Center for Biotechnology Information - NCBI (USA). The primers were obtained using the Primer BLAST (NCBI) program, based on the sequences of selected transcripts. RNA was extracted from the cells, and RT-PCR was performed to evaluate expression profiles of the selected genes. Among the analyzed genes, four genes proved to be highly up-regulated in cells exposed to the nephrotoxic: α-HAV1 (hepatitis A virus, cell receptor 1), GAPDH (gad2 and caspase 1), ICAM1 (intracellular adhesion molecule 1), and EXOC3 (exocyst complex component 3). According to the obtained results, it can be suggested that these genes can be used as early in vitro biomarkers for the identification of nephrotoxicity. The establishment of genomic markers will be useful in the development of safer drugs.

**1212** TCDD Alters Keratin Expression in the Urogenital Sinus during Initiation of Prostatic Budding in Mice

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Prostate development begins in utero in the urogenital sinus (UGS). Buds emerge from the basal surface of UGS epithelium (UGE) and extend into the surrounding mesenchyme. In mice, prostatic buds grow into four pairs of bilateral lobes: anterior, dorsal, lateral, and ventral. Prostatic budding is a transformational process that likely involves disparate differentiation for epithelial cells that do or do not form prostatic buds. Keratins are a large family of cytoskeletal proteins that are expressed in epithelial tissue and used to track and define cellular differentiation. We hypothesized that keratin expression in initiating and elongating prostatic buds differs from the surrounding epithelium. Exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) prevents prostatic bud formation in the ventral UGE. Therefore, we also hypothesized that TCDD alters keratin expression in the ventral UGE. We dosed pregnant C57BL/6J mice with vehicle or TCDD (5 μg/kg, po) at embryonic day (E) 15.0, prior to the initiation of prostatic budding. Male UGSs were harvested at E16.0, E16.5, and E17.5, and sagittal sections of the UGSs were stained by immunohistochemistry to reveal the expression pattern of keratin 5 (KRT5), KRT8, KRT10, KRT14, and KRT18. Robust expression of KRT5, 8, and 18 was observed throughout the UGE except in developing prostatic buds where expression was greatly reduced. Staining for KRT14 was also robust, but was restricted to the basal UGE, and was not decreased in the developing prostatic buds. KRT10 expression was expressed exclusively in the intermediate UGE, and was absent from developing prostatic buds. TCDD did not alter KRT14 expression, but increased KRT10 expression and prevented decreased expression of KRT5, 8, and 18 in the budding region of the ventral UGE. Therefore, TCDD-inhibition of prostatic budding might be due to altered cellular differentiation in the ventral UGE. (Supported by NIH grant E5001332)

**1213** Fit-for-Purpose Validation of Urinary Csb-9 As a Glomerular Injury Biomarker in Rats


With biotherapeutic development increasing in recent years, immunologic effects such as glomerular injury have become a significant risk to public health as well as drug development efforts. In order to monitor the risk of glomerular injury in preclinical and clinical drug development, reliable, sensitive and specific biomarkers are greatly needed. Urinary albumin and total protein are among the most commonly used diagnostic biomarkers for monitoring kidney injury. Rat passive Heymann nephritis (PHN) is a model of glomerular injury characterized by deposition of subepithelial immune complexes and complement fragment C3b-9, with ensuing proteinuria. Using a commercially available kit, we evaluated the performance of rat Csb-9 ELISA on zymosan-activated rat serum samples as a positive control, along with the urine samples from rat PHN studies. Acceptable criteria for a fit-for-purpose validation were set at ≤25 % coefficient of variation, range of 70 to 130 % of recovery, and ≤70% sensitivity and specificity. Moreover, the assay had to clearly distinguish the urine samples from the PHN rats and naive rats. The linear characteristics of the standard curve, the intra- and inter-precision, freeze and thaw stability, long term stability, sensitivity and specificity data generated for this study met acceptable criteria and demonstrated validity and robustness of the assay in monitoring immune-mediated glomerular injury in rat. In addition, significantly elevated urinary Csb-9 was observed in PHN rats compared to naive rats, clearly distinguishing the rats with immune complex-mediated glomerular injury. Furthermore, immune complex-mediated glomerular injury in rats was confirmed immunohistochemically by Csb-9 deposition in glomeruli. We concluded that Csb-9 assay performed robustly and was validated as a reliable biomarker for immune complex-mediated glomerular injury associated with complement activation in rat.

**1214** Assessment of Biomarkers for the Detection of Renal Toxicity following Intraurethral Tobramycin Administration in Male and Female Cynomolgus Macaques


Traditional serum biomarkers of nephrotoxicity, blood urea nitrogen (BUN) and creatinine (CRE), are relatively insensitive to acute renal damage, generally requiring a loss of up to two-thirds of kidney function to induce significant changes. We have evaluated a new generation of urinary biomarkers (Albumin (ALB), beta-2-microglobulin (β2M), Cystatin C (CysC)), epithelial growth factor
(EGF), neutrophil gelatine-associated lipocalin (NGAL), osteopontin (OPN), uromodulin (UMOD), glutathione S-transferase alpha (GSTα), Calbindin (CLB), Clusterin (CST), Kidney Injury Molecule-1 (KIM-1), Osteoactivin (OSA), trefoil factor 3 (TFF3), and vascular endothelial growth factor (VEGF) using MesoScale Discovery® (MSD) kits. Seven male (N) and female (F) non-naïve Cynomolgus macaques (CM) were administered either vehicle (2 M & 1 F) or tobramycin (TBM; 10 M & 2 F) or 18 mg/kg (2 M) via intravenous (iv) daily dosing for 14 days. Histopathology revealed lymphocytic infiltration of the interstitial tissue and minimal to moderate necrosis of the proximal tubule in male CM treated with 10 or 18 mg/kg of TBM and histopathology indicated that CM’s given 18 mg/kg of TBM compared with CM’s given 10 mg/kg. No elevations in BUN or CRE were observed following either 10 or 18 mg/kg of TBM. Only one male, treated with 18 mg/kg of TBM, showed elevated urinary KIM-1, CST, ALB, CLB, GSTα, OSA, and VEGF on Day 2; no other changes were reported. Female CM’s exhibited no histopathology following treatment with either 10 or 18 mg/kg of TBM and the findings are consistent with findings in both traditional and novel biomarkers in female CMs. These results suggest that 14 days of treatment with 10 and 18 mg/kg of TBM induce renal toxicity in male CM as evidenced by histopathology, but that the currently available MSD kits provide limited sensitivity for this endpoint in CMs. Work supported by NIAID Contract N01-AI-70043.

**1215 Urinary Biomarkers for Drug-Induced Renal Toxicities in Cynomolgus Monkeys**


Blood urea nitrogen (BUN) and creatinine (CRN) have been used as biomarkers (BMs) for detecting drug-induced renal toxicities. Since FDA and EMA proposed several BMs in rats, such as urinary protein, albumin (ALB), β2-microglobulin (B2M), clusterin (CLU) and cystatin C (CystC), we examined the availability of these urinary BMs in cynomolgus monkeys. We used 3 renal toxic compounds: gentamicin (GM), cisplatin (CDDP) and paromomycin aminoside (PAN). Three cynomolgus monkeys/group (total 9 animals) were given daily subcutaneous injections of 200 mg/kg/day gentamicin for 1 or 4 days. Blood and urine samples were collected following each day. In parallel to conventional parameters, urinary L-FABP and urinary enzymes were evaluated (after normalization to urinary creatinine) included LDH, γ-GTP and NAG. Concentrations of L-FABP in urine were measured using ELISA. Kidney microscopic findings of foamy eosinophilic globules were slightly observed in proximal tubule whereas BUN and serum creatinine were not changed. Urinary γ-GTP and NAG were significantly increased from 2 days after start of injection, urinary LDH was not changed. Urinary L-FABP elevated significantly as early as 6 hour after 1st injection (both strains). The peak levels of urinary L-FABP in Sprague-Dawley and Wistar rats were >200-fold, whereas that of Wistar rats was >75-fold. Both the speed and magnitude of response detected with urinary L-FABP was superior to other biomarkers included traditional biomarkers and urinary enzymes. There was no difference between both strains in urinary L-FABP response. These results suggest that the urinary L-FABP may be a useful sensitive biomarker of nephrotoxicity not only in humans but also in rats.

**1216 Performance of Urinary Kidney Translational Safety Biomarkers in Tenofovir-Treated Cynomolgus Monkeys and Beagle Dogs**


L-type Fatty Acid Binding Protein (L-FABP) is used in the assessment of kidney injury in clinical and preclinical studies. However, the biological processes associated with L-FABP and ALB increase on Day 7 with hypertrophy of glomerular cells (HP), dilatation of urinary tubules, an increase in urinary levels of ALB, and B2M were analyzed using an automatic analyzer, and CLU and CysC were analyzed using ELISA method. Renal histopathology (HP) and electron microscopy (EM) were also conducted. In the GM group, protein, ALB, and B2M in fresh urine increased from Days 0 to 6; however, BUN and CRN increased on Day 7 only. Degeneration/necrosis, hyaline droplets, basophilia in cortex (HP) enlargement/increase of lysosomes with myelinoid bodies were noted in renal tubules (EM). In the CDDP group, protein and ALB in fresh urine increased from Day 0, and BUN and CRN increased from Day 4. Cellular cast, renal tubular dilatation, basophilic in cortex and medulla (HP) dilatation of intratubular organelles and intercellular gap (EM) were noted. In the PAN group, protein and ALB in fresh urine increased on Day 0, but albumin in preserved urine did not increase. BUN and CRN increased on Day 7 with hypertrophy of glomerular cells (HP), dilatation of rough endoplasmic reticulum, and fusion of foot process in podocytes (EM). These results showed that the urinary BMs reflected renal pathologic lesions and that the change in urinary BMs level could be detected earlier than that in serum BMs. Urinary albumin is the most useful biomarker to estimate any renal lesion in the early stage in cynomolgus monkeys.

**1217 Comparison of Urinary Liver-Type Fatty Acid-Binding Protein (L-FABP) and Other Urinary Nephrotoxicity Biomarkers in Gentamicin-Induced Nephrotoxicity in SD Rats**

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L-type Fatty Acid Binding Protein (L-FABP), which is located in the cytoplasm of basolateral membranes of proximal tubular cells, responds to ischemia and oxidative stress and is excreted in urine. KDIGO (Kidney Disease Improving Global Outcomes) selected L-FABP as one of the major 5 biomarkers (cystatin C, NGAL, Interleukins, Kim-1, L-FABP) in the world for detecting Acute Kidney Injury. L-FABP ELISA kit has been approved as a IVD product in Europe and Japan. The purpose of this research was to evaluate the utility of L-FABP as a nephrotoxicity biomarker in preclinical studies. We used gentamicin-induced (GM) model in Albus Sprague-Dawley and Wistar rats. In the GM model, rats were given daily subcutaneous injections of 200 mg/kg/day gentamicin for 1 or 4 days. Blood and urine samples were collected following each day. In parallel to conventional parameters, urinary L-FABP and urinary enzymes were evaluated (after normalization to urinary creatinine) included LDH, γ-GTP and NAG. Concentrations of L-FABP in urine were measured using ELISA. Kidney microscopic findings of foamy eosinophilic globules were slightly observed in proximal tubule whereas BUN and serum creatinine were not changed. Urinary γ-GTP and NAG were significantly increased from 2 days after start of injection, urinary LDH was not changed. Urinary L-FABP elevated significantly as early as 6 hour after 1st injection (both strains). The peak levels of urinary L-FABP in Sprague-Dawley rats was >200-fold, whereas that of Wistar rats was >75-fold. Both the speed and magnitude of response detected with urinary L-FABP was superior to other biomarkers included traditional biomarkers and urinary enzymes. There was no difference between both strains in urinary L-FABP response. These results suggest that the urinary L-FABP may be a useful sensitive biomarker of nephrotoxicity not only in humans but also in rats.

**1218 Developing a Translatable Molecular Signature for Drug-Induced Kidney Injury**


Drug induced kidney toxicity (DIK) is estimated to account for around 2% of drug attrition in preclinical development and 19% in phase III clinical trials. This study aims at evaluating the in vivo in vitro translatability of a molecular gene signature associated with DIK. A set of nephrotoxicants were selected for this study (cyclosporine A, cisplatin, ochratoxin A). A seven-day in-vivo rat toxicity study was conducted which comprised macroscopic, microscopic, toxicogenomics analysis of the kidney and urinary/plasma biomarker assessment. After compound exposure, in-vivo microscopic findings showed the presence of tubular degeneration/necrosis and basophilia from day 3 of treatment. These findings were associated with decreased body weight and increased levels of urinary protein biomarkers (KIM-1, LCN2 and NGAL). A panel of 16 genes, involved in Wnt and TNF signaling and extra-cellular matrix and focal adhesion pathways, was identified and associated with macroscopic, microscopic and urinary biomarker findings. In-vitro studies were conducted using rat, dog, monkey and human kidney immortalized cells. Cells were exposed to nephrotoxicants for a period of up to 72h, cell viability assessed by Real-Time Cell Analysis, mitochondrial deregulation evaluated using XFe Extracellular Flux Analyzer and a panel of genes analysed by qRT-PCR. A threshold of 20%-40% loss of cell viability was determined for each cell line. Concentrations of nephrotoxicants were selected and no association found with mitochondrial deregulation. Analysis of a subset of genes (8/16) show the potential to be translated to in-vitro models. Further in-vitro studies are ongoing to validate the gene panel in kidney tubular primary cells and determining potential cut-offs
1219 Urinary KIM-1 Detection of Subclinical Nephrotoxicity in Oncology Patients Treated with Cisplatin

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Acute kidney injury can be observed in 8 to 40% of oncology patients treated with cisplatin using traditional clinical markers, including serum creatinine and urinary albumin excretion. However, these indicators of nephrotoxicity are considered insensitive since a significant degree of tubular damage is needed in order to increase their levels. Preclinical studies support detection of kidney injury molecule-1 (KIM-1) in urine as an early and sensitive biomarker of cisplatin-induced injury. Therefore, we sought to characterize urinary levels of KIM-1 protein in patients prescribed cisplatin-containing chemotherapy regimens. Thirty-four patients scheduled for outpatient chemotherapy for solid tumors (head/neck, lung, cervix, bladder, urothelial, breast, or melanoma) with IV cisplatin were recruited. Patients participated in the study during two different cycles of cisplatin therapy. Urine was obtained at baseline as well as 3 and 10 days post infusion and analyzed for KIM-1 protein concentrations using an ELISA assay. During the first cycle of cisplatin therapy, urinary KIM-1 levels were increased 2- and 4-fold at 3 and 10 days post infusion, respectively. A similar time-dependent trend was observed with subsequent cisplatin cycles. Additionally, the baseline KIM-1 levels were increased ~2-fold in patients that had previously received cisplatin chemotherapy, although still within normal ranges. These data suggest KIM-1 is a novel biomarker for detection of subclinical nephrotoxicity in patients treated with cisplatin.

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1220 Technical Best Practices for Urinary Biomarker Evaluation

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There is growing interest in the incorporation of novel urinary biomarkers into rat toxicology studies to monitor drug-induced renal injury. Differences exist between institutions in the procedure for urine collection and data analysis, which may affect biomarker values. A survey of eight companies indicated that the urine collection period, access to food and water, and data normalization was largely consistent among survey participants. Certain variables, such as urine collection temperature, the presence of fecal or food contamination, have been shown to affect urinary biomarker values. Rat urine samples representing baseline, medium, and high range of urinary biomarker concentrations were spiked with 1 or 5% food or feces for 20 hours. The results showed that fecal contamination significantly decreased urinary concentrations of osteopontin (up to 99%) and clusterin (up to 75%) and increased urinary concentration of total protein (up to 50%). Fecal contamination also affected cystatin C, albumin, and NGAL concentrations, particularly in urine samples close to baseline values. KIM1 and beta-2 microglobulin were not affected by fecal contamination. Food had minimal impact on most biomarkers, except urinary total protein (increased by up to 40% in the presence of 5% food contamination). In addition, urinary osteopontin, NGAL, and clusterin concentrations were lower when collected at room temperature as compared to 4°C. The study highlights the importance of identifying and implementing best technical practice for analysis of novel urinary biomarkers.

1221 The Human FRY Gene Is a Novel Biomarker for Breast Cancer Progression and Prognosis

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Our recent studies identified the rat Fry gene, located on the short arm of rat chromosome 12, as a potential mammary carcinoma susceptibility (Mc) gene [Ren, X. et al. 2013]. The amino acid sequence of rodent Fry genes is more than 90% identical to that of human FRY immediately distal to the BRCA2 gene. In this study, we evaluated the correlation of FRY mRNA and protein with breast cancer phenotypes such as tumor grade, estrogen receptor, progesterone receptor, and Her2 status. To investigate the significance of altered FRY levels in breast cancer, we first investigated FRY mRNA expression in >4,800 clinically annotated human breast cancers, representing distinct cohorts entered into the curated Oncomine 3.0 Cancer Profiling Database. Secondly, through the use of a FRY anti-peptide antibody (which we designed and validated), we examined FRY protein levels in >1,100 clinically annotated breast cancers available as breast tissue microarrays from US Biomax, Inc. and from the National Cancer Institute Cancer Diagnosis Program . Semi-quantitative immunohistochemical analysis revealed that decreased FRY expression, especially nuclear expression, was highly correlated with mammary tumor histopathology, clinical grade and the progression of cancer. Additionally, we showed that FRY mRNA and nuclear protein expression are down-regulated by epigenetic silencing in estrogen receptor negative tumors. Together, our results showed that loss of FRY expression is associated with breast cancer progression and poor prognosis, indicating that FRY could be an important new biomarker of breast tumor development and progression, and may serve as a target for environmental toxicants that contribute to aberrant DNA methylation.

1222 Identification and Quantification of MTH1 in Human Tissues As a Cancer Biomarker

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Finding a robust and accurate biomarker for genetically oriented diseases such as cancer is a major concern in today’s prevention-focused clinical approach. DNA repair proteins used as biomarkers in disease etiology or therapeutic response prediction are promising to achieve this goal in the last decade. Thus, the accurate measurement of their expression is of fundamental importance. MTH1 is a DNA repair protein that sanitizes the nucleotide pool so that modified 2-deoxynucleoside triphosphates (dNTPs) cannot be used in DNA replication. Cancer cells require MTH1 to avoid incorporation of modified dNTPs resulting in DNA damage and apoptosis. Inhibition of MTH1 eradicates cancer, validating MTH1 as an anticancer target. Cancer cells may resist therapy that may damage dNTPs. MTH1 is overexpressed in many cancers. Accurate measurement of MTH1 in patient tissues may be essential for the use of MTH1 inhibitors in cancer therapy and for determining patient response. We present a novel approach involving LC-MS/MS with isotope-dilution to accurately measure human MTH1. 15N-labeled full-length MTH1 was produced to be used as an internal standard. Seven tryptic peptides of both MTH1 and 15N-MTH1 were identified following trypsin digestion. These peptides matched the theoretical ones, expected from trypsin digestion and provided a statistically significant protein score that would unequivocally identify MTH1. Product ion spectra of the tryptic peptides and their product ions were obtained. Selected-reaction monitoring was used to monitor the characteristic mass transitions of tryptic peptides to analyze mixtures of both proteins. Using the developed methodology, we positively identified and quantified MTH1 in protein extracts from disease-free breast tissues and malignant breast tumors. This novel approach may help elucidate the role of MTH1 in disease development and treatment responses.
In order to validate the use of troponins I (cTnI) and T (cTnT), fatty acid binding protein 3 (FABP3), and myosin light chain 3 (Myh3) as biomarkers of acute myocardial injury, we have conducted experiments in four species of animals: mouse, rat, dog and non-human primates. Four Beagle dogs were outfitted for continuous ECG monitoring using the DSI JET system. Acute myocardial injury was induced by a bolus s.c. administration of 1 mg/kg of Isoproterenol HCl (ISO). Pulse oximetry parameters and blood samples for determination of plasma concentration of cTnI, cTnT, FABP3, Myh3 and cTnI were collected from each dog prior to and at 2, 4, 6, 8, and 10 hours after ISO administration. ISO produced tachycardy and tachypnea, which were sustained throughout the 10-hour monitoring period. All dogs demonstrated shortening of RR and PR intervals and prolongation of QTc on ECG, as well as transient arrhythmias. Acute myocardial injury was evidenced by a significant increase in circulating biomarkers: cTnI (370-fold), cTnT (70-fold) and Myh3 (20-fold), FABP3 (8-fold) by the end of 10-hour observation. The level of skeletal Tnl remained below detection threshold throughout the experiment. The chemiluminescence Mesoscope Discovery (MSD) platform was used to measure the biomarkers of cardiac injury described. ISO at equivalent doses have also been used to induce myocardial injury in mice, rats and non-human primates in our laboratory. These biomarkers were proven to be useful and were quantified in the ISO-induced cardiac injury model across all 4 tested species. In addition, we developed custom in-house MSD CK-MM and CK-BB MSD assays. The normal and abnormal historical levels of biomarkers, sensitivity, accuracy and precision, LLOQ and freeze-thaw stability in plasma and serum were also determined. Positive plasma and serum controls with high and low levels of cardiac toxicity biomarkers were prepared and frozen in multiple aliquots to verify the assay performance between MSD plates by running a positive biological matrix control on multiple plates.

**Biomarkers of Pathologic Cardiac Hypertrophy: Investigation of NTproANP and NTproBNP in Rats**


Cardiovascular (CV) toxicity is a primary cause of attrition during drug development and following product launch. Better understanding and application of biomarkers of cardiac structure or function during development may aid in the selection of molecules with improved CV safety. Natriuretic peptides (NP) are hormones secreted from the myocardium with a central role in maintaining CV tone, plasma volume, and cardiac growth. Their structure and function, and in the case of ANP, sequence, is well-conserved across veterinary species commonly used in drug development, making them important translational CV biomarkers. In humans with adaptive increases in left ventricular mass (LVMi), for instance as a result of exercise, plasma NP concentrations are normal, whereas patients with increased LVMi as a result of prolonged hypertension have increased NP concentrations. We hypothesized that NTproANP and/or NTproBNP could distinguish between adaptive (physiological) and maladaptive (pathological) increases in cardiac mass in rodents. Male Sprague Dawley rats were administered a PPARY agonist daily or participated in a swimming protocol, working up to two 90 minute swim sessions each day. Heart weights and NP concentrations were compared to control and active control (2 minutes swimming twice daily) groups. Comparably increased heart weights (~15%) were observed in PPARY and swim group rats after 28 days. Increased NPs were observed in rats administered the PPARY agonist, but not in swimming rats. These data support the use of NPs in rats as translational safety biomarkers for detection of pathological changes in cardiac mass during drug development.

**Shotgun Proteomics of Human Sputum and Plasma Identifies Biomarkers of Acute Exposures to Diesel and Biodiesel Emissions**

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Exposures to diesel particulate matter (DPM) are linked to a broad range of illnesses. In recent years, biodiesel has been used to reduce respirable DPM (rDPM) but there are few human studies that analyze the health effects of this fuel. Sputum and plasma are attractive sources of proteins and these bio-fluids have been used to...
monitor airway and systemic responses to a variety of toxicants. The goal of this study was to analyze these bio-fluids for novel protein biomarkers in response to acute diesel and biodiesel emission exposures. Using a cross-over experimental design, 48 subjects operating a load-haul-dump vehicle in an underground mine were exposed on separate days for 200 minutes each alternating use of diesel and 75% biodiesel/25% diesel (B75) blend fuels. Switching to B75 reduced TDP exposure by 20%. Sputum and plasma were then collected before and after diesel and B75 exposures. Proteins from the sputum and plasma were extracted from 6 subjects and were enzymatically digested into peptides that were then run in triplicates using LC-MS/MS. Underivatized peptides were identified. Label-free quantitation was also employed and based on criteria of identification in four or more of the subjects or at least two fold-increase or decrease, 42 and 32 novel candidate biomarkers were selected in the sputum and plasma respectively. Two sputum (matrix metalloproteinase-8 (MMP-8) and growth-regulated alpha protein (GRO-α)) and one plasma (tenasin-C (TN-C)) were further validated in all samples using enzyme-linked immunosorbent assay (ELISA). MMP-8 significantly increased following exposures to emissions from both fuel-types. GRO-α was only significantly elevated in post-B75 exposures. Plasma TN-C was significantly increased following diesel exposure, and, not quite significantly, in the post-B75 exposures. This study gives us a better understanding in evaluating the comparative toxicity of the emissions from diesel and B75. Supported by NIEHS Training Grant T32 ES007091 and NIOSH RO1 OH009878

1228 Comparative Analyses of Methods Used to Prepare Diisocyanate-Protein Conjugates: Implications in Clinical Assay Development

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Exposure to diisocyanates (dNCO), such as methylene diphenylisocyanate (MDI) can cause occupational asthma. Recently we observed differences in MDI-specific mAb reactivity with MDI-protein conjugates prepared using different methods. The consistent preparation of dNCO-protein adducts is crucial for dNCO-specific antibody screening. Therefore, the aim of this study was to identify the dNCO-protein conjugation method that resulted in the most extensive and consistent conjugation. Four methods were used: 1) MDI was slowly dripped into human serum albumin (HSA) while vortexing (drip), 2) MDI was quickly dispensed into HSA and vortexed (fast dispense), 3) MDI was pumped into HSA while vortexing (infusion), and 4) MDI was dissolved in a non-water miscible solvent forming 2-phases and was stirred overnight (2-phase). On average the infusion method resulted in approximately 1.5, 3.7, and 3.9 times more reactivity in a MDI specific sandwich ELISA than the drip, fast dispense, or 2-phase methods, respectively. Interestingly, the amount of crosslinking, as measured by the binding of free amines by trinitrobenzene sulfonic acid, was similar amongst samples prepared using all of the described methods. Intrapersonal variability was assessed by instructing 3 individuals to prepare conjugates using either the drip or fast dispense methods. A high variability in protein conjugation was observed between users, particularly in the drip method. This may correlate to the drip speed of the MDI and vortex speed of the HSA. This work demonstrates intra- and interpersonnel variability in protein conjugation methods. A high variability in protein conjugation was observed between users, particularly in the drip method. This may correlate to the drip speed of the MDI and vortex speed of the HSA. This work demonstrates intra- and interpersonnel variability in protein conjugation methods.

1229 Variability of Cytokine Response following Ex Vivo Stimulation of Blood from Cynomolgus Monkeys

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In order to evaluate endogenous cynomolgus cytokines, whole blood or peripheral blood mononuclear cells (PBMCs) were stimulated with 15 ug/mL of either pokeweed mitogen (PWM), lipopolysaccharide (LPS), or concanavalin A (ConA) for 2-48 hours at 37°C. Sixteen cytokines were evaluated on two multiplex plates (IFN-γ, IL-1β, IL-2, IL-6, IL-8, IL-10, MIP-1α, MIP-1β, Eotaxin-3, TARC, IP-10, MCP-1, MDC & MCP-3). For each cytokine, the lower limit of quantitation (LLOQ) was 0.8 to 17 pg/mL. The upper limit of quantitation (ULOQ) was 201 to 6,520 pg/mL. All of the cytokines were below the LLOQ prior to stimulation. MIP-1α and MIP-1β had the largest increase following 18 hour stimulation, rising to over 70 ng/mL and IL-6 levels increased significantly following stimulation of whole blood or PBMCs with PWM or LPS (11.300 to 13,450 pg/mL) but not ConA. Eight out of 13 of the other cytokines had detectable increases following stimulation. A nominal concentration for NHP derived material was spiked into individual or pooled serum and assessed for precision, accuracy, selectivity/spike recovery, linearity/parallelism, and biomarker stability. Additional validation tests such as suitability of calibration standards, determination of endogenous biomarker levels in matrix were conducted, but did not use the NHP spiked product. Inter-Assay Precision and Accuracy was demonstrated in both the Kit controls and NHP derived QCs for 12 of the 14 cytokines with % CV of 9.9% and 13.4% RE, respectively and 1) TARC did not meet acceptance criteria with precision >23% CV. These results define the limits for measurement of endogenous cynomolgus cytokines in peripheral blood.

1230 Impairment of Skin Function—Defining Biomarkers from Gene Expression Data Sets

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Exposure of skin to chemicals can induce changes on gene/protein levels and affect range of biological pathways responsible for its normal function. Similarly, certain systemic diseases can in addition modify skin molecular composition, leading to changes in its structural proteins, inflammatory mediators, nucleic acids and small molecules. Those affected molecules represent a collection of potential biomarkers valuable for toxicity assessment, diagnosis, or therapeutic outcome. Microarrays are a powerful method to deduct potential sets of genes indicating a particular response, but require additional interpretation and processing to identify true biomarker candidates. We created a hand curated database of over 2,000 gene expression signatures from in vitro and in vivo experiments, manually interpreted from 600 studies in the literature, and an integrated pipeline of computational tools for defining biomarkers from gene expression datasets. We benchmarked the tool, using a variety of datasets: 1) data arisen from exposure to few skin sensitizers and 2) data from few systemic diseases (such as cancer, diabetes and psoriasis), and arrived at sets of high-confidence and mechanistically plausible biomarkers for use in predictive models. Those biomarkers are shown to have capacity in assessing sensitizing potential of chemicals or as endpoints for therapeutic interventions in diseases.

1231 Arsenic (+3) Methyltransferase (AS3MT) and Glutathione S-Transferase Omega (GSTO1) Genetic Variants Associated with Arsenic Susceptibility: Influences on As Metabolism and Skin Lesions

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Geological studies have recently shown that arsenic (As) levels in drinking water ranged from 1 to 500 ug/L in Nevsehir province, Turkey. This study is a part of molecular epidemiology research carried out to collect human data on As exposure in this area. For this purpose, peripheral blood samples were collected from the residents of villages with levels of As > 50 ug L-1 (n=230) and 10-50 ug L (n=151) and from four villages with levels of As <10 ug L-1 (n=182) in drinking water. The polymorphisms in AS3MT and GSTO1 genes were studied by PCR-RFLP method. The influences of these genotypes on As methylation index (as DMA/MMA) and frequency of skin lesions were also investigated. The genotypic distribution of AS3MT in As exposed group were not significantly different from that of controls. The frequencies of GSTO1 genotypes were also similar in As exposed and control groups. The methylation index was significantly decreased (p=0.005) in individuals having the variant 56% B/5 as compared to 44% B/5. The frequency of skin lesions were associated with neither AS3MT nor GSTO1 genotypes. Our results indicate that variations in AS3MT or GSTO1 do not play a major role in individual susceptibility to As-induced health effects. However As metabolism (DMA/MMA) is slightly influenced by AS3MT polymorphism which may result in increased levels of MLL as well.
High levels partial body irradiation (PBI) produce well defined intestinal damage and inflammation. In initial experiments 15.8 Gy (LD60/30) PBI was administered to C57BL/6j mice in the abdominal portion with thorax and head shielded above the sternum. NanoDots as well as the Sel’mex ion chamber used for dosimetry confirmed the dose close to target. Irradiated mice (n=15/group) received either SMA-11 (our proprietary mitochondria-targeted antioxidant for radiation mitigation) or vehicle control. Animals were observed for survival over 15 days (all clinical signs restored to normal by this day). Jejunum samples were collected from moribund sacrificed animals during the observation period as well as from the surviving animals on day 15. Jejunums were gently cleaned using saline, immediately flash frozen in liquid nitrogen and then stored at -80°C freezer until analysis. For analysis a homogenate was prepared from frozen tissues and analyzed using the MSD® electrochemiluminescence (ECL) technology. Two separate 5-plex cytokine /chemokine panels and a TNF assay developed in our laboratory for mice were used for these assays. The cytokines/chemokines evaluated included monocytic chemoattractant protein -1 (MCP-1), monokine induced by gamma interferon (MIG), tumor necrosis factor alpha (TNFα), interleukin-18 (IL-18), thrombopoietin (TPO), interferon-gamma inducible protein-10 (IP-10), erythropoietin (EPO), interleukin-15 (IL-15) and interferon γ (IFNγ). MCP-1 and MIG showed highly significant difference (p<0.01) and EPO, IP-10 and IL-18 as well as IFNγ also showed good potential (p<0.05) to be used as a biomarker. TNF assay showed significant difference only when compared to day 7 moribund sacrificed animals. The profiles of cytokine levels were significantly different in drug-dosed animals compared to the control group animals. These data demonstrate the usefulness of tissue cytokine levels as potential biomarkers for intestinal damage and inflammation and monitoring of disease progression as well as the effect of treatment.
Introduction: Carbon nanotubes (CNTs) are gaining increasing attention due to possible health risks from occupational and environmental exposures. Mice exposed by inhalation to multi-walled CNTs (MWCNTs) develop pleural inflammation and fibrosis. We showed that the application of aluminum oxide (Al2O3) by atomic layer deposition (ALD) alters the pro-inflammatory and pro-fibrogenic effects of MWCNTs in human macrophages (THP-1 cells) in vitro and in the lungs of mice in vivo. The purpose of this study was to determine whether ALD coating of MWCNTs with Al2O3 alters pro-inflammatory and pro-fibrogenic cytokines in a human macrophage/mesothelial cell co-culture system. Methods: Human macrophages (THP-1) co-cultured with mesothelial cells (Met-5a) were exposed to Al2O3-coated (A)-MWCNTs or uncoated (U)-MWCNTs for 24 hr and mRNA expression levels of pro-inflammatory/fibrotic mediators (IL-1β, CCL2, IL-6, OPN, CXCL10, PDGF-A, VEGF) were measured via RT-PCR. Results: All mediators measured were elevated in the co-culture system when compared to THP-1 cells or Met-5a cells alone. CCL2, IL-6, and PDGF-A mRNA levels were increased in co-culture with exposure to A-MWCNTs in comparison to U-MWCNTs. In contrast, OPN, IL-1β, and VEGF mRNA levels were decreased in co-culture with exposure to A-MWCNTs compared to U-MWCNTs. Conclusions: Our findings indicate MWCNTs stimulate a pro-inflammatory and pro-fibrogenic microenvironment in a cell co-culture system designed to model macrophage-mesothelial cell interaction at the pleural lining surrounding the lungs. Furthermore, MWCNTs coated with Al2O3 applied by ALD elicit a different pattern of cytokine expression compared to uncoated MWCNTs. This co-culture should be useful for predicting pleural disease caused by functionalized CNTs. Funding: Supported by NIEHS Grant R01-ES020897.
Previously, we have reported that pharyngeal exposure of C57BL/6 mice to single walled carbon nanotubes (SWCNTs) caused formation of granulomatous bronchial interstitial pneumonia, fibrosis, oxidative stress, acute inflammatory/cytokine responses and a decrease in pulmonary function. In the current study, we used electron spin resonance (ESR) to directly assess whether pulmonary exposure to respirable SWCNTs caused formation of free radicals in the lungs and in two distant organs, the heart and liver. Here we report that exposure to partially purified SWCNTs (HiPCo, CN1, Inc, TX) resulted in the augmentation of oxidative stress as evidenced by ESR detection of a-[4-pyridyl-1-oxide]-N-tetramethyl-pyrindinum (POBN) spin-trapped carbon-centered lipid-derived radicals recorded shortly after the treatment. This was accompanied by a significant depletion of antioxidants and elevated biomarkers of inflammation presented by recruitment of inflammatory cells and an increase in pro-inflammatory cytokines in the lungs, as well as development of multifocal granulomatous pneumonia, interstitial fibrosis and suppressed pulmonary function. Moreover, pulmonary exposure to SWCNTs also caused the formation of carbon-centered lipid-derived radicals in the heart and liver at later time points (day 7 post exposure). Additionally, SWCNTs induced a significant accumulation of oxidatively modified proteins, an increase in lipid peroxidation products, depletion of antioxidants and an inflammatory response in both the heart and the liver. Overall, we provided direct evidence that lipid-derived free radicals are a critical contributor to tissue damage induced by SWCNTs not only in the lungs, but in distant organs.

Graphene (G), graphite nanoplatelets (GP), carbon nanotubes (mwCNT) and low surface area carbon black (CB) are carbon-based nano-materials with broad technological applications. mwCNT and CB possess different inhalation toxicities, whereas less is known about G and GP. In order to compare the inhalation toxicity of these carbon-based nanomaterials, male Wistar rats were exposed head-toe to aerosols for 6h/day on 5 consecutive days. Target concentrations were 0.1, 0.5, or 2.5 mg/m3 for mwCNT and 0.5, 2.5, or 10 mg/m3 for G, GP and CB. Toxicity was determined at the end of exposure and three week later using broncho-alveolar lavage fluid and microscopic examinations of the entire respiratory tract. No adverse effects were observed after inhalation exposure to 10 mg/m3 GP or CB. Increases of lavage markers indicative for inflammatory processes started at exposure concentration of 0.5 mg/m3 for mwCNT and 10 mg/m3 for G. GP and CB. Toxicity was determined at the end of exposure and three week later using broncho-alveolar lavage fluid and microscopic examinations of the entire respiratory tract. No adverse effects were observed after inhalation exposure to 10 mg/m3 GP or CB. Increases of lavage markers indicative for inflammatory processes started at exposure concentration of 0.5 mg/m3 for mwCNT and 10 mg/m3 for G. Consistent with these changes, granulomatous inflammations were observed at 2.5 mg/m3 mwCNT and G. In order to evaluate volumetric loading of the lungs as the key parameter driving the toxicity, deposited particle volume was calculated, taking into account different methods to determine the agglomerate density. However, the calculated volumetric load did not correlate to the toxicity, nor did the particle surface burden of the lung. The inhalation toxicity of carbon-based materials is likely to be a complex interaction of several parameters. Until the properties which govern the toxicity are identified, testing by short-term inhalation is the best option to identify hazardous properties in order to avoid unsafe applications or select safer alternatives for a given application. Ma-Hock, Lan, et al. Comparative inhalation toxicity of multi-wall carbon nanotubes, graphene, graphite nanoplatelets and low surface carbon black.” Particle and fibre toxicology 10.1 (2013): 23.

The aim of this study was to assess differences in the toxic potential of surface modified carbon black particles. To this end a 14-day inhalation study was conducted in rats using nose-only exposure to compare the effects of pristine Printex®90 with surface modified carbon black, i.e. acetylene soot particles and Printex®90 coated with benzo[a]pyrene. This set of particles was also tested in different in vitro and ex vivo systems. On day one after the end of the 14-day inhalation period, acetylene soot alone caused an increase in relative lung wet weights. Furthermore, acetylene soot caused the most frequent histological alterations like interstitial inflammatory cell infiltration and bronchiolo-alveolar hyperplasia. Cytotoxicity tests with human pulmonary cell lines and precision cut lung slices (PLCS) were limited due to solubility of compounds. However, measurement of the transepithelial electrical resistance (TEER) in Calu-3 cells as well as the analysis of reactive oxygen species (ROS) in A549 and 16HBE14o- cells were able to differentiate between the carbon black modifications. Murine ex vivo airway preparations also proved to be a valuable model as acetylene soot was found to be the most toxic carbon black modification for epithelial cells, and the mechanism of action was linked to CYP1A1 induction. In summary, the results of this study suggest that the acute inhalation toxicity of carbon black is low, but increased if the surface is coated with polycyclic aromatic hydrocarbons. In vitro models with proven CYP1A1 inducibility seem to be useful tools to predict the in vivo effects of carbon black, coated with polycyclic aromatic hydrocarbons, on pulmonary epithelia.

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In the current study, we used electron spin resonance (ESR) to directly assess whether pulmonary exposure to respirable SWCNTs caused formation of free radicals in the lungs and in two distant organs, the heart and liver. Here we report that exposure to partially purified SWCNTs (HiPCo, CN1, Inc, TX) resulted in the augmentation of oxidative stress as evidenced by ESR detection of a-[4-pyridyl-1-oxide]-N-tetramethyl-pyrindinum (POBN) spin-trapped carbon-centered lipid-derived radicals recorded shortly after the treatment. This was accompanied by a significant depletion of antioxidants and elevated biomarkers of inflammation presented by recruitment of inflammatory cells and an increase in pro-inflammatory cytokines in the lungs, as well as development of multifocal granulomatous pneumonia, interstitial fibrosis and suppressed pulmonary function. Moreover, pulmonary exposure to SWCNTs also caused the formation of carbon-centered lipid-derived radicals in the heart and liver at later time points (day 7 post exposure). Additionally, SWCNTs induced a significant accumulation of oxidatively modified proteins, an increase in lipid peroxidation products, depletion of antioxidants and an inflammatory response in both the heart and the liver. Overall, we provided direct evidence that lipid-derived free radicals are a critical contributor to tissue damage induced by SWCNTs not only in the lungs, but in distant organs.

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Multi-walled carbon nanotubes (MWCNTs) have many industrial applications. However, the low density and small size of MWCNT makes respiratory exposures in workers likely. Nitrogen-doped MWCNT (ND-MWCNT) has been shown to be less inflammatory in vitro than pristine MWCNT (P-MWCNT) of the same dimensions. In order to investigate the potential for lessened in vitro toxicity of ND-MWCNT we exposed immortalized and primary respiratory epithelial cells to physiological relevant concentrations of single-walled (SW) CNTs (0.08-0.15 μg/cm²) upregulated the expression of podoplanin, a known CAF marker of human lung adenocarcinoma, suggesting that SWCNTs can trigger NHFLs to initiate CAFs. Subcutaneous injection of the SWCNT-exposed NHFLs along with human lung carcinoma H460 cells in NSG mice resulted in a high rate of tumor formation compared with the coinjection of vehicle-exposed NHFLs and H460 cells, indicating the tumor-promoting effect of SWCNT-exposed NHFLs. The mechanism by which SWCNT-exposed fibroblasts promote tumor growth was shown to involve cancer stem cell (CSC) induction, as determined by tumor sphere formation and side population assays. Together, our study unveils a novel mechanism of CNT-promoted lung carcinoma through the acquisition of CAFs that regulate CSC formation.

Inhalation of multi-walled carbon nanotubes (MWCNT) can induce significant pulmonary pathology owing to their nanoscale, fibrous morphology and/or biopersistence. MWCNT were shown to aggravate asthmatic responses using various animal models. However, there is need of validating these findings in more translational relevant models. We sought to elucidate 1) the differential sensitivity of human bronchial epithelial cells from healthy and asthmatic subjects to low dose MWCNT exposures and 2) to elaborate molecular mechanisms through which MWCNT induce toxic effects. HBE cells were collected from healthy (BEC) and asthmatic (ABEC) human volunteers through fiber optic bronchoscopy, exposed to fully characterized MWCNT suspensions (1.5 or 12 μg/mL) for 24 or 48 hours and analyzed for toxic or inflammatory responses. Cells cultured in the presence or absence of IL-13 were exposed to MWCNT to elaborate the effect of asthmatic lung environment. Global gene expression profiling was done using Agilent Whole Human Genome oligo arrays and results were verified using RT-qPCR. ABECs show higher sensitivity to MWCNT and significant toxicity is observed after 24 hours exposure at lower doses as compared to BECs. Interestingly, BECs recover from toxic effects at 48 hours while significant toxicity persists in ABECs. MWCNT are internalized by both types of HBE cells but were seen to localize preferentially in cytoplasm in asthmatic BECs. Microarray revealed significant changes in inflammatory, immunological, cell survival and proliferation pathways. No amplification of toxicity was observed when cells were exposed to MWCNT in the presence of IL-13. IL-13 induced a significant increase in the endoplasmic reticulum stress signaling which was impaired by MWCNT exposures. Our results demonstrate that ABECs are more sensitive to the toxicity of MWCNTs and show differences in cellular signaling as compared to BECs. These findings suggest that asthmatic individuals are at greater risk to the toxic effects of MWCNTs.

**Dose- and Time-Dependent Assessment of Human Mesothelial Cell Neoplastic Transformation Potential after Functionalized MWCNT Exposure**

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MWCNTs are characterized by asbestos-like fiber morphology, large surface area and surface chemistries. Exposure results in pulmonary fibrosis, biopersistence, extrapulmonary transport and promotion of adenocarcinoma and sarcomatous mesothelioma. As MWCNTs become widely used, elevated cancer risk in pleural mesothelium following inhalation exposure is a concern. Long-term exposure risks resulting from surface functionalization of MWCNT on pleural mesothelioma potential is largely unknown, but critically needed. We hypothesized that the effect of MWCNT surface functionalization on human mesothelial cell neoplastic transformation potential depends on dose and duration of exposure. Human immortalized mesothelial cells were continuously exposed to fully characterized prepared (pMWCNT), carboxylated (MW-COOH) and aminated (MW-NH2) for 6 months (M) at 0.002 and 0.02 μg/cm² which are relevant to animal exposure doses. Saline for several cancer hallmarks. Results indicated that 1) low dose MW-COOH and (SAL), dispersant (DISP) and crocidolite asbestos (ASB) exposed cells served as controls. At regular intervals during exposure, each treatment group was assessed for several cancer hallmarks. Results indicated that 1) low dose MW-COOH and MW-NH2 exposure caused significant increases in cell proliferation compared to controls starting at 1 M and persisted over 6 M exposure. High dose exposure alleviated this effect. 2) pMWCNT, MW-NH2, and ASB cells exhibited significantly greater numbers of soft agar colonies at both doses compared to controls starting at 4 M. Low dose pMWCNT treatment resulted in a more potent colony-forming effect than high dose, while both doses of asbestos possessed an equipotent effect. 3) Only MW-COOH cells at 6 M exhibited a significant increase in invasion ability compared to all treatments. Lastly, ASB cells displayed the largest transformation frequency while all MWCNT exposures caused moderate effect. In summary, exposure dose, duration and type of surface functionalization determine MWCNT neoplastic transformation potential in pleural mesothelial cells.

**Lessening Genotoxicity Using Nitrogen-Doping of Multiwalled Carbon Nanotubes**

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S. Hussain¹, P. R. Bushel², K. Gerrish and S. Garantziotis¹, Clinical Research Unit, NIEHS, Research Triangle Park, NC, Biostatistics Branch, NIEHS, Research Triangle Park, NC and Molecular Genomics Core, NIEHS, Research Triangle Park, NC.

Multi-walled carbon nanotubes (MWCNT) have many industrial applications. However, the low density and small size of MWCNT makes respiratory exposures in workers likely. Nitrogen-doped MWCNT (ND-MWCNT) has been shown to be less inflammatory in vitro than pristine MWCNT (P-MWCNT) of the same dimensions. In order to investigate the potential for lessened in vitro toxicity of ND-MWCNT we exposed immortalized and primary respiratory epithelial cells to 0.024, 0.24, 2.4, 24, and 48 μg/cm² MWCNT. Three-dimensional reconstruction of Raman confocal optical images determined that both ND-MWCNT and P-MWCNT were taken up by the cell and localized in the nucleus. Further analysis by enhanced darkfield microscopy (Cytoviva) demonstrated that 4% of the cells had P-MWCNT in the nucleus while only 0.8% contained ND-MWCNT. ND-MWCNT caused significantly less cellular necrosis than P-MWCNT after 72 hours of exposure. Cell cycle analysis of both cell types showed that exposure to P-MWCNT caused a significant G1/S block 24 hours after exposure indicating genotoxicity. By contrast, ND-MWCNT did not induce a similar block in the cell cycle. In the primary cells, P-MWCNT induced a dramatic G1/S block after 72 hours while ND-MWCNT caused a moderate G1/S block. Preliminary studies indicate mitotic spindle aberrations in both P-MWCNT and ND-MWCNT, though a higher dose is required for ND-MWCNT. Further investigation of mitotic spindle disruption and chromosome errors following exposure to P-MWCNT and ND-MWCNT at occupationally-relevant doses is needed. However, these data indicate nitrogen-doping of MWCNT may have ramifications for lessening the toxicity of pristine MWCNT and protecting worker health.
1248 Effects of Pristine and Nitrogen-Doped Multilayered Carbon Nanotubes (ND-MWCNT) on Reactive Oxygen Species (ROS) and Cell Cycle Progression

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ND-MWCNT are modified MWCNT with enhanced electrical properties that are used in a variety of applications including fuel cells and sensors; however, the mode of action of toxicity of ND-MWCNT has yet to be elucidated. Recent in vivo data showed that ND-MWCNT induced inflammation and fibrosis in mouse lungs to a lesser extent compared to pristine MWCNT. In this study, we compared the interaction of ND-MWCNT or Misui 7 MWCNT (MWCNT-7) with human small airway epithelial cells (SAEC) and evaluated their subsequent biological responses. ND-MWCNT were characterized by transmission electron microscopy, X-ray photon spectroscopy, and Raman spectroscopy, which suggested the presence of defects in the nanotube lattice. The nanotubes were determined to be 93.3% carbon, 3.8% oxygen, and 2.9% nitrogen. A dose-response MTS assay showed that low doses up to 12 μg/mL of ND-MWCNT and MWCNT-7 increased cellular proliferation, while the highest dose of 120 μg/mL significantly decreased proliferation. ND-MWCNT and MWCNT-7 appeared to be engulfed by SAEC at 6h and were fully internalized by 24h. ROS was elevated at 6 and 24h in ND-MWCNT exposed cells, but only at 6h in MWCNT-7 exposed cells. Significant alterations to the cell cycle were observed in SAEC exposed either to 1.2 μg/mL of ND-MWCNT or MWCNT-7 in a time-dependent manner, as shown by a decreased percentage of cells in S phase and an increased percentage of cells in G2 phase, respectively, thus suggesting potential damage or alterations to cell cycle machinery. Our results indicate that exposure to MWCNT-7 or ND-MWCNT induces effects in SAEC possibly through different mechanisms that are potentially related to physicochemical characteristics that may alter their toxicity.

1249 Cross-Species Approach Reveals ER-Stress As a Conserved Mechanism of MWCNT-Mediated Biological Interactions

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The raised considerable concern about the possible environmental health and safety impact of multi-walled carbon nanotubes (MWCNTs) originated from its potential widespread applications. We performed a comprehensive study about biological interaction of MWCNTs, specifically in regard to its differential surface functionalization (-OH, -COOH, -NH2 and pristine) on Beas2B cells (at various concentrations, 5 to 200 μg/mL, for 24h). Caenorhabditis elegans (50 μg/mL for 24h) and mouse (treated with 1mg/kg with 50 μl of suspension in PBS for 24h). A conserved ER-stress mediated biological interactions of MWCNTs were evident in all three model systems but with differential mode of action. The IRE1A –XBP1 pathway mediated ER stress was found in Beas2B cells as well as in mouse and the highest effects were observed in COOH functionalized MWCNTs. Conversely, p38-1 (PERK kinase homologue) pathway was the main mechanism of ER stress in the case of Caenorhabditis elegans and the pristine MWCNT showed the highest effects in the worms. In summary, our results present a paradigm for analyzing surface functionality-activity relationship of MWCNT with different model systems which could eventually be utilized for more efficient and innocuous applications, specifically in biomedical field.

1250 MRNAs and miRNAs in Whole Blood Associated with MWCNT-Induced Lung Hyperplasia, Fibrosis, and Bronchiolo-Alveolar Adenoma and Adenocarcinoma following Inhalation Exposure in Mice

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Inhalation exposure to MWCNT in mice results in inflammation, fibrosis, and the promotion of lung adenocarcinoma; however, the molecular basis behind these pathologies is unknown. This study determined global mRNA and miRNA profiles in whole blood from mice exposed by inhalation to MWCNT that are related to the presence of lung hyperplasia, fibrosis and bronchiolo-alveolar adenoma and adenocarcinoma. Six week old, male, B6C3F1 mice received a single intraperitoneal (i.p.) injection of either the initiator methylcholanthrene (MCA, 10 μg/g BW) or vehicle oil. One week after injections, mice were exposed by inhalation to MWCNT (5 mg/m3, 5 hours/day, 5 days/week) or filtered air (control) for a total of 15 days. At 17 months post-exposure, mice were euthanized and examined for lung pathology, and whole blood was collected and analyzed by microarray for global mRNA and miRNA expression. Numerous mRNAs and miRNAs in the blood were significantly up- or down-regulated in the presence of lung pathology after MCA and/or MWCNT inhalation, including fcer5 and miR-122-5p in the presence of hyperplasia, mthfd2 and miR-206-5p in the presence of fibrosis, fam178a and miR-130a-3p in the presence of bronchiolo-alveolar adenoma, and if77 and miR-210-3p in the presence of bronchiolo-alveolar adenocarcinoma, among others. These miRNAs and mRNAs and their respective regulatory networks may be correlated with MWCNT-induced lung pathology and may potentially serve as biomarkers for the presence of lung pathology following MWCNT exposure.

1251 Systematic Evaluation of Carbon Nanotube Toxicity Using the Embryonic Zebrafish to Inform Health and Safety

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The unusual physicochemical properties of carbon nanotubes (CNTs) make them attractive for applications in sporting goods, electronics, water purification and biomaterials. With global CNT production predicted to surpass 12,300 metric tons in 2015, there is increased likelihood of unintended environmental impacts and a need to systematically characterize how CNT physicochemical traits contribute to adverse biological response. We exposed eight-hour post fertilization (hpf), dechorionated embryonic zebrafish to five-fold dilutions of systematically modified multi-walled carbon nanotubes (MWCNTs) for five days and assessed zebrafish for morphological malformations and mortality at 24 and 120 hpf. MWCNTs varied by charge, percent surface oxygen, aggregate radius, aggregate morphology and electrophoresis. Mortality at 24 hpf was the primary adverse response observed in our assay and only occurred with any significance at 50 mg/L. We employed multivariate statistics to develop a predictive MWCNT toxicity model based on that data. Mortality positively correlated with dispersion of MWCNTs and modeling identified surface charge as the primary predictor of zebrafish mortality. A validation set of MWCNTs from a different vendor and systematically treated in the same manner confirmed the model. The predicted mortality of the test MWCNTs generally agree with the actual results. Incorporation of transcriptional and proteomic data associated with MWCNT exposure will improve model predictions and inform safer CNT design. Research support: NIH grant T32 ES07060 and P30 ES000210 and U.S. EPA Assistance Agreement RD83558001-0. This abstract has not been reviewed by the EPA and any views expressed are solely those of the authors and do not necessarily reflect those of the Agency.

1252 Uptake, Translocation, and Stress Effects of Carbon Nanotubes in Drought-Induced Corn

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Carbon nanotubes are one of the most used manufactured nanomaterials. However, these materials are not regulated and there are concerns regarding their behavior in the environment and human health. This study was conducted to evaluate uptake of various types of carbon nanotubes in corn under ideal watering and drought conditions. Corn was exposed to either non-functionalized carbon nanotubes (CNTs) or functionalized carbon nanotubes (COOH-CNTs). Plants were grown for 21 days in soil with 10 mg/kg of CNTs or COOH-CNTs in 1 L or 3 L of soil in a greenhouse with natural day:night conditions. Corn was also grown under conditions simulating a seven-day drought and photosynthesis measurements were taken using a LI-6400XT Portable Photosynthesis System. Following harvest after 28 days, roots, stems, and leaves were dried, ground, and analyzed using a microwave-induced heating technique to quantify CNT and COOH-CNT concentrations in the corn. Photosynthetic rate declined throughout the duration of the drought treatments. CNT uptake was only detected in roots of drought-treated plants exposed to CNTs and COOH-CNTs. Additional plant analyses are currently ongoing.
Despite recent advancement in manipulating nanomaterials and their growing application in a wide variety of fields, sound understanding regarding toxicity associated with potential exposures is still urgently needed. Single-walled carbon nanotubes (SWCNTs), allotropes of carbon with a cylindrical structure that share a resemblance to asbestos, raise concerns regarding long-term adverse health effects associated with inhalation. This underscores the critical need to comprehend how SWCNTs impact the respiratory system. While numerous toxicological studies have focused on fibrosis, cancer, and exacerbation of asthma, the ability of SWCNTs to modulate infectivity of pathogens has been minimally explored. Our recent work has indicated that SWCNTs increase influenza virus infectivity in small airway epithelial cells (SAECs) and suppress anti-inflammatory and viral genes. To decipher the molecular mechanisms driving viral infectivity, we investigated whether SWCNTs modulate TLR3, a receptor that recognizes viral dsRNA as the first line of defense. Our hypothesis was that SWCNTs reduce TLR activity, resulting in inhibition of downstream anti-inflammatory and viral genes mediated by transcription factors NF-κB and/or IRFs. For these studies, SAECs were exposed to Poly (I:C), a TLR3 agonist, and following pre-treatment with SWCNTs. TLR3 activity and gene expression were measured by luciferase reporter assays and eQRT-PCR, respectively. The results demonstrated that SWCNTs did not alter TLR3 activation alone, but suppressed TLR3 activity by Poly (I:C) via NF-κB and IRFs in a dose-specific manner. SWCNTs also repressed genes induced by Poly (I:C), including IFIT2/3, CCL5 while further stimulating IL8. Collectively, these data suggest that SWCNTs suppress the innate immune response to viruses in lung cells, rendering them more susceptible to infections. Our study highlights a novel mechanism of SWCNT toxicity.
Carbon nanotubes (CNTs) are rapidly emerging as occupational and environmental lung toxicants. The increasing manufacture and applications of CNTs has prompted concerns surrounding their potential to cause adverse lung effects in light of asbestos-like characteristics such as high aspect ratio and biopersistance. Because of their novelty, the long-term health outcomes of CNT exposures in humans are unknown. CNTs may become agitated into aerosols in occupational settings, posing an inhalation threat to those who work in places of CNT manufacture, distribution, and usage. However, there are limited studies or inconsistent findings regarding CNT toxicity or their toxicological similarities to asbestos. In this study, we developed mouse models of exposure using repeated, low-dose oropharyngeal aspirations of multi-wall CNTs or crocidolite asbestos. Histopathological analysis of lung sections showed that while granulomatous inflammation was similarly induced in both exposures, CNT caused type II pneumocyte (T2P) hyperplasia, while asbestos caused mixed-cell bronchoaveolar hyperplasia. Both exposures caused increases of fibrotic collagen as shown in Mason’s trichrome stains. Fluorescent immunohistochemistry for T2P-specific proSPC showed that CNT number was substantially increased specifically in CNT-exposed lungs. These observations are significant considering that T2P cells are known to become hyperplastic in response to alveolar epithelial injury. Co-staining for proSPC and IL-1β showed that while both exposures increased IL-1β cells in lung tissue, CNT-induced IL-1β increases were largely specific to T2Ps. These results illustrate that CNT and asbestos exposures may induce related but reproducibly distinct profiles of toxicologic lung pathology, and that T2Ps may be sensitive to CNT-induced toxicity. This suggests that CNTs and asbestos may differ in their mechanisms of toxicity and resultant injury in the distal airway.

1257 Exposures to Carbon Nanotubes and Asbestos Induce Related but Distinct Profiles of Toxicologic Lung Pathology
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Carbon nanotubes (CNTs) are rapidly emerging as occupational and environmental lung toxicants. The increasing manufacture and applications of CNTs has prompted concerns surrounding their potential to cause adverse lung effects in light of asbestos-like characteristics such as high aspect ratio and biopersistance. Because of their novelty, the long-term health outcomes of CNT exposures in humans are unknown. CNTs may become agitated into aerosols in occupational settings, posing an inhalation threat to those who work in places of CNT manufacture, distribution, and usage. However, there are limited studies or inconsistent findings regarding CNT toxicity or their toxicological similarities to asbestos. In this study, we developed mouse models of exposure using repeated, low-dose oropharyngeal aspirations of multi-wall CNTs or crocidolite asbestos. Histopathological analysis of lung sections showed that while granulomatous inflammation was similarly induced in both exposures, CNT caused type II pneumocyte (T2P) hyperplasia, while asbestos caused mixed-cell bronchoaveolar hyperplasia. Both exposures caused increases of fibrotic collagen as shown in Mason’s trichrome stains. Fluorescent immunohistochemistry for T2P-specific proSPC showed that CNT number was substantially increased specifically in CNT-exposed lungs. These observations are significant considering that T2P cells are known to become hyperplastic in response to alveolar epithelial injury. Co-staining for proSPC and IL-1β showed that while both exposures increased IL-1β cells in lung tissue, CNT-induced IL-1β increases were largely specific to T2Ps. These results illustrate that CNT and asbestos exposures may induce related but reproducibly distinct profiles of toxicologic lung pathology, and that T2Ps may be sensitive to CNT-induced toxicity. This suggests that CNTs and asbestos may differ in their mechanisms of toxicity and resultant injury in the distal airway.

1258 Single-Walled Carbon Nanotubes (SWCNTs) Induce Vasodilation in Isolated Rat Aortic Rings

Single-walled carbon nanotubes (SWCNTs) are used in biological systems with impact in medicine to continue improving in drug development and treatment of diseases. However, their effects upon the vascular system are not fully understood. Endothelium and smooth muscle cells (SMC) communicate through the release of vasodilatory factors such as nitric oxide (NO) to maintain vascular tone. The aim of this study was to evaluate the effect of SWCNTs on vascular tone using an isolated rat aortic rings model. Aortic rings were exposed to SWCNTs (0.1, 1 and 10 μg/mL) both in presence and absence of endothelium. SWCNTs induced vasodilation in both conditions, indicating that this effect was not dependent on endothelium. Moreover, blockage with L-NG-Nitroarginine methyl ester (L-NAME) did not modify the observed effect suggesting vasodilation was independent on NO production. Further investigation is required to understand the mechanisms of action and mediators involved in the signaling pathway induced by SWCNTs on the vascular system components under specific biological conditions.

1259 Role of Stem-Like Cells in Carbon Nanotube-Induced Pulmonary Fibrosis
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Carbon nanotubes (CNTs) have generated great interest commercially with their diverse applications however, the risk of their adverse health effects is not well understood. Studies have shown that CNTs can induce pulmonary fibrosis in animal models. Since fibrosis is associated with aberrant tissue repair and extracellular matrix (ECM) accumulation, identifying the cells that are responsible for the repair and ECM production is fundamental to the understanding of fibrosis mechanism. We hypothesize that CNTs induce fibroblast stem-like cells (FSCs) and that such induction is essential to the development of fibrosis. Fluorescence activated cell sorting was used to isolate FSCs from CNT-treated normal human lung fibroblasts (NHLFs). The expression of stem cell markers and fibrogenic markers was examined using western blotting, immunofluorescence staining and confocal microscopy. Our results demonstrated for the first time that CNTs can induce FSCs from NHLFs as evidenced by their side population (SP) property and expression of stem cell markers ABCG2 and ALDH1A1. These cells, isolated from NHLFs and FSCs, showed strong expression of markers of stem cells and tropo-onc smooth muscle actin, which are key biomarkers of fibrosis, as compared to non-SP cells. The induction of FSCs by CNTs was redox-sensitive since inhibition of oxidative stress by antioxidants including N-acetyl cysteine and catalase effectively inhibited the FSC induction. Together, our results support the existence of FSCs induced by CNTs and their putative role in fibrogenesis. This novel finding provides a new insight into the mechanism and may aid the development of early detection biomarkers and treatment strategies for the disease. [Supported by NIH grants R01-HL095579 and R01-E0029368 and by NSF grants EPS-1003907 and CBET-145490]
follows: KT >> Bz-OX-MX >> SB-DO. The predicted phototoxic risk was almost in agreement with the results of the 3T3 NRU PT and the \textit{in vivo} photocytotoxicity test, whereas OX seemed to be non-phototoxic in these phototestirg tests. Phototoxic skin reactions are known as main phototoxic reactions of OX, and thus, there appeared to be the data discrepancy between the predicted phototoxic risk of OX upon the proposed strategy and the observed phototoxic events of OX in the \textit{in vitro in vivo} photocytotoxicity tests. The combined use of photochemical and cassette-dosing PK data would provide reliable predictions on phototoxic risk of candidates with high productivity.

1262 Differential Effects of Some Natural Compounds on the Transdermal Penetration of Caffeine and Salicylic Acid

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Many natural compounds have the potential to modulate the transdermal penetration of topicaly applied drugs and chemicals. We studied the effect of five natural compounds (Hydroxycitronellal, Limonene 1, 2-epoxide, terpinyl acetate, p-coumaric acid, transferrulic acid) and ethanol on the transdermal penetration of two marker drugs (14C Caffeine and 14C Salicylic acid) in a flow through in-vitro porcine skin diffusion system. Caffeine and Salicylic acid were topically applied either in 10% solutions of natural compounds or ethanol at a concentration of 1.6 µg/µl as finene (25µl) volume to 1 cm² of skin. The receptor fluid was a Krebs-Ringer bicarbonate buffer with dextrose, heparin and bovine serum albumen. Levels of radioactivity were determined in receptor phase, skin surface by swabbing, stratum corneum by tape stripping and remaining skin using liquid scintillation counting. The parameters of flux, permeability coefficient, diffusivity, and percent dose absorbed/retained were calculated and compared. The dermal absorption of 14C-Caffeine was significantly higher with limonene epoxide (93.2%) and terpinyl acetate (29.54%) as compared with ethanol (3.24%). In contrast, the dermal absorption of 14C-Caffeine was significantly lower with coumaric acid (0.78%) and transferrulic acid (0.61%) as compared with ethanol (3.24%). Absorption was similar in hydroxycitronellal and ethanol. Similar trends were observed for flux and permeability parameters. Interestingly, significant differences in transdermal penetration of 14C-Salicylic acid were not seen with any of the natural compounds. These results emphasize the potential transdermal penetration enhancement effects of some natural compounds on a hydrophilic drug such as caffeine as compared to a hydrophobic drug such as salicylic acid. Dermal absorption decreasing effects of some natural compounds in this study might be further explored with toxic marker compounds. (Supported by Kansas Bioscience Authority)

1263 Stratum Corneum Proposed Water Domain Role in Percutaneous Absorption and Decontamination


Compounds with varying physical and chemical properties of compounds may have different affinities to stratum corneum (SC) and/or its intercellular lipids, keratin protein, and possible water domains. Attempting to better understand why chemical skin decontamination is often problematic, we utilized 25 carbon-14 labeled chemicals, which are hydrophilic (P<0.15), lipophilic (P>0.15), and quantified their binding and partitioning properties in regards to intact SC membrane, delipidized SC membrane, and SC lipid. A facile method was developed for lipid partitioning, providing a more equivalent procedure and comparable data with intact SC and delipidized SC binding assays. SC lipid/water partition coefficient (PClip/w) of chemical solutes positively correlated with log Po/w (log PClip/w = 0.32 Po/w - 1.33, R2=0.7306). Differences between the percent dose of chemi- cal binding to intact SC and total percent dose contributed by protein and lipid domains suggest the likely possibly and significance of a water domain (0.15 – 58.16%). For instance, assuming a unit mass of SC is composed by 0.54 (mass fraction) of protein, 0.35 of water and 0.09 of lipid, % dose of methyl in unit mass of SC, % dose contributed by protein domain and lipid domain were 56.4%, 0.5% and 1.2%. Chemical binding ratio showed a longer lag time for intact SC than for delipidized SC or SC lipid; lag times of terbinafine HCl and salicylic acid binding to intact SC, delipidized SC, SC lipid were 3.0, 0.0 min, and 4.4, 0.0 min, respectively. This suggests that a water domain may delay chemical binding to protein and lipid domains, and may be a factor in the resistance of many chemicals to practical decontamination. This project is supported by Defense Threat Reduction Agency (DTRA) Grant: HDTRA1-14-0005 UCSF (BRBAA11-PerC-9-2-0054 – Base)

1264 Chemical Skin Contamination: Effects of Vehicles on Penetration and Stratum Corneum Binding

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This study evaluated effects of three vehicles—ethanol, isopropyl alcohol, and isopropyl myristate (MW/ClogP: 46/0.19, 60/0.16, 270/4.33, respectively)—on skin penetration and stratum corneum (SC) binding of model compounds: benzonic acid, butanenitrile HCl and terbitatine HCl (MW/ClogP: 122/1.98, 354/6.6, and 328/5.9, respectively) – to better understand the role of vehicles (excipients) in decontamination. The C14-labeled chemicals were prepared in above vehicles, applied to human skin \textit{in vitro}, and 30 minutes later washed with soap/water. Absorption was continuously measured for 24 hours, using flow-through diffusion cells. For instance, lag times of terbinafine HCl in ethanol, isopropyl alcohol, and isopropyl myristate were 0.6 h, 0.6 h and 0.9 h, respectively. Absorption data was compared to the results of chemical binding into SC, performed via 30 minutes of chemical exposure to the SC sheets, followed by water wash. % dose of terbinafine HCl in isopropyl myristate binding to SC (59.9%) was significantly higher than those of in ethanol (37.9%) and isopropyl alcohol (45.1%) (P<0.05), suggesting higher binding rate to SC may increase retention of chemical in SC and lead to a longer lag time of skin penetration. SC binding experiments with soap/water wash showed significantly lower binding rate of terbinafine HCl/isopropyl myristate (25.1%, P<0.01) to SC, but no significant different when ethanol (39.1%) and isopropyl alcohol (49.32%) were used as vehicles. Results suggest chemical skin penetration, stratum corneum binding and decontamination efficiency are vehicle dependent; decontamination without detergent increases binding of chemical to SC, when hydrophobic vehicle is used. These data provide mechanistic insight that may provide options for more efficient decontamination schema. This project is supported by Defense Threat Reduction Agency (DTRA) Grant: HDTRA1-14-0005 UCSF (BRBAA11-PerC-9-2-0054 – Base)

1265 The Development of a Simple \textit{In Vitro} Human Skin Model for Dermal Absorption Investigations

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Skin tissue engineering has gained significant momentum of the last decade fuelled mainly by the demand to move away from the use of \textit{ex vivo} human and animal skin models. Rigorous evaluations regarding the dermal absorption prediction potential of the currently available tissue engineered constructs have demonstrated poor correlations against established \textit{ex vivo} gold standards. Thus, there is a real need to develop a model which consists of definitive keratinocyte tight junction formation and distinguished epidermal differentiation. Electrospinning polyethylene terephthalate (PET) onto commercially available AZO® wipes for varying times (minutes): 15, 30, 45 and 60, generated four bi-phasic scaffolds exhibiting both nano- and macro-scale architectures. SEM images revealed two distinct pores layers consisting of fibre diameters ranging between 220 nm and 270 nm and 13 µm to 26 µm. Tensile stress/strain curves illustrated increased strength with increased whole scaffold thickness. Mono-cultures of iHaC epithelial and iBf6 fibroblasts on the four scaffolds generated good proliferation rates throughout the whole construct range, with the 60 minute nano fibre scaffold demonstrating highly favourable characteristics for both cell types. Additional investigations on polycarbonate membranes generated higher transepithelial electrical resistance (TEER) readings for the co-culture than that observed for the iHaC mono-culture. Based on these preliminary findings, it is hoped to translate these protocols into a suitable bi-phasice scaffold to ultimately generate a simple co-culture \textit{in vitro} skin model exhibiting the much needed barrier function for dermal absorption investigations.

1266 Dermal Exposure and Skin Notation—Important to Account for Evaporation


A so-called skin notation assigned to an occupational exposure limit (OEL) warns about the potential of the currently available tissue engineered constructs have demonstrated poor correlations against established \textit{ex vivo} gold standards. Thus, there is a real need to develop a model which consists of definitive keratinocyte tight junction formation and distinguished epidermal differentiation. Electrospinning polyethylene terephthalate (PET) onto commercially available AZO® wipes for varying times (minutes): 15, 30, 45 and 60, generated four bi-phasic scaffolds exhibiting both nano- and macro-scale architectures. SEM images revealed two distinct pores layers consisting of fibre diameters ranging between 220 nm and 270 nm and 13 µm to 26 µm. Tensile stress/strain curves illustrated increased strength with increased whole scaffold thickness. Mono-cultures of iHaC epithelial and iBf6 fibroblasts on the four scaffolds generated good proliferation rates throughout the whole construct range, with the 60 minute nano fibre scaffold demonstrating highly favourable characteristics for both cell types. Additional investigations on polycarbonate membranes generated higher transepithelial electrical resistance (TEER) readings for the co-culture than that observed for the iHaC mono-culture. Based on these preliminary findings, it is hoped to translate these protocols into a suitable bi-phasice scaffold to ultimately generate a simple co-culture \textit{in vitro} skin model exhibiting the much needed barrier function for dermal absorption investigations.

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exposure at the OEL (only applicable for chemicals with systemic toxicity as critical effect). Obviously, dermal uptake is less important for chemicals with high volatility, as evaporation lowers the amount available for absorption. Nevertheless, no organization explicitly includes evaporation in their skin notation assessments. The aim of this study was to compare the contribution of dermal uptake and evaporation to systemic dose and toxicity of industrial chemicals. Experimental data on skin uptake was compiled from the literature, evaporation rates were calculated theoretically from molar weight, molar volume, wind speed (0.6 m/s) and temperature (305K = skin temperature), and toxic doses were calculated from AEGL-3 values. Dermal uptake, evaporation, absorbed fraction and toxicity all varied by several orders of magnitude in a largely uncorrelated manner. Thus, between the 73 chemicals with a skin notation on the Swedish OEL list: evaporation and dermal uptake rates varied 10^7-fold, whereas the fraction absorbed varied 10^5-fold, between 99.7% (pentachlorophenol) and 0.001% (tetradecylfuran) of the applied dose. A rough comparison against AEGL-3 values suggested a 10^5-fold range in time to reach toxic systemic dose (extremes 40s to over 100000s) (DEHP). We concluded that evaporation should be taken into account in a systematic manner when setting skin notations. The study was financed by the Swedish National Board of Health and Welfare and the Swedish Council for Working Life and Social Research.

1267 Dermal Uptake of Tetrabromobisphenol A (TBBPA) by Female Wistar Han Rat or Human Skin

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TBBPA, a brominated analog of Bisphenol A, is the highest production volume brominated flame retardant in production and human exposure is ubiquitous. Although the major route of exposure to TBBPA is oral uptake, skin penetration is possible. In the studies presented here, the dermal penetration of TBBPA was determined using humans and female Wistar Han rat skin in vitro and compared to that of rat skin exposed in vivo. Split-thickness human and rat skin samples were administered a dose of 100 nmol/cm² skin (~1 in vivo determined using human and female Wistar Han rat skin). Dermal uptake, evaporation, absorbed fraction and toxicity all varied by several orders of magnitude in a largely uncorrelated manner. Thus, among the 73 chemicals with a skin notation on the Swedish OEL list: evaporation and dermal uptake rates varied 10^7-fold, whereas the fraction absorbed varied 10^5-fold, between 99.7% (pentachlorophenol) and 0.001% (tetradecylfuran) of the applied dose. A rough comparison against AEGL-3 values suggested a 10^5-fold range in time to reach toxic systemic dose (extremes 40s to over 100000s). We concluded that evaporation should be taken into account in a systematic manner when setting skin notations. The study was financed by the Swedish National Board of Health and Welfare and the Swedish Council for Working Life and Social Research.

1268 Skin Permeability of Ortho-Phenylphenol in Metalworking Formulations


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Over 10 million workers worldwide are exposed to metal working fluids (MWF). Phenols are common constituents of MWF's and have been known to cause irritant contact dermatitis in humans. This study assessed the in vitro dermal absorption of [14C]-radiolabeled ortho-phenylphenol (OPP). Twenty-eight porcine skin sections from the dorsum of an adult pig were used in a flow-through diffusion cell system. A dosing solution of [14C]-radiolabeled OPP in water, water + 5% soluble oil, 5% semi-synthetic, or 5% synthetic MWF was applied to each skin surface (n=7 each). Timed perfusate samples were collected for 24 hours and the level of [14C]-radiolabeled OPP was measured via liquid scintillation counting. At termination, mass balance samples were taken of the skin sample, stratum corneum, surface swabs, dosing device and the remaining dose left on the skin's surface. OPP is a lipophilic compound (log Ko/w = 3.09) and it was expected to permeate the skin at its highest levels in the water, then the synthetic oil, followed by the semi-synthetic oil, and least in the soluble oil. Data analysis showed that the log permeability (log Kp) values were -1.70 cm/h for water, -2.20 cm/h for synthetic oil, -2.62 cm/h for semi-synthetic oil, and -2.76 cm/h for soluble oil. This indicates that the safest way to use OPP in MWF's is in a semi-synthetic oil solution or soluble oil solution to minimize dermal absorption in the workplace. Other related in vitro and in vivo studies will make it possible to estimate in vivo dermal absorption in human skin for various MWF formulations. This information can then be used to help formulate and regulate occupational safety rules to benefit those working with MWF's. Funding: National Institute of Occupational Safety and Health (NIOSH).

1269 A Multicompartment Mathematical Model of the In Vitro Percutaneous Absorption of Nerve Agent VX

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Measurement of diffusion in vitro to estimate how chemicals penetrate the skin is well established and many mathematical models have been published describing this process, often from first principles of diffusion or considering the skin as compartments. The models enable an understanding of the processes involved in percutaneous absorption that is particularly important with extremely toxic chemicals such as VX. The diffusion of VX has been reported previously and the work described here used a multi-compartment model written in Microsoft Excel to demonstrate good predictions of amounts absorbed through guinea pig skin over a 24 hour period. The skin was full thickness or cut to 0.5mm with a dermome; receptor fluid 50:50 ethanol/water (continuously stirred); area available for diffusion 2.54 cm² and skin surface temperature of 32 ± 1°C. Oculated and unoccluded conditions were investigated. The model consisted of a surface donor, superficial skin, skin, peripheral skin (modelling lateral diffusion) and receptor compartments. The general equation for diffusion between compartments was based on Fick’s law of diffusion dP/dt = k (P1 – P2). Where P = penetrant and k is the diffusion constant between compartments 1 and 2. The model was manually fitted then optimised by least squares using the Microsoft Excel Solver that was set to vary the values of the constants (k) to minimise the sum of squares. This estimated the diffusion constants between each compartment that could be used to inform an input algorithm for a pharmacokinetic model. The models were able to describe the relationship between amount absorbed and time for occluded and unoccluded conditions when the agent was applied undiluted or diluted in isopropanol to full thickness and damnetomed guinea pig skin. Some assumptions made to construct the model require further testing (e.g. instantaneous spread, minimum sustainable continuous thickness). This approach represents a good method of fitting lines to in vitro diffusion data based on a conceptual model of diffusion. © Crown Copyright 2014.

1270 Nr2 Controls Skin Inflammation Provoked by Chemical Allergens Regardless of the Chemical Reactivity of Contact Sensitizers


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Chemical sensitizers inducing contact hypersensitivity (CHS) are known to induce reactive oxygen species (ROS). The Nr2/Keap1 pathway is central for detoxification. Nr2 plays a central role in protecting cells from ROS and other electrophiles. Recently, we have demonstrated that allergic skin inflammation induced by chemical sensitizers was controlled by Nr2. In order to study the role of Nr2 in response to chemicals that react with different amino acids, various compounds were tested using the Mouse Ear Swelling Test (MEST) and the Local Lymph Node Assay (LLNA). These studies were performed in nrf2 knock out (KO) and in wild type (WT) mice. Eleven chemicals were used: two molecules known to react with cysteine residues, trinitrochlorobenzene (TNCB) and diphenylcyclopropenone (DPC); four molecules known to exhibit mixed reactivity to cysteine and lysine residues, isophorone diisocyanate (IPDI), 4, 4’ methylene diphenyl diisocyanate (4, 4’ MDI), tolune diisocyanate (TDI) and 1-phenyl-1,2-propanedione (P2P); three molecules reacting specifically with lysine residues, phthalic anhydride (PA), trimellitic anhydride (TMA) and 3,4-dihydroxycoumarin (DHC); one pro-hapten, eugenol and one pro/pre-hapten p-phenylenediamine (pPD). The MEST results showed that all tested compounds induced a greater increase in the ear thickness in KO mice than in nr2+/+ mice (WT). Furthermore, the swelling increase was dose dependent. The non-sensitizing dose of all compounds in WT mice efficiently induced CHS in KO mice. Results obtained in the LLNA showed that lysine compounds (PA & TMA) and isocyanate compounds (IPDI & TDI) induced an increase of lymphocyte proliferation in KO and WT mice. Regardless of the chemical used, the stimulation index (SI) for a similar concentration was higher in KO mice than in WT mice. Nr2 controls the inflammation response and the lymphocyte proliferation, involved in allergic response to chemical sensitizers having different reactivities to aminoacids.
Reactive carbonyl species originating from endogenous chemical processes such as lipid peroxidation and glycation are potent mediators of environmental electro-phile tissue damage, but their involvement in skin photocarcinogenesis and photo-toxing remains poorly understood. Here we demonstrate for the first time that the glycolysis-derived reactive carbonyl species methylglyoxal (2-oxopropanal), apart from causing protein damage through formation of advanced glycation endproducts (AGEs), displays potently activity as a sensitizer of UVA-induced oxidative damage in human skin cells. Photodynamic inhibition of proliferation was observed in human skin fibroblasts and keratinocytes exposed to the combined action of low doses of solar simulated UVA (9.9 J/cm2) and physiologically relevant concentrations of MG in the low micromolar range. MG-induced photosensitization caused oxidative stress and upregulation of stress response gene expression (HMox1, Hspa1A, Hspa6). Related compounds containing the α-dicarbonyl-chromophore (2,3-butanedione, phenylglyoxal) were equally active UVA-photosensitizers. Consistent with UVA-driven singlet oxygen formation by MG, ΦX174-plasmid cleavage assays revealed induction of DNA damage that was enhanced by formamidopyrimidine-DNA glycosylase (Fpg)-digestion indicative of photodynamic introduction of 8-oxo-dG lesions. Comet analysis in keratinocytes revealed MG-potentiation of UVA-induced genomic damage, and MG-photosensitotoxicity was also observed in human reconstructed epidermis exposed to solar simulated UVA. The occurrence of markers of solar insult (TUNEL-positivity) was significantly enhanced in skin reconstructs exposed to the combined action of UVA and MG. Taken together, these data demonstrate that reactive carbonyl species containing an UVA-active α-dicarbonyl-chromophore represent a novel class of endogenous photosensitizers, suggesting a heretofore unrecognized phototoxic activity of glyca-tion-intermediates in skin.

Skin sensitization remains a major environmental and occupational health hazard. Till recently, only animal tests were accepted by regulations. Since March 2013, the 7th Amendment of the Cosmetics Directive prohibits in Europe the marketing of cosmetic products containing ingredients which were tested on animal-based assays and prompted the implementation of Integrated Approaches to Testing and Assessment (IATA) for Skin Sensitization. While there is a common understanding of the Adverse Outcome Pathways (AOP’s) leading to skin sensitization, it is still well as a wide appropriation of a core battery of assays addressing these AOP key events, the ways of integrating such data to allow risk assessment of new ingredients is still in its early experimental phase. We generated a complete training set of data from in silico predictions (Derek, TIMES, Tintree), from DPRA, MUSST, Nrf-2 and PGE-2 assays as well as numerous physico-chemical experimental or calculated parameters on 168 substances having an LLNA-based N/N classification. We submitted it to statistical analysis: from the large number of supervised classification models proposed in the literature, we chose five different methods: Boosting, Naïve Bayes, SupportVectorMachine (SVM), Sparse PLS-DA and Expert Scoring. These methods have strong differences, but they all produce posterior probability of belonging to the group of interest (‘sensitizer’). We combined them by the stacking methodology of Wolpert and Breiman, in order to obtain a specific ‘stacking’ meta-model. Results from this two classes (Sensitizer/NonSensitizer) prediction meta-model obtained on a validation data set show predictive performances of 86 % concordance, 81% sensitivity and 94 % specificity. Based on this experience on cosmetic case studies we will conclude on the opportunities and remaining challenges to support the ongoing OECD IATA initiative to reach the final goal of safety evaluation and risk assessment of new ingredients.
cell-line activation test (h-CLAT) as well as the (modified) modelled uroid 937 skin sensitization test (mUSST; 2nd cellular response: ‘dendritic cell activation’). To assess the overall skin sensitizing potentials of the substances, a simple ‘2 out of 3’ integrated testing strategy (ITS) was applied (Urbisch et al., submitted). In order to facilitate regulatory acceptance of these methods, test results of 213 substances were compared to both local lymph node assay (LLNA) and human data. The individual test methods provide predictivities between 73 and 76% or 78 and 84%, when compared to LLNA or human data, respectively. The ‘2 out of 3’ ITS shows accuracies of 79 or 90%, when compared to LLNA or human data, respectively. These results demonstrate that the non-animal test methods correlate better with human data than with LLNA data and confirm their utility for reliably discriminating skin sensitizers from non-sensitizers. In addition, different mechanistic domains were identified by probabilistic binding mechanisms of the 213 substances. This approach shows that all assays predict Michael acceptors with the highest accuracies of at least 80%. In the domain of acylating agents, the keratinocyte based assays show decreased predictivities of 58%. Taking mechanistic domains into account offers a more accurate estimation of the predictive performance of the individual non-animal test methods as well as the overall ITS prediction.

### 1276 Examining the Role of In Silico Assessment in Skin Sensitation-Integrated Testing Strategies


The prediction of skin sensitisation potential is an important requirement for a variety of chemical safety assessments, including submissions for REACH, the toxicity assessment of new cosmetic ingredients, and risk assessments associated with occupational exposure. The use of in vitro testing is required by regulatory bodies. However, this has been restricted in recent years, and this has driven the development of new in vitro tests as potential replacements. These tests, either alone or as part of an integrated testing strategy (ITS), offer the potential to assess toxicity without the need for animal studies. However, it is important to recognize that the in vitro assays measure components of the toxicity pathway(s). In contrast, in silico models derived from animal or human data are, in principle, capable of modelling the end-to-end biological process of sensitisation. With this in mind, in silico models may offer a valuable complement to the current battery of in vitro tests. To assess this, two datasets, comprising of 146 compounds with LLNA, DPRA, h-CLAT and KeratinoSens assay data were compiled from published sources. The datasets were curated and evaluated against a Derek Nexus knowledge base containing 73 alerts for skin sensitisation, based on human and animal data. We report that using an in silico system in combination with one or more in vitro assay(s) improves predictive performance against the LLNA dataset, versus the use of in vitro tests alone. The structural alerts model a different but overlapping applicability domain to the in silico models and this methodology may be applied as part of an ITS approach.

### 1277 Vascularized Skin Models and Impedance Spectroscopy for the Assessment of Skin Toxicity

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Despite advances in the development of in-vitro-tissue-equivalents such as reconstituted human epidermis (RHE), the number of endpoints in toxicity-testing, which can be addressed with these models is limited. One reason for that is that key cellular components are lacking and the live time of the models is restricted. In addition, the analysis of the models is still dependent on invasive methods such as histological processing or 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) staining. To overcome these pitfalls, we achieved advanced culture systems and biosensors which allow long term culture of complex tissue-equivalents. Using these technologies, we have developed the first full thickness skin model with a perfused vascular network. Furthermore, as an alternative for classical methods, we have established a non-destructive technology to analyze the integrity of the epidermal barrier based on impedance spectroscopy. Although similar to obtaining a trans-epithelial-electrical-resistance value, impedance spectroscopy is using multiple measurements in a specific frequency range instead of just measurement at 1 frequency. RHE typically exhibits characteristic impedance spectra in a frequency ranging between 1 Hz and 100 kHz, which is comparable to the spectra of freshly isolated human epidermal biopsies. From these spectra, we extracted electrical parameters of the RHE such as the capacitance and the ohmic resistance. These parameters change significantly during epidermal differentiation and were used to quantify the effects of mechanical and chemical disruption of the epidermal integrity. Most relevant, impedance spectroscopy shows a sufficient sensitivity to detect a transient decreased ohmic resistance caused by 2-propanol, which is classified as a non-irritant by MTT assays. This result indicates that impedance spectroscopy can be employed as an additional method to assess mild irritative effects.

### 1278 In Vitro Skin Metabolism of Cosmetic Ingredients: Experimental Influencing Factors, Comparison with In Silico Generated Data

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Risk assessment in cosmetology requires the use of in vitro, in silico and in chemo approaches, as animal based testing has been totally prohibited (7th Amendment of the Cosmetics Directive). A relevant risk assessment requires robust data on exposure, thus it relies on two main factors: skin absorption and skin metabolism. As a result, a bioavailability factor can be established. Few data exist on skin metabolism and extrapolation from data obtained from other routes is difficult. Personal care products are not expected to lead to systemic exposure; however skin absorption and metabolism are two critical parameters to be evaluated. Moreover, in the final cosmetic product interactions among the different components may alter basal metabolism as some compounds may be enzymatic inducers or inhibitors. Skin metabolism was investigated in this study using phenytoin, a preservative commonly used in personal care. Phenytoin was used for enzymatic induction studies. Both 2 D and 3 D cultures have been performed. The model used was a 3D reconstructed human epidermis, developed in house (VitroDerm). In parallel, computerized generation of metabolites has been conducted using OECD QSR Toolbox and Tox tree. This work presents both metabolism data and influencing factors which play a role when studying in vitro skin metabolism. The following critical points have been identified, quantified and are discussed: Type of culture: 2D vs 3D. Vegetal medium vs animal medium. Protein level. Influence of enzymatic inducers. Changes in these parameters lead to qualitative and quantitative differences in cell metabolism. Intrinsically clear and bioavailability may be affected. Discrepancies between in vitro and in silico analysis have been identified. Differences are mostly due to the poor enzymatic capacity of skin compared with liver capacity taken as reference for simulation.

### 1279 VEGF Expression on 3D Skin Equivalent Plays an Active Role in Maintaining Epithelial Integrity by Inducing Capillary-Like Structure and Keratinocyte Proliferation

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Introduction: Skin equivalent (SEs) were developed to reproduce key aspects of natural skin and they can be used in different contexts, e.g. chemical safety assessments. There are several types of SE and consequently a variety of protocols for constructing them, having in common the aim to achieve a skin substitute that more closely resemble in vitro skin. Keratinocytes (KC) and fibroblasts (FB) can secrete cytokines, like vascular endothelial growth factor (VEGF) that is related to tissue maintenance and repair as well as vascularization. Objective: A capillary-like structure (C-LS) was observed in an in-house SE by histological staining (H&E). Aimed to verify whether such structures could be generated on SE by KC and/or FB, an in vitro experiment using indirect peroxidase technique. Normal human skin biopsy was incubated for 13 days. 5-fluorouracil (5-fluorouracil) and anti-VEGF and anti-CD34 immunohistochemistry were carried out. Methodology: SE was constructed from HaCaT cells (immortalized human KC) seeded (1x106/insert) onto contracted collagen type-I gel with 3x105 primary human FB. After 48 h at submersed culture conditions, the cultures were placed at air–liquid interface and incubated for 13 days. 5-mm sections were processed for immunohistochemical staining using indirect peroxidase technique. Normal human skin biopsy was used as negative control (NC). Results and Discussion: The C-LS was observed in an in-house SE by histological staining (H&E). Aimed to verify whether such structures could be generated on SE by KC and/or FB, anti-VEGF and anti-CD34 immunohistochemistry were carried out. Methodology: SE was constructed from HaCaT cells (immortalized human KC) seeded (1x106/insert) onto contracted collagen type-I gel with 3x105 primary human FB. After 48 h at submersed culture conditions, the cultures were placed at air–liquid interface and incubated for 13 days. 5-mm sections were processed for immunohistochemical staining using indirect peroxidase technique. Normal human skin biopsy was used as negative control (NC). Results and Discussion: The C-LS was observed in an in-house SE by histological staining (H&E). Aimed to verify whether such structures could be generated on SE by KC and/or FB, anti-VEGF and anti-CD34 immunohistochemistry were carried out. Methodology: SE was constructed from HaCaT cells (immortalized human KC) seeded (1x106/insert) onto contracted collagen type-I gel with 3x105 primary human FB. After 48 h at submersed culture conditions, the cultures were placed at air–liquid interface and incubated for 13 days. 5-mm sections were processed for immunohistochemical staining using indirect peroxidase technique. Normal human skin biopsy was used as negative control (NC). Results and Discussion: The C-LS was observed in an in-house SE by histological staining (H&E). Aimed to verify whether such structures could be generated on SE by KC and/or FB, anti-VEGF and anti-CD34 immunohistochemistry were carried out. Methodology: SE was constructed from HaCaT cells (immortalized human KC) seeded (1x106/insert) onto contracted collagen type-I gel with 3x105 primary human FB. After 48 h at submersed culture conditions, the cultures were placed at air–liquid interface and incubated for 13 days. 5-mm sections were processed for immunohistochemical staining using indirect peroxidase technique. Normal human skin biopsy was used as negative control (NC). Results and Discussion: The C-LS was observed in an in-house SE by histological staining (H&E).
Toxicity Study of a Three-Dimensional (3D) Organotypic Skin Model Using Keratinocytes and Mesenchymal Stem Cells Immortalized by hTERT

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Primary keratinocyte cultures are important as a cell model for the study of normal and pathological biology of the cutaneous epithelia. They can form skin equivalents that mimic the architectural features and behavior of normal skin in a three-dimensional (3D) organotypic culture model in an air-liquid interface (ALI). However, primary keratinocytes have finite lifespan in culture, which greatly restricts their use as in vitro cellular models. Previous studies with hTERT immortalized keratinocytes have demonstrated that wound healing is equivalent to primary cells. In this study, we compared primary keratinocytes to hTERT immortalized keratinocytes co-cultured with hTERT immortalized mesenchymal stem cells (MSCs). We confirmed that both primary keratinocytes and hTERT immortalized keratinocyte cell line, Ker-CT, are able to fully differentiate into skin equivalents in an ALI 3D culture model, when co-cultured with hTERT immortalized mesenchymal stem cells (MSCs). To confirm the functionality of the co-culture models, both primary keratinocytes and Ker-CT ALI co-cultures were subjected to a toxicity study using 1% Triton X-100 and 5% SDS. IC50 for primary keratinocytes and Ker-CT are similar and have the expected IC50 for both compounds. The immortalization of Ker-CT cell line makes it an invaluable model for the research of keratinocyte biology, as it eliminates the issue of short life span and donor variation seen with primary cells.

Metabolic Oxidation of Rhododendrol and Enhanced Cytotoxicity in Melanocytes

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[Objectives] Whitening cosmetic products containing rhododendrol (4-(4-hydroxyphenyl)-2-butanol) have caused skin degeneration in some users. Occupational leucoderma has been associated with raspberry ketone (4-(4-hydroxyphenyl)-2-butanol) used as a raw material in the manufacture of rhododendrol. Therefore, we expected that rhododendrol, residual raspberry ketone or their cytotoxic metabolites would decrease epidermal melanocyte viability. Analysis of impurities of the rhododendrol and raspberry ketone on epidermal cells were performed. The metabolism of rhododendrol and raspberry ketone with tyrosinase was also investigated. [Methods] Methanol extracts from rhododendrol were analyzed using HPLC with a chiral or ODS column. The cytoxicities of rhododendrol and raspberry ketone were determined by MTT assay. The catechols were oxidized to their corresponding catechols [4-(3,4-dihydroxyphenyl)-2-butanol and 4-(3,4-dihydroxyphenyl)-2-butanol] on normal human epidermal melanocytes and HaCaT cells were evaluated using an ATP assay. The cell culture media were analyzed after incubation with the test compounds using LC/MS. Each compound was incubated with mushroom tyrosinase, and the reaction mixtures were analyzed using LC/MS. [Results and Discussion] The rhododendrol that was extracted from the products was a 1:1 mixture of R and S enantiomers. Raspberry ketone detected in the products was little in quantity. The catechols showed greater cytotoxicity to both melanocytes and HaCaT cells compared to rhododendrol and raspberry ketone. In the culture media incubated with melanocytes and rhododendrol or raspberry ketone, corresponding catechols were determined by LC/MS analysis. Rhododendrol and raspberry ketone were oxidized to their catechols using mushroom tyrosinase with molecular oxygen. These results suggest that rhododendrol was metabolized to 4-(3,4-dihydroxyphenyl)-2-butanol by intracellular tyrosinase, which resulted in the death of melanocytes.

Decon. Gel for Skin Decontamination In Vitro Human Skin Model


After a half century of extensive study, it remains difficult to effectively decontaminate skin post chemical exposure, especially for chemical warfare agents. Here, we developed a novel skin decontamination polymeric gel—Decon. Gel—and assayed its decontamination efficiency for model lipophilic and hydrophilic chemicals, including chemical warfare simulants. Results showed that Decon. Gel effectively inhibited chemical penetration through skin. In experiments examining 5 minutes delayed decontamination, C14-labeled chemicals were applied on skin surface in vitro and decontaminated with Decon. Gel or traditional methods. Percent dose of butenafine remaining in skin and receptor fluid for soap/water, bleach/water, dry fuller’s earth, and Decon. Gel were 34.2%, 14.3%, 44.4% and 2.2%, respectively (P<0.01). In addition, Decon. Gel’s decontamination efficiency did not deteriorate significantly over time, for 1, 5, 15 and 30 minutes delayed decontamination (percent doses of butenafine remaining in skin and receptor fluid were 0.9%, 2.4%, 2.5% and 2.4%, respectively, P<0.05). These findings suggest Decon. Gel holds the potential to be a significant advance in effective decontamination over traditional methods. This project is supported by Defense Threat Reduction Agency (DTRA) Grant: HDTRA1-14-0005 UCSC (BRBA11-PerC-9-2-4084 A Base).
Antimicrobials are commonly prescribed for control of surgical site infections (SSI) which frequently occurs despite strict adherence to aseptic surgical conditions leading to increased morbidity and mortality. The patient ends up with various complications, incomplete recovery, prolonged hospital stay and increased health care cost. Analgesics are concomitantly prescribed to relieve post-surgical pain and suffering. The objective of the study was to analyze the prescribing pattern of antimicrobials and analgesics drugs and the incidence of adverse drug reactions in patients who have undergone surgery. The data was collected from the case records of 400 surgical patients over a period of one year following institutional ethical approval. The prescribed drugs and ADRs were recorded before surgery, post-surgery and at discharge. Antimicrobials were used in 75% patients, whereas analgesics were used in all patients following surgery and/or at discharge. Cephalosporins, Diclofenac and paracetamol topped the list among all drugs prescribed. Minor adverse effects such as Nausea, vomiting (23%), Bad taste, anorexia (18%) and Diarrhea (11%) were recorded. GIT drugs were prescribed to minimize these adverse effects. Hypersensitivity (0.5%) and Thrombophlebitis (0.6%) was also observed in a small number of patients which were successfully managed. The results reflected appropriate use of antimicrobial and analgesic agents in department of surgery with minimum adverse drug reactions and post-operatives complications. The average hospital stay following surgery was 3.2 days suggestive of good recovery. The limitations of the study were that the findings represent overall usage irrespective of nature of surgery and the antimicrobial sensitivity pattern was not correlated to support the choice of antimicrobial prescribed. Moreover, the multiple antibiotics usage for individual patients got masked.

Antitoxic Effect of *Veratrilla baillonii* Franch on Acute Toxicity of Mice Induced by *Aconitum brachypodum* Diel, One of the Genus *Aconitum*

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*Aconitum brachypodum* Diels (Family Ranunculaceae) is well known for its both therapeutic and high-toxic activities in Yunnan and Sichuan provinces in China. The present study was conducted to observe the detoxication effect of *Veratrilla baillonii* Franch on mice induced by *Aconitum brachypodum* Diels. The acute poisoning effects of *Aconitum brachypodum* diei (CFA) in mice showed that toxicological symptoms such as retching, hyperventilation, hypoxia, scratching mouth, diaphoresis, dribbling, diarrhea, protopis, writhing and even hyperspasmia were observed, resulting in an LD50 of 41 mg/kg. Water extraction of *Veratrilla baillonii* Franch (WVBF) (25-200 mg/kg) could attenuate the acute toxicity induced by 40 mg/kg of CFA. Histologically, distinct degenerative changes of the heart, liver and kidney were observed after treatment of CFA single or along with WVBF. WVBF could attenuate the pathological changes to some extend. For biochemistry detection, CFA could lead to significantly increased of ALT, AST, ALP, TP, CREA, BUN and Tg in the serum, which could be decreased by WVAF. Further, the integral values of different groups indicated that WVBF treatment could properly regulate concentration alterations for some metabolites in the heart and brain of CFA-induced mice. Notably, total creatine and taurine, metabolites respectively involved in bioenergetics and synaptic efficiency, were both up-regulated in cardiac and brain tissue. WVBF could markedly attenuate the increase of the above metabolites. The results showed that WVBF could obviously reduce the onset of CFA toxicity. It may provide in depth understanding to the toxicological and pharmacological profiles of *Aconitum brachypodum* Diels and *Veratrilla baillonii* Franch. Footnotes: Supported financially by the National Natural Science Foundation of China (81102897 and 81374064).

Many efficacious cancer treatments cause significant cardiac morbidity but we lack biomarkers or functional indices of early damage to allow monitoring and intervention. Here, we utilised a rat model of progressive doxorubicin-induced cardiomyopathy to provide the most comprehensive characterisation to date of the timecourse of serological, pathological and functional events underlying this toxicity. Rats were dosed with 1.25mg/kg doxorubicin weekly for 8 weeks followed by a 4 week recovery period. Electron microscopy of the myocardium revealed subcellular degeneration and marked mitochondrial changes after a single dose. Histopathological analysis revealed progressive cardiomyocyte degeneration, hypertrophy/cytomagla and extensive vacuolation after 2 doses. Extensive replacement fibrosis (quantified by Sirius red staining) developed during the off-dosing period. Functional indices assessed by cardiac MRI (including left ventricular ejection fraction (LVEF), cardiac output and E/A ratio) declined progressively, reaching statistical significance after 2 doses, culminating in ‘clinical’ LV dysfunction by 12 weeks. Significant increases in peak myocardial contrast enhancement and serological cardiac troponin I (cTnI) emerged after 8 doses, importantly preceding the LVEF decline. Troponin I levels correlated with gadolinium contrast enhancement, histopathological grading and diastolic dysfunction. In summary, subcellular cardiomyocyte degeneration was the earliest marker, followed by progressive functional decline and histopathological manifestation. Myocardial contrast enhancement and elevations in cTnI occurred later. However, all indices pre-dated ‘clinical’ LV dysfunction and thus warrant further evaluation as predictive biomarkers.

In most cases, the safety evaluation of new anti-cancer small molecule pharmaceuticals includes repeat dose toxicity studies in rodents and non-rodents. In order to evaluate the impact of data from the non-rodent species, we analysed retrospectively data from the AstraZenea oncology portfolio over 13 years (1999-2012). There were 53 projects that provided at least one compound that progressed into dose range finding studies in non-rodents. In approximately 90% of these projects the non-rodent species was beagle dog, with the remaining 10% using cynomolgus monkey. The development of 12 compounds (approximately 23%) was stopped prior to clinical studies due to non-clinical safety signals which were considered to have an unacceptable negative impact on the risk/benefit profile of the drug. Of the 12 programs halted, in 7 instances (13%) there was non-rodent data which was the most significant contributor to the stop decision. In addition to informing the decision to stop a compound, in several instances non-rodent toxicology data had a significant influence on the clinical safety monitoring strategy during early human trials. In several cases, data from non-rodent studies was the primary driver to include additional clinical monitoring. Safety considerations identified from non-rodent data included the potential for ocular, liver, pulmonary, cardiovascular and testicular toxicity. However, for those compounds that reached the clinical phase, the dose and exposures achieved in patients showed no consistent relationship to that achieved in rodents and non-rodents in GLP testing. Overall, a retrospective analysis of our recent oncology portfolio illustrates that toxicology data from non-rodent studies makes an important contribution to human risk assessment.
doses for sunitinib, but is seen in animal models only at higher equivalent doses. By contrast, sorafenib treatment results in liver toxicity in animal models at HEDs considerably lower than the therapeutic dose. This approach allows for identifying those drugs where the endpoints in non-clinical studies do or do not predict the clinical safety concerns, and hence areas for research in improving methods for safety assessment.

**1290 Effects of Vitamin C on Tobacco Smoking-Related Gene Expression**

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Chemopreventive effects of vitamin C (vt C) may be different due to the cell environment. For example, tobacco smoking induces reactive oxygen species (ROS) and this mechanism has been suspected as a main cause of the tobacco toxicity. However, effects of vt C on tobacco smoking are not completely understood, yet. Therefore, we performed a randomized single blind crossover trial to evaluate detoxification effects and mechanisms of vt C in 25 healthy smokers (age= 25.0 ± 3.9 yrs). The subjects consumed placebo or vt C (3g/day) for 2 weeks with 2-week wash out period. As results, we found a positive association between urinary cotinine, a main metabolite of nicotine, and malondialdehyde (MDA), a biomarker for oxidative stress (p<0.05). However, there were no significant changes in levels of urinary cotinine or MDA by consumption of vt C. Global methylation, or expression of TP53, MTHFR and MTHFR were not changed by consumption of vt C, either. Interestingly, we found strong positive associations between urinary MDA levels and expression of TP53 or MTHFR, when they consumed vt C. In conclusion, our study suggests that vt C modulates expression of TP53, MTHFR and Nrf2, depending on high oxidative stress of tobacco smokers.

**1291 Assessment of Ethanol Effects on Pulmonary Antimicrobial Peptide (Cathelicidin/LL-37) Levels and Vitamin D Metabolism**

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The relationship between Vitamin D levels and severe respiratory infection, especially among patients with Alcohol Use Disorder, is of serious concern and being investigated by scientists all over the world. Approximately 6% (18 million) Americans heavily consume ethanol (> 14 drinks/week for men and > 7 drinks/week for women) and are susceptible to different complications and adverse health outcomes, especially bacterial pneumonia. Chronic ethanol abusers have been observed to have reduced levels of Cathelicidin/LL-37, active vitamin D, (1,25(OH)2D3) and associated increases in incidence and prevalence of respiratory infections. Human Cathelicidin/LL-37 is an endogenous peptide with bactericidal, bacteriostatic and antiviral properties and has been associated with protective pulmonary functions in humans. Preliminary data supports the theory of an ethanol-mediated inhibition of Cathelicidin/LL-37 function via a metabolic pathway mediated by CYP2E1, a specific cytochrome in the alveolar epithelium. Our research has been investigating the relationship between chronic ethanol over-exposure, pulmonary vitamin D speciate levels, levels of aldehyde anti-microbial peptides (Cathelicidin/LL-37) among susceptible populations with alcohol use disorder (AUD) and implications of all these relationships on public health. In a cohort, n=48, we analyzed the biological response of alveolar and broncho-epithelial cells to physiologically relevant levels of ethanol and CYP2E1-inhibiting compound like DADS (Diethyl Disulfide). We also quantified ethanol-mediated vitamin D speciate and Cathelicidin/LL-37 levels in biological samples from subjects with alcohol use disorder (AUD). Preliminary results revealed levels of Cathelicidin/LL-37, inactive vitamin D, 25(OH)D3 and active vitamin D 1, 25(OH)2D3 were reduced by 80%, 40%, 30% respectively among the subjects when compared to healthy controls.

**PS 1292 Acrolein Exacerbates HAART-Induced Apoptotic Death in Hepatocytes by Enhancing Transcriptionally Permissive Epigenetic Modifications at the Fasl Promoter**

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Highly active antiretroviral therapy (HAART) has significantly increased the life expectancy of patients with HIV infection. However, HAART-associated hepatotoxicity is becoming a major clinical problem leading to morbidity, mortality and treatment discontinuation in HIV patient. Environmental/dietary factors are known to significantly impact general health as well as therapeutic outcomes. Acrolein is one such common dietary and environmental pollutant and is also generated endogenously by cellular metabolism. The present study examines the interaction of acrolein with HAART drug, azidothymidine (AZT) in relation to the development of hepatotoxicity. Human hepatoma cells (HepG2) as well as rat primary hepatocytes were used to investigate the cytotoxic effects of acrolein (ACR) along with AZT treatment either individually or in combination (AZT+ACR). Data showed that acrolein sensitizes AZT treated cells to undergo enhanced apoptotic cell death. The underlying mechanism of increased apoptosis was increased expression of pro-apoptotic gene, Fasl, a major contributor to hepatocyte death. The induction of Fasl expression in response to acrolein and AZT combination was mediated by increased Fasl, promoter histone H3K9 acetylation leading to recruitment of NFκB and RNA Polymerase II and transcriptional activation. Notably, acrolein scavenger, hydralazine significantly attenuated acrolein induced transcriptionally permissive epigenetic modifications at the Fasl promoter preventing Fasl expression and hepatocyte cell death. Overall, the data suggest that environmental and/or dietary exposure to acrolein can induce chromatin modifications and further exacerbate the hepatotoxic effects of HAART medication affecting treatment options for HIV patients. This work was supported by NIH grants.

**PS 1293 P. gingivalis Modulates the Antiviral Immune Response in Oral Epithelial Cells**

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Polymicrobial bacterial and viral infections are common to multiple body compartments. Innate immunity represents the first line of defense against these invading microbial pathogens. It has recently been demonstrated that histone deacetylase (HDAC) inhibition is critical for down modulation of innate antiviral type 1 interferon gene expression. Previous studies from our laboratory, have determined that bacterial end products are capable of facilitating HDAC-associated epigenetic modifications and initiation of viral reactivation. Based on our findings, we hypothesize that bacterial metabolites decrease type 1 interferon antiviral responses thus enhancing viral pathogenesis in the setting of polymicrobial infection. 293 cells were transfected with Interferon promoter-luciferase reporter constructs. 293 cells, 2fTGH, and OKF6 (oral epithelial cells) were treated with P. gingivalis spent media or Wilkins-Chambers media alone. Elisa assay and immunoblot were used to determine modulation of Acetylated Histone 3, IFN-β, and/or STAT3 expression. One to 6 hours post treatment, bacterial spent media, dosed dependently, decreased the activation of the IFN promoter in a reporter assay. Following bacterial spent media treatment, there was decreased secretion of IFN-β from 2fTGH cells in the presence of bacterial spent media. However, at 24 hours post bacterial treatment of oral epithelial cells, immunoblotting detected enhanced histone 3 acetylation, enhanced expression of IFN-β that decreased with PG concentration and STAT 3 that increased with PG concentration. In conclusion, oral bacteria transiently suppressed the type 1 antiviral response then over time (24hr) enhance the response. These observations are important in the setting of oral polymicrobial infections and indicate that bacteria may significantly modify the host response to viral infection.
Reactive dicarbonyls, such as methylglyoxal (MG), are elevated in type-2 diabetes mellitus (T2DM) patients and the ability of dicarbonyls to covalently modify proteins contributes to a number of diabetic complications. The T2DM first-line drug metformin (MF) significantly reduces diabetes-related endpoints and mortality more effectively than other glucose-lowering medications. We have examined whether, in addition to its ability to reduce hepatic gluconeogenesis, MF directly scavenges dicarbonyls as an additional mechanism to reduce T2DM complications. We synthesized a MF/MG cyclized product (183 mw) and characterized the product by 13C, H and HMBC NMR (Bruker DRX-600), X-ray diffraction analysis (Bruker APEX-II CCD) as well as ESI-MS/MS mass spectrometry (MH+, 184 m/z; Agilent 6490). Using an LC-MS-based multiple reaction monitoring analysis we measured MF and the imidazolinone product (IMZ) in human urine with nM sensitivity of detection. The IMZ was detected in all MF-treated T2DM subjects analyzed to date. Subjects examined that have not taken metformin show no IMZ peak, as expected. Quantitation of IMZ in a cohort of >90 MF-treated subjects is ongoing, utilizing specific gravity normalization. The data reveal that urine from every T2DM patient treated with MF contains this IMZ product as a result of direct reaction with MG, and increased levels of MF correlate with elevations in IMZ. In addition to lowering hepatic gluconeogenesis, MF may play a role in scavenging the highly reactive MG in vivo. The role of the IMZ in the reduction of diabetic complications warrants further study. (DK090958, ABRC1115, T32ES007091, P30ES006694).

Mechanistic Role of PDE4/cAMP in Regulating HIV Drugs-Induced Fas/Fasl Expression and Hepatotoxicity

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Highly active antiretroviral therapies including HIV-protease inhibitors (HIV-Pis) are being successfully used in the treatment of HIV-1 infection. However, HIV treatment related liver injury can be a significant cause of morbidity, mortality and treatment discontinuation in HIV-infected patients. Hence, there is high clinical relevance to examine the mechanisms involved in HIV-PI-induced hepatotoxicity. Fas/Fasl mediated apoptosis is a significant mechanism that can contribute to hepatotoxicity and hence, was examined in HIV-PI induced hepatotoxicity. The combinatorial treatment of HIV-Pis, Ritonavir (R) and Lopinavir (L) (R+L), significantly up-regulated Fas and Fasl expression in H411RE rat hepatoma cells as well as primary rat hepatocytes. Since, our recent work showed that PDE4/cAMP metabolism plays a significant role in hepatic injury, we investigated its potential contribution to R+L induced Fas/Fasl expression. The data showed that in R-L treated hepatocytes there was a significant induction of PDE4, Fas and Fasl, mRNA and protein expression. Significantly, inhibition of PDE4 activity by a highly specific inhibitor, rolipram, markedly attenuated Fas and Fasl expression and prevented hepatocyte death. Furthermore, the role of PDE4 regulated, CAMP activated EPAC (Exchange Proteins Activated by cAMP) and PKA (Protein Kinase A) was also examined. Notably, suppression of Fasl expression afforded by PDE4 inhibition was significantly prevented by PKA and EPAC inhibitors. Moreover, inhibition of both EPAC and PKA exacerbated Fasl expression induced by HIV-Pis. These data strongly suggest that induction of PDE4 expression and a decrease in CAMP-dependent downstream signaling constitute the critical pathogenic mechanisms underpinning HIV-PI-induced Fas/Fasl expression and hepatotoxicity. Importantly, PDE4 inhibition can be an effective treatment strategy in HIV infected patients.

Zinc Reduces the Detection of THC by ELISA Urine Testing, While Copper May Cause a False-Positive Result


In today’s work place, many adulterants are used in an attempt to pass a routine drug test. Zinc sulfate has been observed to have such an adulterating effect on urine in occasional marijuana smokers and may cause a false negative in a standard urine drug test. Because zinc does not interfere with the integrity of the urine, it is an effective adulterant. This study focuses on testing the effect of zinc sulfate on THC levels in urine obtained from human subjects. Using a standard ELISA detection kit, THC levels appear to be reduced following consumption of zinc supplements, often enough to yield a false-negative result in a THC drug test. Higher concentrations of zinc in the urine samples were observed to have a stronger adulterating effect. Because zinc is known to cause cestovitis, we hypothesize that zinc supplementation may also affect urine excretion of cannabinoids, which may also affect standard ELISA drug testing. We are currently testing this hypothesis in both simulated conditions and in urine from human subjects.

Male B6C3F1 mice were given a weekly dose of 3 mg/kg doxorbacin (DOX; an anti-cancer drug) or saline via tail vein for 2, 3, 4, 6, and 8 weeks. Mice were euthanized one week after the last dose. Plasma levels of cardiac troponin T indicated cell injury at week 6 while cardiac lesions were observed at week 8. Metabolites in plasma were evaluated by LCMS and NMR and in cardiac tissue by LCMS. Biogenic amines, ciniline and ornithine of the urea cycle, putrescine, kynurenine, serotonin and 14 amino acids were increased in heart tissue at week 2 while 7 short chain acylcarnitines including carnitine were decreased in heart tissue at week 2. Citrulline, ornithine, 16 short, medium, or long chain acylcarnitines including carnitine, and 14 amino acids were increased in plasma at week 2. Twelve of the 14 amino acids were increased in both plasma and heart tissue at week 2, while 3 of 7 short chain acylcarnitines including carnitine were increased in plasma but decreased in the heart. Changes in acylcarnitines may be a signal of mitochondrial injury and altered capacity for beta oxidation of fatty acids. The increases in branched chain amino acids could be due to protein catabolism for producing energy. Putrescine is produced during the breakdown of amino acids while kynurenine and serotonin are produced from tryptophan. Levels of acetylornithine and arginine were significant...
cantly altered by DOX at all times in the heart while glycine and hexadecadienylcarnitine were altered in plasma at weeks 2–4. Transcriptomics of the heart tissue using whole genome and mitochondria-related genes were evaluated. Pathway analysis of omics data suggested protein catabolism, fatty acid metabolism, apoptosis and oxidative phosphorylation were altered at week 2, before plasma cardiac troponins were observed at 6 weeks. Metabolomics data were consistent with the transcriptomics data and helped understand pathways involved in DOX-induced cardiac injury. These findings also may aid in identification of potential early metabolomics biomarkers of cardiac tissue injury.

**1299 Solid-Phase Microextraction As Rapid Direct Postmortem Sampling Tool**


Postmortem drug concentrations do not necessarily reflect concentrations at the time of death due to the process of postmortem redistribution (PMR). As a consequence, postmortem concentrations are often difficult to interpret and may vary according to the drug involved, sampling site and time interval between death and sample collection. To investigate PMR, a solid-phase microextraction (SPME) method is developed and optimized for various pharmaceutical and illicit drugs.

With SPME as sampling tool, the sample concentration is not depleted, making time-dependent studies possible as the internal distribution and partitioning equilibria are not disturbed. The current SPME set-up employs a mixed-mode fiber, coated with hydrophobic C18 chains and a strong cation exchange phase. This fiber has an increased affinity for basic and neutral compounds, making it applicable in forensic samples with a pH of 5.5. This SPME method was tested under different conditions including changes in pH, ionic composition and temperature. Sorption of basic drugs to our SPME fiber is slightly altered with different pH and differences in ionic composition, due to the competitive effects of electrolytes. Also, temperature (420:37°C) has a small influence on sorption. Additionally, SPME was used to study free concentrations in solutions containing plasma proteins, from which protein binding and binding affinities could be calculated. The current SPME method is applicable in concentrations ranging from (sub)therapeutic to lethal concentrations of various drugs in blood and performs well in different media mimicking in vivo and postmortem circumstances. In future, this analytical tool will be applied to study the partitioning of drugs to proteins and cell membranes as well as the effect of postmortem changes on drug concentrations.

**1300 Arsenic Methylation Is Associated with Body Mass Index among US Adults**


Human exposure to arsenic has been associated with a wide array of adverse health effects including cardiovascular disease, diabetes, skin lesions, and cancers. Evidence suggests that methylation of inorganic arsenic to monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) is a critical pathway for urinary arsenic excretion. Body mass index (BMI) has been linked to arsenic methylation. However, limited human data are available on this issue. We tested the hypothesis that BMI may play a role in metabolic transformation of inorganic arsenic by examining the data from the National Health and Nutrition Examination Survey (NHANES, 2003-2010) among US adults aged 20 years or older. We assessed the relationship of BMI to urinary total arsenic and its metabolites including MMA and DMA, as well as the secondary methylation index (ratio of DMA to MMA in urine). Logarithmic transformations were performed to normalize the continuous data whenever necessary and regression analysis was used to examine the association of interest. Preliminary results suggest that higher BMI was inversely correlated to the ratio of MMA-to-total arsenic in urine (p < 0.001). Conversely, secondary methylation index (ratio of DMA to MMA in urine) was positively correlated to BMI. Similar findings were observed from multivariate-adjusted regression analyses (adjusting for age, gender, and race). The current findings support recent studies suggesting that BMI may play a role in arsenic methylation efficiency. Disclaimer: The views expressed in this abstract are those of the authors and do not necessarily represent the views or policies of the U.S. EPA.

**1301 Marketed Drugs with Nonclinical Testicular Toxicity and Concordance with Clinical Semenology Findings: A Survey in Pharmapendium™**


Testicular toxicity continues to be a challenging area for pharmaceutical safety assessment scientists for a number of reasons, including lack of accurate biomarkers and predictive screens, limited in vitro screening methods, poor understanding of mechanisms and uncertainty of concordance between animal models and humans. A survey of marketed drugs was done in Pharmapendium™ (1) to determine those with evidence of nonclinical testicular toxicity and (2) to evaluate the concordance between rodent, non-rodent and human findings. The “safety data search” function within Pharmapendium™ was used to tabulate (1) testicular toxicity and epididymal disorders and (2) spermatogenesis and semen disorders. The output included a list of approved drugs, testicular adverse effect, species affected, dose, route, and source document (hyperlinked FDA and/or EMA approval document or literature citation). The source documents and other literature references were searched for nonclinical testicular toxicity and clinical semenology/hormonal abnormally information. Semenology results and/or hormonal abnormally in clinical trials were compared to the nonclinical testicular toxicity findings. Of 743 drugs with an animal testicular toxicity and/or spermatogenesis/semen finding, there were 22, from a variety of pharmacological classes, which had clinical trials with semenology and/or hormonal investigations. Of these 22, 11 had effects on semenology and/or hormones in humans. For these 11 “human positives”, 4 were positive in rodent only, 1 was positive in non-rodent only, and 6 were positive in 2 or more animal species. For the 11 animal positives for which human semenology/hormonal assessments were negative, large exposure margins, shorter duration of exposure in man than in the animal studies, species specificity and/or the design of the clinical study could be factors.

**1302 The Effect of Crinkle Paper Nesting Material on Basic Toxicology Parameters**

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Nesting material for mice has been shown to be an ethologically relevant environmental enrichment, which reduces consequences of thermal (cold) stress, resulting in improved feed conversion and larger litters. We hypothesized that mice not provided nesting material are at risk of cold stress with consequential immune function parameters affected. A 90 day toxicity study was performed to assess the following parameters in a factorial design: Nesting (0g or 10g of crinkled nesting material); Drug (50mg/kg cyclophosphamide or 10ml/kg saline IP weekly) used by both sexes. Mice were housed in groups of 3 and data was averaged per cage (n=4; 32 cages total). Detailed examinations and body weights (BW) were performed weekly; clinical pathology and immunology parameters were collected at termination. Fecal pellets were collected at 0, 4, 6, and 12 weeks to analyze cortisol metabolites as a measure of stress. Male BW were highest in nested mice injected with saline and lowest in nested males injected with cyclophosphamide (p=0.05). No effect on BW was observed in females. There were no significant differences in hematology, clinical chemistry, or relative % of T lymphocytes between nested and non-nested groups. Relative % of B lymphocytes was increased (p=0.045) in groups provided nesting material, regardless of drug treatment. Males, but not females, that received nesting material had decreased fecal cortisol metabolites compared to non nesting groups (p=0.023). Metabolites were also reduced in nesting mice receiving saline injection (p=0.005). This study illustrates that access to 10g of nesting material does not interfere with clinical pathology parameters on a standard toxicology study, but may support immune function and buffer stress in male mice. These results suggest that nesting material provides improved welfare and may be an important enrichment consideration for conduct of immunotoxicology studies.
1303 Successful Combination of Scheduled Plasma Exchange with Continuous Veno-Venous Hemofiltration in Treatment of Fulminant Hepatic Failure Due to Ochratoxin A

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Aims: To describe a successful combination of scheduled plasma exchange (PEX) with continuous veno-venous hemofiltration (CVVHF) and other supportive therapies in treatment of fulminant hepatic failure due to ochratoxin A poisoning from an outbreak after in eating stale maize cake. Object: A 13-year-old boy live in Can Ty village, Quan Ba district, Ha Giang province, Vietnam. His family had 6 other victims eating stale maize cake (4 persons had died: 01 at home, 01 in the Can Ty district hospital, 02 in Ha Giang province hospital; 2 alive other persons had mild symptoms, who were followed and treated in Ha Giang hospital). Methods of collecting information: (1) Asking history from the patient, his family members, doctors and medical specialists who directly gave first aid and treated these patients, (2) Medical records of two death cases at Ha Giang province hospital, (3) Observed clinical manifestation and laboratory test at PCC Bach Mai hospital, (4) Ochratoxin A was detected in the patient urine by high-performance liquid chromatography. Conclusion: Poisoning from ochratoxin A in maize cake occurs commonly when eating stale maize cake at Quan Ba district, Ha Giang province, Vietnam. It was an acute disease, leading to fulminant hepatic failure, renal failure, even death in hepatic coma with gastrointestinal bleeding, other organ hemorrhage and multiple organ dysfunction syndromes. Early combination of scheduled plasma exchange with continuous veno-venous hemofiltration and other supportive therapy was an effective treatment for the case.

1304 Confidently Predicting Cardiac Liabilities in Drug Discovery, Applications of Conformal Prediction and Teaching Schedules

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Cardiovascular (CV) safety liabilities are a major cause of drug attrition in all stages of drug discovery and development. In early stages of drug discovery compounds can be screened to reveal potential ECG risk using in vitro assays of selected ion channels that regulate heart function, hERG (Kv11.1), NaV1.5, CaV1.2, Kv3.3 and Kv7.1. Inhibition of these ion channels is an early indicator of ECG risk so the assays can be used to rank compounds. Quantitative Structure-Activity Relationship (QSAR) models have been used to predict the outcome of these assays for specific compounds. In practice screening data has been preferred since such predictions generally lack quantifiable confidence. We have utilized the conformal prediction framework developed by Vovk, Gammerman and Shafer and applied it to CV QSAR models resulting in predictions with associated confidence. The framework uses past experience to determine precise levels of confidence in new predictions. Furthermore, the models have been evaluated using teaching schedules2 to mimic the drug development process. Teaching schedules give a good indication of how models behave with new data and can be used to describe the risk at any given point in time. We show that conformal prediction in conjunction with teaching schedules applied to QSAR is an effective way to deliver enhanced decision making capabilities to projects. Predictions with high confidence can reduce the number of tested compounds thus saving both money and time. As a consequence conformal prediction coupled with teaching schedules provides predictions with reliable confidence and a realistic view of the expected model performance over time. References: 1. Vovk V. Algorithmic learning in a random world 2005 Springer-Verlag NY. 2. M Ekeland, U Norinder, S Boyer, I. Carlsson, The application of conformal prediction to the drug discovery process, Sept 2013, Ann Math Artif Intell, Springer.

1305 Implementation of New Cardiac Proarrhythmia Safety Paradigms in the eTOX Prediction System


Current practice for an early assessment of torsadogenic drug candidates is based on in vitro studies, mainly hERG channel assay. The application of the ICH E15/S&B non-clinical guidance can be considered a success, since it resulted in no drugs with unrecognized risk being approved. Unfortunately, this protocol is too sensitive and has contributed to the withdrawal of many drugs candidates unlikely to be proarrhythmogenic. For this reason, a change in paradigm for assessing the proarrhythmogenic liability of new drugs has been proposed [1]. Here we present a multiscale in silico model for the prediction of proarrhythmic risk that incorporates some of the principles of the proposed new paradigm. It is a novel version of a model previously published by us [2], developed within the eTOX project [http://www.etoxproject.eu] with the aim of producing high quality predictions of in vivo toxicity of drug candidates. The core of our approach is a computational framework that simulates the effect of drugs on a virtual cardiac tissue composed by different types of cardiomyocytes, based on the O’Hara model. The input of this model, a set of three ion channel blockades (IKr, IKs and ICaL), can be obtained experimentally or predicted using advanced 3D-QSAR models. In order to facilitate risk assessment, the system allows the prediction of the drug effect at different drug concentration levels. The new system has been implemented in the integrated eTOX prediction system allowing easy prediction of the proarrhythmogenic liability of new drugs. The method has been validated by comparing the results of the proposed assessment with those produced by standard approaches yielding better results. 1. Sager PT et al. Rechanneling the cardiac proarrhythmia safety paradigm: a meeting report from the cardiac safety research consortium. Am Heart J. 2014; 167: 293-300. 2. Obiols-Pardo C et al. A multiscale simulation system for the prediction of drug-induced cardioxicity. J Chem Inf Model. 2011; 51: 483-92.

1306 In Silico Prediction of Off-Target Related Adverse Drug Effects: Cardiotoxicity, Hepatotoxicity, and Reproductive Toxicity

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Most drug candidates are active against more than a single target. Some inter-actions can lead to toxicology, such as the hERG potassium channel and the seroton receptor 5HT2B have been linked to severe cardiovascular side effects of drugs. Combined in silico in vitro off-target profiling strategies are most effective to identify critical liabilities in drug discovery. A stepwise approach is required to optimally support drug safety profiling. Computational models for numerous off-targets allow for systematic prediction of drug-target interactions. Target engagement in adverse pathways can be analyzed with pathway databases, supporting the toxicity hazard prediction and mode-of-toxicity evaluation of drugs. - The G-Link method for off-target prediction offers several algorithms to link new chemical samples to a curated reference set of several million biologically active samples. Prospective validation for in vitro assay data revealed a predictive accuracy of up to 81%. More than 4000 different protein targets can be predicted this way, each limited by an individual model applicability domain. - Quantitative QSAR models were built for > 400 important off-targets (200 kinases, >200 receptor/channel) in alignment to corporate in vitro panels. These models were extensively validated and the best have good r2/z2(cv)/q2 >= 0.6. - A biological network analysis approach, based on pathway databases (IPA®, MetaCore®, WikiPathways and Reactome), provides signals for potential drug toxicity. This approach links validated predictions to pathways that explain the observations exceeded a given threshold. In silico predictions help to design experimental follow-up studies and to enhance compound selection. Relevant application examples will be presented, focusing on organ toxicities (e.g. cardiotoxicity, hepatotoxicity and nephrotoxicity) and reproductive toxicity.

1307 Computational Comparison of the Anti-Inflammatory Targets of the Traditional Chinese Medicine Sargentodoxa Cuneata (Hong Teng) and Western Therapeutics for the Treatment of Osteoarthritis


Osteoarthritis is a major public health concern in any country with an aging population. Current Western therapeutics focus on relieving inflammation through the prostaglandin synthase (PTGS) pathways, with the most current drugs being PTGS2 selective. The PTGS2 selective drugs have a reduced risk of damage to the upper digestive tract, but carry a higher risk of cardiovascular incidents. After consulting with several Chinese medical professionals we found on an alternative methods of treatment, ie. Hong Teng (S. cuneata) a commonly used Traditional Chinese Medicine (TCM) that to date has not been reported to have the same side effects as Western NSAID therapeutics. Computational tools and databases used
to form a comparison of these two types of therapy included, TCM Database® Taiwan, Comparative Toxicogenomics Database, DrugBank, STITCH 4.0, and specific chemical-protein docking using Pharmmapper. Hong Teng consists of 40 phytochemicals. Eight proteins were shown to be common targets between the TCM and NSAIDs, and 4 (AKT1, BIRC, PTGS1, ALB) with known evidence for anti-inflammatory activity. We also found that Hong Teng is a modulator matrix metalloproteinase 2 and 9 (MMP2 and MMP9) enzymes. Matrix metalloproteinase enzymes are a large component of the degradation of cartilage, and therefore the onset and worsening of osteoarthritis. We believe that the phytochemicals in Hong Teng or newly discovered analogues may have a potential for future use in osteoarthritis treatment.

1308 Computational Analysis of Coffee Constituents and Their Potential Neuroprotection Action in Alzheimer’s Disease

Coffee has been shown to have a neuroprotective role against Alzheimer’s Disease (AD). Constituents evaluated for these effects include caffeine, chlorogenic acid and trigonelline. Our analysis indicates that another coffee constituent theophylline, synergizes with caffeine to increase plasma G-CSF. The increase in G-CSF/SCF3 induces JAK-STAT mediated PI3K-AKT signalling, NFE2L2/NRF2 antioxidant pathway, and the inhibition of the β- and γ-secretase, providing evidence of syner-gism in remediation of AD progression. This would be achieved by increasing proli-feration and differentiation of neurons, reducing oxidative damage and prevent-ing Amyloid β-plaque formation. We created a Chemical-Gene-Protein database within Cytoscape 3.1.0 compiled using database mining searches such as STITCH 4.0 and the Comparative Toxicogenomics Database. All reported chemical-pro-tein-gene interactions of coffee substituents and certain active metabolites were merged onto a shared network. We compared known interactions of individual components with their targets to determine novel interactions that may elucidate neuroprotective benefits. Chlorogenic Acid and Diterpenes (Cafestol/Kahweol) are known to initiate the antioxidant response by stimulating Type 2 Nuclear Receptors. Past research indicates that chlorogenic acid is an antioxidant and has also been shown to play a role in inducing microglial activation. Additionally, research performed on rat models have also indicated that chlorogenic acid has an inhibitory effect on acetylcholinesterase in the brain suggesting a possible mech-anism for it’s neuroprotective effect. Chlorogenic acid is transported across the blood brain barrier and induces transcription factor by binding NFE2L2, which is responsible for the cellular defense to oxidative stress. We believe that theophylline warrants further investigation and is a promising avenue of future research for understanding the mechanism behind coffee’s neuroprotective effect against AD.

1309 Computational Analysis of Active Phytochemicals and Potential Synergism with Western Therapeutics in the Treatment of Parkinson’s Disease
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Parkinson’s disease is the second most common neurodegenerative disease, and treatments have been complicated by undesired effects such as dyskinesia. Herbal formulations might be able to alleviate parkinsonian symptoms and re-place Western drugs to avoid the side effects. In this study we analyzed Zeng-xiao An-shenZhi-chan 2 (ZAZ2), a Traditional Chinese Medicine (TCM) recipe of 14 herbs shown to improve parkinsonian motor symptoms in a previous clinical study. We sought to further investigate the neuroprotective effects of component phytochemicals with a computational systems pharmacology/toxicology approach. We used TCMID and TCM database @ Taiwan to compile the list of active phytochemicals and screen for predicted mechanisms of action, including syner-gism with Western therapeutics, general neuroprotection, and improvement of pharmacokinetic profile of Levodopa, a drug often complicated with dyskinesia. Literature review, STITCH, ChemMapper, and PharmMapper were utilized to screen the chemicals with existing experimental evidence and predictive in silico structure docking. Using both literature and predictive approaches, we identified some important therapeutic targets, including caspase-3 and MAO-B. The power of prediction has been confirmed as the predicted targets for curcumin agree with the published results. Interactions with Levodopa were inconclusive, but we have shown that phytochemicals in ZAZ2 may be combined in a cocktail formulation to achieve synergistic effects and possibly replace the Western therapeutics. More im-portantly, the novel computational analysis of herbal medicines can be extrapolated to the field of TCM study and fill data gaps for newly discovered phytochemicals.

1310 A Computational Analysis of Ethnicity-Specific Polycystic Ovarian Syndrome Treatments Using Western and Traditional Medications
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Polycystic Ovarian Syndrome (PCOS) is the most common endocrine disorder found in women, and can lead to development of a number of reproductive and metabolic problems such as acne, hirsutism, infertility, and type 2-diabetes. As a worldwide disorder, PCOS can also affect women in different ways based on ethnicity, which plays an important role in phenotypic expression, making a gen-eralized treatment for PCOS in all women difficult. Because of this, we developed computational systems pharmacology/toxicology approaches to explore multiple 1st, 2nd, and 3rd line treatments. In addition, second-group treatments of herbal medications combined with dietary changes were identified that could encompass all ethnicities displaying PCOS as an overarching method of controlling various symptoms. Different software and databases such as STITCH, TCMID, yED, PharmGKB, DrugBank, SIDER, and KEGG were used to connect the two under-lying PCOS causes, insulin resistance and increased androgens, to molecular path-ways that could be targeted by different Western and traditional medications. To decrease androgens, CYP19A1 mediated inhibitors of 5α reductase were explored to lower dihydrotestosterone (DHT) levels and the mTOR pathway. To decrease insulin resistance, the activation of MAPK8 and PPARY signaling was explored. Different types of treatment were grouped with respect to ethnicity, and dietary implementation with the combination of herbal medications, such as palmetto, Korean angelica, and scutellaria baicalensis. These were combined and summa-rized with respect to controlling different symptoms of PCOS. Because PCOS is such a complex disease that can manifest itself differently in varying populations, we believe that choosing from more specific treatment plans will be more effective in controlling PCOS in women.

1311 A Computational Analysis of the Potential Environmental and Lifestyle Risk Factors Associated with Breast Cancer Incidence in South Napa, California
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A recent report from the California Breast Cancer Mapping Project shows four areas in California, where the age-adjusted incidence of invasive breast cancer (BC) appears to be 10-20% higher than the rest of the state. This study centered on the South Napa region where environmental and lifestyle factors could be studied in relation to extensive grape and wine producing areas. This included researching water sources/quality and pesticide use. Key sources used were the Napa 2010 Urban Water Management Plan, EPA Tri, the California Department of Pesticide Regulation and T3DB to identify compounds of concern. These included glyphosate, sulfate, 1,3-dichloropropene, and methyl bromide. The Comparative Toxicogenomics Database (CTD) was used to investigate gene-chemical interactions; key genes potentially associated with breast cancer included ARF4, BCL2L1, CXL12, CD63, EGR1, PP3CD, and TRAF4 which were primarily associated with glyphosate. Although biomonitoring studies have not been conducted in South Napa we used data from glyphosate Farm Family Exposure Studies where farmers, spouses, and children were monitored in South Carolina and Minnesota. In these studies subjects from all groups had detectable urinary levels of glyphosate; whereas exposures were estimated as below EPA’s reference dose for glyphosate. Absent from these studies are potential additive effects of multiple chemical expo-sures. Using a systems toxicology approach with other known chemicals in the area we discovered that glyphosate may have an additive estrogenic effect with resveratrol, a phytosterogen, found in grapes. In terms of lifestyle effects, a California Health Profile found that Napa has a higher rate of binge drinking and smoking compared to most other counties in California. The discoveries from our investigation have led us to infer that there are preventative measures that Napa county women can take that could potentially decrease their chance of developing BC.
Computational Investigation of the Combination of Traditional Chinese Medicine (TCM) and Western Therapeutics for the Treatment of Non-Small Cell Lung Cancer (NSCLC)

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Standard treatments for NSCLC include EGFR inhibitors (eg, erlotinib, afatinib) and chemotherapeutics (eg, cisplatin). Several patients also use TCM alone or in combination to reduce side effects and/or boost the immune system. Since Jin Fu Kang (JFK) is a TCM formulation that has been studied in clinical trials, we conducted a computational systems pharmacology/toxicology analysis of JFK and its constituents to explore both potential positive and negative interactions with Western therapeutics. Databases and tools used included KEGG, TCIM Taiwan, TCIM, CTD, MyGeneVenn, DrugBank, SIDER, STITCH, and yED. A disease pharmacology network was established using the 5 drugs above, and pharmacometrics in JFK were incorporated. Significant direct interactions were found between 22 of the 43 important phytochemicals within JFK and important protein targets of the Western Therapeutics including metabolic enzymes, transporters, and therapeutic targets. Several potentially counter interactions were apparent such as activation vs. inhibition of CYP enzymes, UG1A1, and ABC transporters. Additionally, a counter interaction concerning pharmacological activity was apparent with the effect of Quercetin which acts in an opposing manner to afatinib and erlotinib by activating EGFR; however, it also decreases the transcription of ATP7A, which in excess can cause a resistance to platinum-based drugs such as Cisplatin. One of the primary uses of TCM in cancer patients is supplementation to increase their immune systems and three herbs found in JFK have been shown to boost immunity. These include Ligustrum lucidum, Trigona foemina graecus, and Astragalus membranaceus. It is concluded that using JFK in conjunction with chemotherapy may help alleviate drug side effects such as decreased immunity and increased susceptibility to infections. We have shown that a computational systems pharmacology/toxicology approach using open access tools can be a useful method for both professionals and patients to explore combination treatment regimens.

Integrated Panel of QSAR Models Representing Key Events in Skin Sensitization


Adverse outcome pathway (AOP) for chemical-induced skin sensitization response has been well characterized. Multiple in vitro systems have been developed to reflect its different stages and, as such, to provide alternative to animal tests for the assessment of skin sensitization potency. Consequently, substantial body of experimental data has been generated from in vitro assays and should be further generalized by application of computational techniques. In this study we report Quantitative Structure-Activity (QSAR) models developed for the major stages in skin sensitization AOP using CASE Ultra expert system. The models were trained on data from relevant in vitro assays as well as on historical data from in vivo tests. The resulting panel of models includes reactivity to skin proteins, oxidative stress response, denitrictic cell activation, and T-cell proliferation, as well as the ultimate allergic contact dermatitis. External cross-validation (10-fold) for these models achieved 67-87% accuracy (models based on stricter potency thresholds tended to be more accurate). The developed models can be used as a part of the integrated testing strategy for early assessment of skin care products, topical drugs and environmental chemicals. We demonstrate computational screening applications of the reported models on chemicals with skin route toxicity from RTECS and ChemID databases, as well on cosmetic substances from CosIng database of European Commission.

Updated to an Integrated Testing Strategy for Skin Sensitization Potency


Scientists at the Procter & Gamble Company and the NTP have developed an integrated testing strategy (ITS) for skin sensitization potency. The ITS is based on a Bayesian network and uses in silico and in vitro models that map to the OECD adverse outcome pathway for skin sensitization. The ITS predicts four skin sensitization potency categories, based on LLNA results (nonsensitizer, weak, moderate, strong). The ITS model can determine, based on the existing data for a substance, which additional data are the most important for decreasing the uncertainty of the skin sensitization potency prediction. Such a strategy maximizes efficiency in determining potency and adheres to 3Rs concepts because it requires no animal testing. We recently revised the model, referred to as ITS-3, to (1) add more physiochemical parameters that affect skin penetration; (2) include % plasma protein binding, which affects the in vitro response; (3) replace the U937 test with the human cell line NCI-H1299, which has been validated; and (4) consider variability in the LLNA response. The ITS-3 model was trained on 134 substances and tested on 33. Five predictions in the test set were inconclusive and, upon further investigation, out of the ITS domain (oAD). For the remaining 28, overall accuracy was 89% (25/28). Performance by category: for 9 nonsensitizers, 9 correct; for 7 weak: 1 inconclusive, 5 correct, 1 class off; for 8 strong: 2 oAD, 5 correct, 1 class off. For perspective, the overall accuracy for the ITS-2 model (with a different test set) was 86% (18/21). Performance by category: for 6 nonsensitizers, 6 correct; and for 5 weak sensitizers, moderate sensitizers, or strong sensitizers, 4 correct, 1 class off. The ITS-3 model will be reproduced using open source software and made available on the NTP website at http://ntp.niehs.nih.gov/its/. This project was funded in whole or in part with Federal funds from the NIEHS, NIH under Contract No. HHSN273201400003C.

Impact of Local Lymph Node Assay Uncertainty on Predictions of a Bayesian Network Integrated Testing Strategy for Skin Sensitization Potency


As toxicity testing moves away from traditional animal models towards cell-based assays and in silico methods, computational models integrating such data are being developed and improved. An example is the Bayesian network (BN) model used to predict local lymph node assay (LLNA) potency classification of substances in the NICEATM LLNA database. Datasets used to build such models may include multiple values for some combinations of assays and compounds. Using standard Bayesian network methods, it is difficult to build a model that makes use of all the available data. Instead, the data are either collapsed or selected from to produce a single value, which eliminates all distributional information. Using a published BN integrated testing strategy (ITS-2) for skin sensitization, we compare predictions of the original model and those of two methods that incorporate multiple LLNA values. In the first method, the potency class probabilities assigned by the BN are modified using an empirically derived conditional distribution. In the second method, Markov chain Monte Carlo is used to calculate results for a large number of BNs generated under distributional assumptions on the LLNA variable. This method propagates the uncertainty through all model building steps. On a test set of 21 compounds, agreement on the most probable class prediction was 81% for the two new methods, 80% for the original BN ITS and the second method, and 95% for the original model and the first method. The most probable class predictions were similar, but the distributions of the predictions differed. These more transparent methods enhance risk assessment by describing the uncertainty from the data and the model and better represent the reliability of the predictions. This project was funded in whole or in part with Federal funds from the NIEHS, NIH under Contract Nos. HHSN273201400005C and HHSN273201400006U.

Assessing Skin Sensitization Potential by Combining Multiple Information Types (Chemotype Alerts, QSAR, Chemical Reactivity and Metabolite Generation, and Biological Assay Data) in a Quantitative Weight-of-Evidence Approach

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We present a workflow for evaluating skin sensitization potential in which evidence from multiple sources is rigorously and quantitatively combined to arrive at a weight-of-evidence prediction with associated estimation of uncertainty. In silico approaches, based on chemical structure and physicochemical properties are combined with experimental reflecting key events of the adverse outcome pathway (AOP). Covalent modification of proteins represents the molecular initiating event (MIE). ToXPrint chemotypes, structural fragments encoded with physico-chemical properties and electronic system information, were used to categorize chemicals into MIE classes, including: alkyd halides, carbohydrates, Michael acceptors, Schiff base formers, and phenols. Skin metabolic rules, also coded in chemotypes, were developed and added to the workflow to include pre- and prohaptens. Predictive QSAR models were developed from these descriptors and a number of chemical type alerts were identified. While structure- and property-based in silico methods...
are suitable for molecular events, they are somewhat limited in their ability to adequately address subsequent biological processes. Experimental data from biological assays relevant to the AOP were therefore included in the workflow: DPRA (direct peptide reactivity assay), KeratinoSens and LuSens (activation of Keap1/Nrf2 signaling pathway), and h-CLAT (desensitized cell activation). The results of these biological assays enhanced the reliability and reduced the uncertainty of skin sensitization predictions; for binary hazard prediction, a sensitivity of 90% and specificity of 85% was achieved. Even for compounds correctly predicted by the in silico approach alone, integration of biological assay data reduced the uncertainty of the prediction.

PS 1317 Verification of a Skin Sensitization Assessment Neural Network Model by Fragrance Materials

[Objects] In cosmetics development, the assessment of the skin sensitization potential is very important and it has mainly been assessed using animal tests for example murine local lymph node assay. Recently many skin sensitization alternative methods are developed, and some of them are evaluated as an OECD guideline. Moreover, the risk assessment by the combination of some in vitro tests have been also reported. The interest in the risk assessment is increasing. We have developed a prediction model of skin sensitization using artificial neural network (ANN) based on h-CLAT, DPRA and KeratinoSensSM as a result of the collaborative study with P&G, Givaudan and Kao. In this study, we verified this model using a dataset of fragrance materials which were newly acquired. [Methods] The ANN prediction model was constructed with a dataset (a training dataset) of 134 compounds. A dataset of 36 fragrance materials, which were not used for construction of the model, was used to verify the model as a testing dataset. [Results and Discussion] Among 80% or more fragrance materials, the difference between actual EC3 values and predicted EC3 values was less than 10 times. This result suggested that the ANN model constructed with general compounds could also be useful for evaluating fragrance materials in terms of risk assessment. [Acknowledgement] We thank the working group of skin sensitization evaluation in Japan Cosmetic Industry Association for providing data from the collaborative study. We also thank P&G, Givaudan and Kao for providing data from the collaborative study. [Reference] Hirota et al., WCC, Session 2.12B-160, 2014

1319 From Individual Datasets to Big Data: Developing Mechanism-Driven Predictive Liver Toxicity Models
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High Throughput Screening studies have provided the scientific community with rich toxicity data that is currently so complex that it is difficult to process using traditional data analysis techniques. However, all toxicity-related studies, including bioassays retrievable through big data sources, should be evaluated as a possible alternative to animal tests. The goal of this project was to develop novel computational approaches and predictive oxidative stress-induced liver toxicity models using publicly available big data sources. Since compounds that are both biologically and chemically similar are considered likely to have similar toxicity mechanisms/effects, their response profile was used to predict animal toxicity and/or prioritize toxicants for in vivo tests. This was done using a Weighted Estimate of Biological Similarity (WEBs) tool, which uses the toxicity pathways constructed from the biological and chemical response profiles to calculate the similarity between two compounds. One of the major causes of liver toxicity is oxidative stress induced by electrophilic compounds. Therefore, assays related to oxidative stress, such as the Antioxidant Response Element reporter gene assays, were used to develop mechanism models for liver toxicity. The validation procedure showed that the resulting models have high predictivity (Correct Classification Rate=0.6-0.85) for hepatotoxicity, especially for electrophilic compounds with certain structural features. The mechanism profiles and predictive models can be used to predict the toxicity of environmental compounds (e.g., the Tox21 library). Furthermore, this study illustrates the benefits of using multiple toxicity bioassays in the current big data era.

1318 Cheminformatics Approaches to Tailor In Silico Profilers for Refined Category Formation to Support Chemical Safety Assessment
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Chemical safety assessment is increasingly supported by cheminformatics methods. In particular, the grouping of chemicals into categories allows for data gaps to be filled via read-across. In order to form meaningful categories, it is crucial to define chemical and biological similarity robustly. Consideration of mechanistic information is important, where possible anchored by Adverse Outcome Pathways. Based on organic reaction mechanisms, structural fragments have been defined previously to predict potential covalent binding to proteins or DNA, key Molecular Initiating Events for endpoints such as skin/respiratory sensitization, genotoxicity and organ toxicity, e.g., hepatotoxicity. In this study, they have been refined to chemotypes, the next generation of structural alerts: a more flexible format allowing for the inclusion of physicochemical property information or even quantitative structure-activity relationships. The advantage of the coding of these properties is the extension of similarity-based grouping beyond structural information to form improved robust categories and tailors the profilers to specific applications. A total of 219 chemotypes have been developed (freely available at ToxPrint.org) and applied to inventories such as the COSMOS Cosmetics Inventory (>5,000 with defined chemistries-related structures, based on EU CosIng and US PCPC lists) and eRepeatToxDB (in vitro oral repeated dose toxicity database for 228 cosmetics-related compounds). The substances were grouped into mechanistic categories to compare the chemical/biological space. More hits were obtained for DNA than protein binding alerts in both datasets, Michael addition and S01 prevailing, respectively. The coding of molecular weight and logP calculations into the chemotypes allows in addition an estimation of the skin permeability, especially relevant for cosmetics safety assessment, to be taken into consideration. Supported by the EU FP7 COSMOS Project.

1320 Predicting Hepatotoxicity Using ToxCast In Vitro Bioactivity and Chemical Structure
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The U.S. EPA ToxCastTM program is screening thousands of environmental chemicals for bioactivity using hundreds of high-throughput in vitro assays to build predictive models of toxicity. A set of 677 chemicals were represented by 711 bioactivity descriptors (from ToxCast assays), 4,376 chemical structure descriptors, and three hepatotoxicity categories (from animal studies), then used supervised machine learning to predict their hepatotoxic effects. Hepatotoxicants were defined by rat liver histopathology observed after chronic chemical testing and grouped into hypertrophy (161), injury (101) and proliferative lesions (99). Classifiers were built using six machine learning algorithms: linear discriminant analysis (LDA), Gaussian Naive Bayes, support vector machines (SVM), classification and regression trees (CART), k-nearest neighbors (KNN) and an ensemble of classifiers (ENSEMB). Classifiers of hepatotoxicity were built using chemical structure, ToxCast bioactivity, and a hybrid representation. Predictive performance was evaluated using 10-fold cross-validation testing and in-loop, filter-based, feature subset selection. Hybrid classifiers had the best balanced accuracy for predicting hypertrophy (0.78±0.08), injury (0.72±0.10) and proliferative lesions (0.72±0.09). CART, ENSMB and SVM classifiers performed the best, and nuclear receptor activation and mitochondrial functions were frequently found in highly predictive classifiers of hepatotoxicity. ToxCast provides the largest and richest data set for mining linkages between the in vitro bioactivity of environmental chemicals and their adverse histopathological outcomes. Our findings demonstrate the utility of high-throughput assays for characterizing rodent hepatotoxicants, the benefit of using hybrid representations that integrate bioactivity and chemical structure, and the need for objective evaluation of classification performance. This abstract does not represent EPA and FDA policy.

1321 Analysis of Human and In Vivo Data for Hepatotoxicity Modelling

Drug induced liver injury (DILI) is one of the major causes of compound failure during late stage development, and in the withdrawal of marketed drugs. The complexity of mechanistic causes of toxicity observed in in vitro or in vivo assays presents a challenge for the development of in silico prediction methodologies. We analysed a repeat dose dataset to ascertain if relationships between reported
Drug-induced liver injury (DILI) has been a frequent cause of safety-related withdrawal of drugs from the market over the past 50 years. Hepatotoxicity is also a factor in the failure for continued drug development during R&D. Often, hepatotoxicity is not detected until later stages of drug development, at which point significant time and resources have been expended. In silico screening for hepatotoxicity provides a fast, high-throughput, and low-cost alternative to in vitro and in vivo testing without the need for test materials or animals. The purpose of this study was to evaluate and validate the ability of in silico models to predict hepatotoxicity. Initially, 149 marketed drugs were screened by in vitro methods with hepatocytes to classify compounds as DILI or non-DILI. Of the 149 compounds, 83 were identified as DILI and 66 were identified as non-DILI. After classifying the compounds, all 149 drugs were evaluated in silico using three different computational programs: Derek Nexus, Leadscope, and MultiCASE. Each program evaluated the compounds by various endpoints associated with hepatotoxicity, including liver damage, liver enzyme abnormality, and jaundice. Lisha Derek Nexus analysis showed 57% and 85% concordance with DILI and non-DILI drugs, respectively; Leadscope showed 54% and 86% concordance with DILI and non-DILI drugs, respectively; and MultiCASE showed 70% and 44% concordance with DILI and non-DILI drugs, respectively. By combining the data from the three in silico programs, there was 98% and 91% concordance with DILI and non-DILI drugs, respectively. Across all three in silico programs, there were 3 DILI compounds and 3 non-DILI compounds that were incorrectly classified. The most common class of compounds that were incorrectly classified was antibiotics (2 of the 4 compounds). Overall, predictability of hepatotoxicity in silico is best when multiple programs are used together, and antibiotics may be a difficult class of compounds to correctly predict hepatotoxicity using in silico methods.
1326 Adverse Outcome Pathways for the Nephrotoxicity of Nonsteroidal Anti-Inflammatory Drugs

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Adverse outcome pathways (AOPs) describe the mechanistic link between a molecular initiating event and an adverse toxicity outcome. Non-steroidal anti-inflammatory drugs (NSAIDs), a class of compounds categorized as one of the most toxic in the FDA CDER urantr tract toxicity dataset, were investigated to identify the mechanisms leading to renal toxicity. Literature review returned several structural sub-classes of NSAID for development into toxicity alerts. Each structural sub-class was evaluated for publicly available in vitro reports of nephrotoxic events in man and other mammals. The harvested information was analyzed and synthesized into knowledge to include plausible mechanisms for the renal toxicity of NSAIDs. Six structural toxicity alerts describing the nephrotoxicity of NSAIDs were implemented in the Derek Nexus knowledge base. These alerts were validated against the FDA CDER urinary tract toxicity dataset, which is based on the post-marketing adverse events of 1609 chemicals in man. This showed a positive predictivity of between 50–100% for each of the implemented alerts. Tentative AOPs to describe the in vivo mechanisms of renal toxicity for the generalized NSAID class were developed which show a dependence on the inhibition of the cyclooxygenase (COX) enzyme in renal tissues. These AOPs are particularly relevant in those individuals with underlying ‘at-risk’ physiology. As such, COX inhibition leads to the development of several nephrotoxic events, including acute renal failure and renal papillary necrosis. The mechanistic AOPs proposed may aid read-across to other chemical classes which act through mechanisms analogous to those described.

1327 Understanding Oxidative Stress Responses via Nrf2-Related Pathways and ToxCast Data

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Reactive oxygen Species (ROS) and oxidative stress (OS) are associated with adverse outcomes such as immune suppression, cancer, aging, rheumatoid diseases, cardiovascular disease and Parkinson’s and Alzheimer’s diseases. OS is associated with an array of structurally diverse chemicals and may proceed by a variety of toxicity pathways. An important element in OS response is the Nuclear factor (erythroid-derived 2)-like 2 (Nrf2) leucine zipper (bZIP) transcription factor. Nrf2 degradation, and localization controls the transcription of many OS-response genes. This analysis aims to map the Nrf2-mediated OS response pathways via the ToxCast database, and related bioassay response patterns to chemical classes. ToxCast phase II contains high throughput toxicity data for 1,865 commercial chemicals, pesticides, and pharmaceuticals. The effects of these compounds on gene and protein expression in multiple cell lines is evaluated via 700 bioassays. The unprecedented collection of toxicological data is ideal for in silico elucidation of toxicity mechanisms and AOPs. We identified all ToxCast assays pertaining to the Nrf2 OS-response pathway and evaluated the relationships within the data. Several bZIP transcription factors exhibit similar patterns of expression. Furthermore, the activity of chemicals activating multiple bZIP factors tend to lie outside the region of general cytotoxicity and burst effects. The patterns of pathway-specific gene and protein expressions collected from ToxCast and associated structural information will help unravel the diverse network of OS toxicity pathways.

1328 Association between Health Effects and Chemical Structure of Environmental Pollutants

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Agency for Toxic Substances and Disease Registry (ATSDR) develops Minimal Risk Levels (MRLs) for hazardous waste substances (HWS) as mandated by the Comprehensive Environmental Response, Compensation, and Liability Act and amended by the Superfund Amendments and Reauthorization Act [9 U.S.C. 9604 et seq]. MRLs are based on critical health effects that affect target organs. Because in vivo animal or human studies that support MRLs exist only for a fraction of HWS, ATSDR also develops cross-extrapolation Quantitative Structure-Activity Relationship (QSAR) techniques in order to fill data gaps in the database of adverse health effects caused by exposures to HWS. These models numerically extrapolate from available tested chemicals to untested ones based on structural similarity/distance, specific to the descriptor space of the model. Previously we found that reliability of extrapolation correlates with pairwise distance metric between compounds, however, it was unclear why. In the present study we tested if the same distance metric may be associated with toxicant-induced health effects in target organs. Using a set of 123 chemicals with well-established oral chronic toxicity in rats, we found that similar chemicals (within the first 25% of the maximum pairwise distance) also shared similarity in affected target organs. The greater the similarity between chemicals, the greater was the effect. This result suggests scientific foundation for QSAR modeling of MRLs (and other health-guidance values) using diverse hazardous chemicals. Furthermore, using a similar approach the full range of health effects and target organs summarized in the Levels-of-Significant Exposure tables of ATSDR toxicological profiles can be examined.

1329 Acute Oral Toxicity Modeling Accounting for Mechanism and Toxicological Mode of Action

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Based on a category approach, quantitative structure activity relationships (QSAR) were developed for acute oral toxicity (AOT) of chemicals (in rats). Chemicals were grouped in different toxicological categories based on their chemical interaction mechanism and toxicological mode of action. Analyzing the toxic potency of chemicals, two general types of toxicity were identified: basic and event-specific. Basic toxicity is considered as the minimum toxicity caused by non-reactive chemicals. The toxicity of chemicals destroying some critical processes in the cells or the whole organism deviates from the basic toxicity. These chemicals revealing an excess toxicity usually have highly reactive groups or act by specific interaction mechanism. Solubility parameters were found to correlate adequately with the potency of these chemicals. However they can also reveal just basic toxicity because of their limited bioavailability. Fast hydrolyzing chemicals having well known highly reactive groups have been analyzed more closely. It was found that AOT could be significantly reduced due to abiotic or biotic hydrolysis. Alteration of physical and chemical properties due to abiotic hydrolysis is expected to affect toxicity of chemicals including AOT. As a result abiotic (biotic) hydrolysis of target chemicals are now being simulated within the AOT modeling system. This AOT model is implemented in the TIMES platform. Currently it contains about 2500 training chemicals classified in 67 toxicological categories. This model overcomes the limitations of current models by integrating multiple but specifically derived QSAR models for each toxicological category. Moreover all predictions are to be supported by mechanistic justification for the mode of action, example chemicals and an applicability domain indication. Future work will include expansion of the training set and thus creation of additional toxicological categories.

1330 High-Content Screening of ToxCast Compounds Using Vala Sciences’ Complex Cell Culturing Systems

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US EPA’s ToxCast research program evaluates bioactivity for thousands of chemicals utilizing high-throughput screening assays to inform chemical testing decisions. Vala Sciences provides high content, multiplexed assays that utilize quantitative cell-based digital image analysis. Twelve assays, with a wide variety of early response targets, were selected for analysis in ToxCast and initially tested across the 311 ToxCast Phase I chemicals. Targets include those important in embryonic stem cell differentiation, neuronal function, pancreatic beta cell differentiation, germ layer proliferation, adipogenesis, adipocyte lipolysis, hepatic steatosis, and toxicological mode of action. Early response targets in this data set provide toxicological and mechanistic insight to complement other ToxCast assays leading to stronger predictions. Collectively, these results add important data to ToxCast
that will help identify and prioritize possible developmental toxicants, endocrine disruptors, liver toxicants, and carcinogens to inform target testing strategies. This abstract does not necessarily represent U.S. EPA policy.

1331 Computational Modeling of Thyroid Hormone Regulated Neurodevelopment for Chemical Prioritization


Thyroid hormones (TH) are critical for normal brain development. Environmental chemicals may disrupt TH homeostasis through a variety of physiological systems including membrane transporters, serum transporters, synthesis and catalytic enzymes, and nuclear receptors. Current computational models provide rich descriptions of some aspects of TH regulation and transport, but do not address the complex cellular dynamics invoking downstream adverse outcomes. The goal of the current research is to develop a sophisticated computational model that predicts chemical effects on known signaling pathways of TH-mediated brain development coupled with estimated chemical exposures. The model has two major components: (1) a biologically-based dose-response/physiologically-based toxicokinetic model used to calculate TH levels in both the euthyroid state and in the presence of xenobiotic thyroid disrupting chemicals; and (2) fetal TH levels were used as input into a cell-agent based model where TH levels regulate endodermal, glial, and neuronal interactions necessary for brain development. Chemicals used in the model included positive hits from ToxCast/Tox21 TH receptor and thyroid peroxidase assays. The model outputs the daily dose required to activate biological targets for both rat and human. These doses were compared to exposure estimates generated from ExposureLower (Wambaugh et al., 2013) for the majority of chemicals, the predicted exposure levels were much lower than doses predicted to trigger biological activity. Future work will include characterization of chemicals with both low and high margins-of-exposure to further validate the model. Computational modeling that combines both hazard and exposure hold great promise in facilitating efficient screening of thyroid disrupting chemicals. This abstract does not necessarily reflect the policy of the US EPA.

1332 Causal Inferences from Mining ToxCast Data and the Biomedical Literature for Molecular Pathways and Cellular Processes in Cleft Palate


Sixty-five chemicals in the ToxCast high-throughput screening (HTS) dataset have been linked to cleft palate based on data from ToxRefDB (rat or rabbit prenatal developmental toxicity studies) or from literature reports. These compounds are structurally diverse and thus likely to perturb prenatal development in mechanistically diverse ways. Integration of the HTS in vitro profiling data with information from chemotype profiling and automated literature survey provides a generalizable approach for adverse outcome pathway (AOP) elucidation. We generated a heatmap by clustering the 65 chemicals using as attributes 204 ToxCast Gene Scores and 233 chemical structural elements (chemotypes). Hierarchical relationships in the heatmap revealed several coherently bioactivity-chemotype clusters. For example, the conazolexol, retinoids, and phthalates formed clusters, as did the chemicals that hit GPCRs, angiogenic targets, and neuro-active targets. Identification of cohesive clusters enables focused literature mining for cellular and tissue effects linked to the ToxCast targets, and extension and enhancement of AOPs for cleft palate. This abstract does not necessarily represent U.S. EPA policy.

1333 Integrated, Multiscale Analysis of Behavioral and Morphological Data from High-Throughput Screening of Environmental Chemicals in Developing Zebrafish

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We describe an integrated analysis of behavioral and morphological data from high-throughput screening (HTS) of all 1060 ToxCast™ chemicals across 5 concentrations in an in vivo zebrafish system. For the in-vivo measurements of behavior (spontaneous movement in response to a series of light pulses) and mortality observed at 24 hours post-fertilization (hpf) plus measurements of behavior (movement in light/dark conditions) and morphology (18 specific endpoints) observed at 120 hpf. We develop a novel, visual analytic for summarizing chemical-elicited effects that integrates all data. We use this analytic to address the relationships between early (24 hpf) and later (120 hpf) measurements, as well as characterizing connections between behavioral abnormalities and observed morphological endpoints. Comparing behavioral data, we find more unique chemicals deemed active at 120 hpf (254 chemicals) versus 24hpf (110 chemicals). While this may be expected given the physiological developments occurring between 1 and 5 days, the sensitivity to detect morphological endpoints when considering both behavioral assays was significantly higher than either alone, suggesting that including both adds value within an integrated testing strategy for diverse compounds. These results present a systematic characterization of behavioral and morphological endpoints associated with chemical exposures in a model of vertebrate development. Importantly, 42% of these chemicals have never been tested in any duration of guideline, in vivo study, so these integrated results can provide a starting reference for characterizing the whole-organism, developmental hazard of all ToxCast™ chemicals.

1334 Hazard Evaluation Support System (HESS): Development of a Category Approach to Predict the Testicular Toxicity of Chemical Substances Structurally Related to Ethylene Glycol Methyl Ether

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We propose a category approach to assessing the testicular toxicity of chemicals with a similar structure to ethylene glycol methyl ether (EGME). Based on toxicity information for EGME and related chemicals and accompanied by adverse outcome pathway information on the testicular toxicity of EGME, this category was defined as chemicals that are metabolized to methoxy- or ethoxyacetic acid, a substance responsible for testicular toxicity. A Japanese chemical inventory was screened using the Hazard Evaluation Support System (HESS), which we have developed to support a category approach for predicting the repeated-dose toxicity of chemical substances. Quantitative metabolic information on the related chemicals was then considered, and seventeen chemicals were finally obtained as a shortlist for the category. Available data in the literature shows that five chemicals for which information is available on the metabolic formation of EGME, ethylene glycol ethyl ether, methoxy- or ethoxyacetic acid do in fact possess testicular toxicity, suggesting that testicular toxicity is a concern, due to metabolic activation, for the remaining chemicals. Our results clearly demonstrated usefulness of AOP-based category approach for hazard assessment of chemicals. HESS can be downloaded free of charge from the website of NITE (https://www.safe.nite.go.jp/english/kasinn/qsar/ Tess/hess.e.html).

1335 Using ToxCast/Tox21 Assays and QSAR Modeling to Predict Androgen Receptor Pathway Activity


The Tox21 and ToxCast programs include in vitro assays conducted in a high-throughput screening (HTS) format. Many are relevant to the androgen receptor (AR) pathway and can identify substances with potential androgenic/anti-androgenic activity in vivo. Here we used nine of these assays to build a mathematical model to distinguish true AR pathway activity from technology-specific assay interference. The assay battery probed perturbations of the AR pathway at multiple points (receptor binding, coactivator recruitment, gene transcription and protein production) in multiple cell types. We compiled a list of putative AR reference chemicals from the ICCVAM and OECD reference chemical lists. Chemicals included agonists, antagonists, selective androgen receptor modulators (SARMs), and inactive chemicals. The model showed 96% (23/24) concordance with reference data, including successfully identifying multiple SARMs with both agonist and antagonist activity. The model identified as agonists or antagonists all chemicals in the Tox21 panel, except for two specifically targeted AR. However, bupropion, a SARM, was active in the coactivator recruitment assays but none of the other AR pathway assays, and was therefore mispredicted by the model as acting via an assay-specific interference pathway. We will discuss patterns of assay activity and pathway predictions across 1846 ToxCast chemicals and identify those predicted to be active against the AR pathway. The results from the AR pathway model were used to train and build a cross-validated quantitative structure activity relationship (QSAR) model for AR binding and used to make predictions for 30,000 chemicals. Where available, we compared in vitro and in vivo predictions to toxicity data from the website of NITE (http://www.safe.nite.go.jp/english/kasinn/qsar/ Tess/hess.e.html).
In Vitro/In Silico Approach to Address Safety Concerns of Zearealenone Metabolites

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Within the framework of reduction, refinement and replacement of animal experiments, new approaches for the identification characterization of health hazards of chemicals have been developed. The Adverse Outcome Pathway (AOP) approach is increasingly acknowledged as a tool for expanding the use of mechanistic data in risk assessment. AOP gives a framework to relate information linking a Molecular Initiating Event (MIE) to adverse effects. Toxicity studies have identified estrogen receptor binding as the MIE of the estrogen mycotoxin zearealenone (ZEN), a common food and feed contaminant. ZEN is extensively metabolized; those formed by reduction have various estrogenic potencies, while the estrogenic behavior of the oxidized metabolites is as yet uncharacterized. An in vitro in silico approach was applied to determine the level of concern for ZEN and its metabolites in two steps: i) an in silico model (by docking and re-scoring procedures) was developed and validated using in vitro data (ERTX-CALUX® and ERTX-Redistribution assay) for the commercially available ZEN metabolites; ii) the validated in silico model was applied to rank the uncharacterized oxidized metabolites (not commercially available). From a close analysis of the docking poses (ligand-receptor interaction) a Structural Alert for binding to ERTX was built for ZEN metabolites. It contains a schematic representation that highlights the functional groups required for the estrogenic activities of ZEN-metabolites. This work gives an example of how to apply the Integrated Testing Strategies (ITS) to reduce animal testing in risk assessment and it highlights the utility of similarity searches driven by MIE to identify analogs for read across in hazard characterization.

Effectopedia: An Open Collaborative Platform for AOP Development and Application

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Effectopedia is a knowledge aggregation and modeling platform designed for collaborative development and utilization of Adverse Outcome Pathways (AOPs). Aimed at a broad range of users, Effectopedia has a visually expressed modular structure which captures semantically annotated knowledge, computational models, algorithms, and supporting evidence. Knowledge is organized in nested layers with different descriptions stored internally (e.g. test methods) and some as references to external systems (e.g. computational modules, databases). Knowledge is structured by its biological context. The context is represented as multidimensional organizational space, which can visualize AOPs against pairs of dimensions (e.g. time to effect vs. level of biological organization or sex vs. life stage). The proximity of different effects and test methods in the pathway space reflects similarities in biological context. Thus, pathway space governs scientists with different backgrounds to establish where their knowledge belongs, and aids them in identifying the larger scope of their research and experts who may be interested in it. New contributions are instantly distributed to interested parties, keeping all information current, documented and open for discussion, while crediting original authors and reviewers. Biological responses and test methods are defined once and shared across pathways that include them. The overarching goal is to provide access to transparent executable AOPs which can be used to improve toxicity predictions by maintaining a single body of knowledge with multifaceted interfaces for users with different backgrounds, including scientists, regulators, industry, and the general public. Quantitative knowledge for two AOPs in fish (estrogen binding and aromatase inhibition mediated population reduction) and one in humans (skin sensitization) serve as proofs of concept and demonstration of the platform’s utility.

Using Mode-of-Action (MOA) Data to Guide the Development of Local Quantitative Structure-Activity Relationship (QSAR) Models for Molecular and Early Cellular Events in an Adverse Outcome Pathway (AOP)


The development of local QSAR models for specific health outcomes within a chemical class holds promise for predicting the toxicity of unstudied compounds in support of Green Chemistry and safer alternative efforts. The utility of MOA data for developing local QSAR models was evaluated in the following steps: 1) publicly available databases were queried to identify well-studied chemicals (i.e., chemicals with toxicity values from EPA, ATSDR or CalEPA) with MOA information available; 2) these chemicals were subsequently clustered for read-across using a structure-based analog approach; 3) clusters were selected where all members are expected to undergo a similar MOA; 4) the potential for QSAR model development for the cluster and MOA was evaluated. The clustering exercise, performed on 877 chemicals using our Chemical Assessment Clustering Engine (ChemACE), resulted in approximately 90 clusters containing between 2 and 18 chemicals per cluster. The candidate cluster selected for evaluation contained seven compounds that were structurally similar to 2-butoxyethanol, for which a well-described hema-topotency MOA evaluation is available. Our Analog Identification Method (AIM) software identified an additional 78 structural analogs of 2-butoxyethanol that have toxicity data available in the public domain. An AOP was described for 2-butoxyethanol-induced hematoxicity, where the molecular initiating event (MIE) involves interaction between 2-butoxy acetic acid and the erythrocyte membrane. Erythrocyte hemolysis is an early cellular event that eventually leads to decreased

Computational Alternative Analysis: Network Relationship of Structural Motifs for Chemicals of Concern Correlated with Health, Ecological, and Lifecycle Impacts


Green chemistry initiatives attempt to identify and prioritize chemicals of concern (CoC) and establish methods to replace or eliminate these from consumer products, the workplace, and/or the environment. Impacts (hazard traits) of chemicals on health and eco-systems and the process of evaluating and/or creating alternatives requires a detailed analysis of structural motifs that correlate with specific hazard traits. In this study, a network and pathway visualization process was employed on a test dataset of 153 CoC listed in the CalEPA DTSC Safer Consumer Products regulations. Carcinogenicity, neurotoxicity, and reproductive toxicity were the initial hazard trait endpoints. Reactive motifs of parent compounds and metabolites were classified using structural alert motifs from OpenTox and Derek (Lhasa Ltd) and these were connected into gene-chemical-disease pathways. For example, the carcinogenicity network was established with tools such as KEGG, CTD, and TTD, as well as detailed metabolic pathways, such as from IARC and NTP, and detailed data searches. The established networks allow placement of individual chemical compounds into the structural-related classifications of cancer (animals and human) and importantly provides a computational methodology to predict the safety of potential alternative chemicals as an initial screen.
hematocrit, hemoglobinuria, hemoglobinemia, and hemolytic anemia with secondary effects in the kidney, liver and spleen. Information-rich descriptors that could form the basis of new QSARs for hematotoxicity of related compounds were evaluated statistically for their ability to address potency trends for cluster members and their analogs.

1341 Prenatal and Postnatal Exposure to Concentrated Ambient Particulate Matter Alters the Developing Immune System of Mice


The deleterious effects to the immune system arising from exposure to ambient air pollution have only begun to be investigated. Prenatal exposure to certain air pollutants have been shown to cause immunological dysfunction in offspring, however previous experimental studies have yet to evaluate the vulnerability of the immune system throughout the pre- and early postnatal period. Therefore, to more accurately reflect human exposure, mice were exposed to concentrated fine-sized ambient particulate matter (PM$_{2.5}$), both pre- and postnatally and parameters of immune status were assessed in offspring. Timed-pregnant dams were exposed 6hr/d throughout gestation (GD 0-17) to filtered air or PM$_{2.5}$. After birth, dams and pups were exposed 2hr/d (PND 1-10) and body weights and offspring crown-to-rump lengths were measured daily. At 5 weeks-of-age, blood and spleen were collected from a subset of male and female offspring, and serum samples were analyzed for cytokine levels. Splenies were weighed and splenocytes isolated to assess changes in immune cell profiles by flow cytometry. Additional male and female offspring were challenged with transplanted tumors in vivo using EL4 mouse lymphoma cells. Of the endpoints analyzed, only female offspring were significantly affected with pre- and postnatal PM$_{2.5}$ exposure resulting in an 11% reduction of normalized splenic weight and decreased matured status of splenic B-cells (MHCI and CD45R expression) compared to their control counterparts. These sex-specific alterations demonstrate gaps in previous developmental studies that focused only on PM$_{2.5}$-induced immune effects on male offspring. Together, these results suggest that particulate air pollution negatively impacts the developing immune system, proposing critical consequences for offspring exposed during the pre- and early postnatal period. As this ubiquitous public health threat could have lasting consequences, additional public health policies need consideration.

1342 Regulation of Macrophage Activity by Histone Deacetylases during Nitrogen Mustard-Induced Lung Injury

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NM is a bifunctional alkylating agent which causes acute lung injury and fibrosis. Proinflammatory/M1 and antiinflammatory/M2 macrophages have been implicated in NM-induced lung toxicity. M1 and M2 macrophage activation is controlled at the chromatin level by histone deacetylases (HDACs) and histone acetytransferases. Herein, we analyzed the role of chromatin modification in NM-induced lung macrophage activation and toxicity. Rats were exposed to PBS or NM (0.125 mg/kg) followed 30 min later by valproic acid (VA, 300 mg/kg/day), a class I HDAC inhibitor. VA suppressed NM-induced upregulation of HDAC2 in the lung, but increased histone H3 lysine9-acetylase (H3K9Ac). This was associated with decreased expression of functional cytokines and reduced immune cell infiltration in the lung; these cells were F4/80+ and expressed CD206, a marker of anti-inflammatory MP migration. Supported by NIH Grants ES004738, CA132624, AR055073, ES007148 and ES005022.

1343 Tracking Inflammatory Macrophage Accumulation in the Lung during Ozone-Induced Lung Injury in Mice

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Ozone induced lung injury is associated with an accumulation of pro- and antiinflammatory macrophages (MP) in the lung which have been implicated in tissue injury and repair. In these studies, we used in vivo tracking techniques to investigate the origin of cells. Initially we generated bone marrow (BM) chimeric mice by adoptive transfer of BM cells from GFP+ mice into irradiated C57BL/6 mice. After 4 weeks, mice were exposed to air or ozone (0.8 ppm, 3 h). Macrophages were isolated from lungs 24-72 h later, stained with fluorescent labeled antibodies and, analyzed by flow cytometry. Approximately 98% of BM cells were found to be GFP+ while only 5% were GFP+ in control lungs. Ozone exposure resulted in a marked increase in infiltrating macrophages (MP; CD11b+ F4/80+) into the lung. Two populations, Ly6C$\text{hi}$ proinflammatory and Ly6C$\text{lo}$ antiinflammatory were identified. Proinflammatory GFP+Ly6C$\text{hi}$ MP increased rapidly after ozone and remained elevated, increases in antiinflammatory GFP+Ly6C$\text{lo}$ were transient. To assess potential mechanisms mediating the accumulation of these MP subpopulations in the lung, we used mice lacking Ccr2, a chemokine receptor involved in proinflammatory MP trafficking. Loss of Ccr2 resulted in decreased numbers of infiltrating CD11b$\text{+}$ MP in the lung. This was due to a selective reduction in proinflammatory Ly6C$\text{hi}$ MP. Loss of Ccr2 also resulted in an increased expression of the fractalkine receptor Cx3CR1; this was correlated with an increase in Ly6C$\text{lo}$ anti-inflammatory MP in the lung and reduced toxicity. To further characterize these cells, we used Cx3CR1 GFP+ mice. After ozone exposure, Cx3CR1 GFP+ MP infiltrated into the lung; these cells were F4/80+ and expressed CD206, a marker of anti-inflammatory MP trafficking. Taken together, these results demonstrate that following ozone exposure, inflammatory MP cells enter the lung and may contribute to tissue damage. Cx3CR1 is involved in inflammatory MP trafficking and is supported by NIH Grants ES004738, CA132624, AR055073, ES007148 and ES005022.

1344 Alterations of Immune Parameters in HPBMC following Hardwood Smoke Exposures in Human Volunteers

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The burning of wood in stoves, fireplaces, campfires and forest fires leads to the formation of particulate and non-particle products that have been associated with a variety of health effects in those exposed to emissions. For example, previous studies have shown an increased risk for lung diseases in chronically exposed individuals. Chemicals such as polycyclic aromatic hydrocarbons and dioxin, and fine particulate matter such as carbon monoxide, formaldehyde, sulfur dioxide and various irritant gases are components of hard wood smoke (HWS). Our studies have shown that wood smoke and cigarette smoke exposure by inhalation in humans results in suppression of T-cell dependent immune responses. In our current studies, HPBMC T-cell proliferation and immunomodulatory, pro-inflammatory cytokine and chemokine responses were measured in human volunteers both pre and post exposure to either HWS (500 μg/m3), 2 hr per day for 4 days) or clean air. Significant variabilities in pre and post exposure responses were seen in all 15 individuals. Thus, nonspecific factors such as stress may play a role in peripheral T cell immunosuppression induced by inhalation exposures. However, a trend towards a decrease in T cell proliferation and selective cytokine production (e.g., IL-17) was seen in the HWS exposed individuals.

1345 The α7 Nicotinic Acetylcholine Receptor Agonist GTS-21 Improves Hyperoxia-Compromised Bacterial Clearance from the Lung by Decreasing Hyperacetylation and the Release of Nuclear HMGBl

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Mechanical ventilation with supra physiological concentrations of oxygen (hyperoxia) is routinely used to treat patients with respiratory distress. However, these patients exhibit enhanced susceptibility to infections and develop ventilator-associated pneumonia (VAP). Previously, we have shown that prolonged exposure to hyperoxia induces the release of nuclear protein high mobility group box 1 (HMGB1) into the airways of hyperoxia-exposed mice. Extracellular HMGB1 can impair macrophage phagocytosis and increase mortality of mice infected with Pseudomonas.
aeruginosa (PA). Here we showed that hyperoxic exposure leads to hyperacetylation of nuclear localization signal sites of HMGB1, resulting in its pronounced translocation to the cytoplasm and its release from lung cells into the airways. We then determined whether GTS-21 (3(2,4 dimethoxybenzylidene) anabaseine), an α7 nicotinic acetylcholine receptor (α7nAChR) agonist, could inhibit hyperacetylated HMGB1 and its subsequent release into the airways and improve bacterial clearance from the lungs in a mouse model of VAP. GTS-21 (0.04, 0.4, and 4 mg/kg) or saline were administered intraperitoneally to mice that were exposed to hyperoxia (99% O2) and subsequently challenged with PA. We found that GTS-21 at 4 mg/kg of GTS-21 inhibits both hyperacetylated hyperacetylation of HMGB1 and its subsequent accumulation in the airways of these mice. GTS-21 also significantly increased bacterial clearance and decreased acute lung injury. Additionally, GTS-21 dose dependently increased hyperacetylation-compromised phagocytic activity of macrophages. This study shows that GTS-21 is effective in improving bacterial clearance by inhibiting the hyperacetylation and release of nuclear HMGB1 and implicates the α7α4nAChR as a possible pharmacological target for improving hyperacetylation-compromised innate immunity in patients with VAP.

PS 1346 Ascorbic Acid Improves Hyperoxia-Compromised Host Defense against Pseudomonas aeruginosa Infection
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Oxygen therapy using supraphysiological concentrations of oxygen (hyperoxia) is associated with compromised host defense and greater susceptibility to bacterial infections, causing ventilator-associated pneumonia (VAP). Hyperoxia-induced excessive ROS production and elevated levels of extracellular HMGB1 in the airways play critical roles in impairing macrophages’ ability to phagocytose. Ascorbic acid (AA), an antioxidant, has been shown to be beneficial in various animal models of ROS-mediated diseases. The aim of this study was to determine whether AA could improve hyperacetylation-compromised host defense and macrophage functions. C57BL/6 male mice were exposed to hyperoxia (99% O2, 48 h) followed by intratracheal inoculation with gram-negative bacteria, P. aeruginosa, and simultaneous administration of AA. AA, at 50 mg/kg, effectively improved bacterial clearance in lungs and airways of the animals and reduced the accumulation of HMGB1 in the airways. To better understand the molecular and cellular mechanisms underlying AA-improved bacterial clearance in the lung, RAW 264.7 cells (a macrophage-like cell line) were treated with AA prior to the exposure to 95% O2. ROS levels, phagocytic activities and extracellular HMGB1 accumulation were analyzed in the macrophages. AA significantly rescued hyperacetylation-compromised macrophage function of phagocytosis. The improved phagocytic activity was accompanied by reduced ROS levels as well as decreased accumulation of extracellular HMGB1. Our study suggests that AA, a cost-effective dietary supplement, can provide a rewarding aid in the prevention of VAP.

PS 1347 Activation of Nr2f by tBHQ Upregulates IgM Production by LPS-Activated Mouse Splenocytes
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Nuclear factor erythroid 2-related factor 2 (Nr2f) is a transcription factor activated by cell stress, including oxidative and electrophilic stimuli, resulting in the upregulation of cytoprotective genes. Nr2f can be activated experimentally by a number of different pharmacological agents, including tert-butylhydroquinone (tBHQ), which is also used commercially as a food additive. We recently showed that activation of Nr2f by tBHQ promotes Th2 differentiation and inhibits Th1 differentiation in isolated CD4+ T cells. Given that tBHQ is a food additive and promotes Th2 differentiation, the purpose of the present studies was to determine the effect of tBHQ in a mouse model of food allergy. Mice were administered a diet either with or without tBHQ (0.001%) for two weeks prior to sensitization with ovalbumin. Notably, this dose of tBHQ is lower than that normally present in standard rodent chow (0.0016%) and in human food. The mice were sensitized weekly transdermally for 4 weeks. Sensitization to ovalbumin caused an increase in ovalbumin-specific IgE and IgG1 in plasma, which was greater in animals exposed to tBHQ. After sensitization, the mice were orally challenged with ovalbumin, causing mild/moderate anaphylaxis, which was exacerbated by tBHQ. We also observed a significant decrease in body temperature after oral ovalbumin challenge in mice on the tBHQ diet. Overall, these studies suggest that low doses of the food additive, tBHQ, increase IgE and IgG1 response to food allergen and exacerbate clinical signs of anaphylaxis. (This work was funded by NIH grant: ES018885.)
Cancer chemotherapy is often associated with adverse tissue injury and organ dysfunction. Cisplatin is a chemotherapeutic agent used against a wide range of solid tumors. A major toxic effect of cisplatin chemotherapy is acute kidney injury. In earlier studies, we reported the presence of a dense network of dendritic cells (DCs) in kidneys and their protective function against nephrotic nephritis. Here, we studied the function of two major subsets of dendritic cells that are regulated by colony stimulating factor 1 (CSF1) and receptor tyrosine kinase Flt3 ligand (Flt3L) in mice. Confocal imaging showed widespread presence of CSF1-dependent DC11c+Flt3+CD38+ cells in the cortex and medullary regions of the kidneys, which are responsible for about 75% of total renal DCs. We used antibody against CSF1R to deplete these DCs to investigate their functional significance in cisplatin nephrotoxicity. CSF1R antibody caused almost complete depletion of CD11c+Flt3+DCs in kidneys but not in spleen. Flt3R deficient mice were more susceptible to cisplatin induced renal dysfunction and tubular injury, indicating that these Flt3-dependent DCs protect from cisplatin nephrotoxicity. We further compared cisplatin nephrotoxicity in mice deficient in Flt3L-dependent DCs with mice depleted of all subsets of DCs using CD11c-DTR mice. Whole body total DC-depleted CD11c-DTR mice showed more extensive renal dysfunction and tubular injury compared to Flt3 deficient mice, suggesting the presence of other renal subsets of protective DCs and/or extra renal tolerogenic DCs. These data demonstrate the protective function of Flt3L-dependent DCs in cisplatin nephrotoxicity, and a unique experimental approach to investigate the role of two important DC subsets in different models of kidney injury.

Idiosyncratic, drug-induced liver injury (IDILI) is a challenging problem that can lead to death of patients and removal of otherwise efficacious drugs from the market. Previous studies in animals have shown that IDILI-associated drugs cause hepatotoxicity when coupled with a nontoxic dose of an inflammasome such as lipopolysaccharide. The inflammatory mediators, tumor necrosis factor-alpha (TNFα) and interferon gamma (IFNγ), are involved in the development of liver injury in animal models of IDILI. In this study, we tested the hypothesis that two drugs associated with IDILI, diclofenac (DCLF) and bromfenac (BRO), synergize with the cytokines TNFα and IFNγ to cause cytotoxicity in vitro. We treated human-derived HepG2 cells with DCLF or BRO alone or in combination with TNFα, IFNγ, or both. DCLF and BRO each synergized with TNF to kill HepG2 cells, and IFNγ enhanced the cytotoxic interaction. Calpain is a calcium-dependent protease that is activated during endoplasmic reticular stress. An inhibitor of calpain against αCSF1R to deplete these DCs to investigate their functional significance in cisplatin nephrotoxicity. αCSF1R antibody caused almost complete depletion of CD11c+Flt3+DCs in kidneys but not in spleen. Flt3R deficient mice were more susceptible to cisplatin induced renal dysfunction and tubular injury, indicating that these Flt3-dependent DCs protect from cisplatin nephrotoxicity. We further compared cisplatin nephrotoxicity in mice deficient in Flt3L-dependent DCs with mice depleted of all subsets of DCs using CD11c-DTR mice. Whole body total DC-depleted CD11c-DTR mice showed more extensive renal dysfunction and tubular injury compared to Flt3 deficient mice, suggesting the presence of other renal subsets of protective DCs and/or extra renal tolerogenic DCs. These data demonstrate the protective function of Flt3L-dependent DCs in cisplatin nephrotoxicity, and a unique experimental approach to investigate the role of two important DC subsets in different models of kidney injury.

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GrB, Gr3/K, and GRN in NK-92CI cells in a dose-dependent manner. However, the strength of the effect differed among the pesticides, and the order was thiram > maneb > carbaryl. In addition, it was also found that the degree of the reductions differed among the five proteins, with perforin more sensitive to pesticides than GRN, Gr3/K, and GRN > Gr3/K ≈ GrA. These findings suggest that determining the effect of chemicals on the levels of perforin, GrA, GrB, Gr3/K, and GRN in NK cells by flow cytometry can assist in the evaluation of the immunotoxicity of the chemicals. References: 1. Li et al. Arch Toxicol. 2012;86:475-81. 2. Li et al. Toxicology 2007;239:89-95. 3. Li et al. J Biol Regul Homeost Agents. 2014;28:23-32. Acknowledgements: This work was supported by a Grant-in-Aid for Scientific Research in Japan.
The mechanism of idiosyncratic drug induced liver injury (IDILI) remains poorly understood, in part due to the lack of a valid animal model. Clinical evidence suggests that most IDILI is immune mediated, and the major factor preventing liver injury in most patients is immune tolerance. Recently we reported an animal model of amodiaquine (AQ)-induced IDILI that utilized PD1-/- mice and an anti-CD4-/- antibody. Both PD1 and CTLA-4 are important for the induction of immune tolerance. Treatment of these mice with AQ resulted in sustained increases in ALT rather than the adaptation that was observed in wild type mice. In addition, the histology was characterized by piecemeal necrosis, which is the pattern observed in clinical IDILI. In the present study we further characterized the changes in hepatic and splenic leukocytes to gain further mechanistic clues. PD1/- mice were treated with 0.2% w/w AQ in food and 250 ug of anti-CD4-/- by IP injection weekly for 10 weeks. Mononuclear cells were isolated from the liver and spleen, stained with specific antibodies, and analyzed by flow cytometry. As before, we observed a sustained increase in ALT levels as well as significantly elevated levels of total bilirubin. We found that these mice had significant increases in percentage of hepatic CD4, CD8, Th17, and Treg cells after 10 weeks of AQ treatment. We also found these mice had significantly increased B cells in the spleen and significantly decreased NK cells in the liver and spleen. These results suggest that Th17 and CD8 T cells are important in the development of liver injury in this model, while NK cells are not. The results also suggest that there are many overlapping immune tolerance mechanisms in the liver as evidenced by the increased percentage of Treg cells. Although this liver injury did not result in frank liver failure, the increase in bilirubin indicates a decrease in liver function. Supported by grants from the Canadian Institutes for Health Research.

**Investigation of Immune Response to OVA Challenge in PD-1 Deficient Mice**


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An investigative study in programmed death 1 (PD-1) KO mice was conducted to determine if deficiency in PD-1, an important negative regulator of immune responses, alters OVA-induced respiratory inflammatory responses. PD-1 KO and corresponding WT mice were injected IP with OVA or vehicle (0.9% saline) on Days 0 and 7 for sensitization. On Days 14 and 28, mice received OVA or vehicle by pharyngeal aspiration and were necropsied 4, 24, or 72 hours after the last challenge. Analyses included lung PD-L1 (PD-1 ligand) and cytokine/chemokine gene expression; cytokine protein levels in lung and blood; lung pathology and cellular infiltrate; and lung and circulating mucin-1 (MUC-1; an indicator of mucous production associated with lung injury) protein levels. Expression of PD-L1 was increased in OVA-challenged PD-1 KO mice compared to vehicle-treated PD-1 KO mice and vehicle or OVA-treated WT mice. OVA-treated PD-1 KO mice demonstrated increased lung TNFa, IL1b, IL10, CCL2, CCL4, and IL10 gene expression relative to OVA-treated WT mice. In addition, TNF-α, MCP-1 (CCL2 encoded), MIP-1α, MIP-1β (CCL4 encoded), IL-12p40, IL-6, GM-CSF, IFNγ, GM-CSF (CCL2 encoded), and RANTES protein levels were higher in lung, BALF, and/or serum of OVA-treated PD-1 KO mice relative to OVA-treated WT mice. There were no overt microscopic differences in lungs between OVA-treated PD-1 KO and WT mice, which correlated with similar MUC-1 levels amongst all mice. Thus, although, in this model, differentiation of PD-1 deficiency on immune response to OVA was demonstrated at molecular and cellular levels, no discrimination was observed at a disease state level.

**Effects of Di-(n)-Butyl Phthalate (DBP) on Immune Cells In Vitro**


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Phthalates are commonly used plasticizers present in numerous consumer products. Epidemiological studies suggest that exposure to phthalates in indoor environments is associated with development and exacerbation of asthma and allergy. Phthalates interact with several cell signaling pathways, but which interactions are important for functional changes of immune cells are not well known. The aim of this study was to evaluate effects of di-n-butyly phthalate (DBP) on macrophages in vitro. DBP was chosen since some of the highest indoor air levels have been reported for this phthalate. DBP also appears to have greater inflammatory potential relative to other phthalates in vitro. The human monocytic cell line THP-1 was differentiated to macrophages by phorbol myristate acetate (PMA) to study effects of DBP on macrophage differentiation process and release of pro-inflammatory cytokines TNFα and IL-1β from the differentiated cells. Toll-like receptors (TLRs) play a key role in the innate immune system. To assess if DBP could modulate an inflammatory response, cells were either primed (3h pre-exposure) or challenged (last 3h of exposure) with lipopolysaccharide (LPS) or Zymosan, known to be TLR4 and TLR2 ligands respectively, and effects on differentiation and cytokine release evaluated. A 24h exposure to 20-80 μM of DBP accelerated the differentiation process (morphological observation) and increased the release of TNF-α and IL-1β (ELISA) in differentiated cells and cells primed with low concentrations of TLR ligands. After TLR challenge with LPS and/or Zymosan, DBP reduced the TNFα release, but had no marked effects on IL-1β. In preliminary data the TNFα mRNA levels show a similar pattern as the protein levels, suggesting involvement of pre-transcriptional events. In conclusion, DBP exposure accelerated macrophage differentiation, increased release of pro-inflammatory cytokines and changed functionality of macrophages in vitro.

**Investigation of the Role of B Cells in the Pathogenesis of Hydrocarbon Oil-Induced Lung Hemorrhage**

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Background: Exposure to toxic substances (isocyanates/pesticides), recreational drugs, pharmaceuticals, and inflammatory diseases may result in inflammation/hemorrhage in lungs, called diffuse alveolar hemorrhage (DAH). With no effective treatment, it is invariably fatal. Limited access to human tissue and/or lack of animal models have prevented investigations into its pathogenesis, until recently, when investigators detected DAH in two otherwise normal mouse strains, C57BL/6 (B6) and C57BL/10, exposed to hydrocarbon oil (TMPS). Here, we specifically focused on B cells, since previous studies have shown reduced DAH
in B cell-deficient mice. Results: We have found that an i.p. injection of TMPD led to DAH in B6 mice within 2-4 weeks in 72% of 29 TMPD-exposed versus none of 23 controls (saline or control oil n-hexadecane). 50% of animals died within 4 weeks post-TMPD exposure. None of the nine animals tested at 1 week post-TMPD showed any hemorraghe. Analysis of immune cells at this stage, prior to any pathology, revealed significant changes in the proportions and numbers of innate B cells, namely B1 and marginal zone B cells that are the first B cell populations to encounter antigens acquired through peritoneum and blood. Contrary to our expectation, we found reduced proportions and numbers of marginal zone B cells, defined as CD19+CD21+CD19+CD22+ cells, in the spleen and reduced numbers of B1a (CD19+CD11b+CD5+) and B1b (CD19+CD11b+CD5-) cells in the peritoneum of TMPD-injected mice as compared to control mice. Gene microarray analyses of the lungs of these mice revealed significant differences in 1,751 genes between PBS and TMPD, and 409 genes were different between hexadecane and TMPD groups. Summary: Unexpectedly, we found reduced numbers of innate B cells in TMPD injected mice as compared to controls. Ongoing study will investigate mechanisms underlying changes in these B cells and their relevance to the development of DAH. We will further analyze our microarray data for genes that may confer these B cell changes and DAH.

1364 Investigating the Role of Inflammase Activation in the Proinflammatory Response Induced by Clozapine
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Clozapine causes a relatively high incidence of agranulocytosis while the closely related olanzapine does not. The difference appears to be due to an immune mediated reaction and clozapine is known to induce an inflammatory response in patients at the start of treatment. We have found that the ability of a drug to activate NLRP3 inflammasomes appears to predict the potential of a drug to induce an idiosyncratic drug reaction. Therefore, we studied the ability of clozapine and olanzapine to activate inflammasomes. Methods: THP-1 macrophages were incubated with clozapine or olanzapine (1-10 μg/mL) for up to 24 hours. Z-VAD-fmk (10 μg/mL) was employed to block caspase activation. Cell media was analyzed for IL-1β, TNF-α, CXCL1, and IL-6 by ELISA. The proinflammatory effect of clozapine was also studied in vivo by administering a single dose of the IL-1 receptor antagonist anakinra (50 mg/kg, i.p.), followed by clozapine (30 mg/kg, i.p.). Leukocytes were counted and serum IL-1β, CXCL1, G-CSF, and cT-α1-glycoprotein (cT-1-AGP) were measured by ELISA. All animal studies were carried out by the University of Toronto and were approved by the Animal Care Committee. Results: Clozapine stimulated THP-1 macrophages to secrete significant amounts of IL-1β and CXCL1 in a caspase-dependent manner, whereas olanzapine did not. Similarly, CXCL1, G-CSF, cT-1-AGP, and neutrophil counts were significantly elevated by clozapine in vivo, and these elevations were suppressed by anakinra. These results suggest that inflammasome activation is the route by which clozapine stimulates a proinflammatory response and that IL-1β is a likely key inflammatory mediator in this process. The observation that olanzapine did not activate inflammasomes suggests that inflammasome activation represents a biomarker of the potential for a drug to cause idiosyncratic drug reactions. This research was supported by grants from the Canadian Institutes of Health Research.

1365 Activation of the NLRP3 Inflammasome As a Potential Biomarker of Idiosyncratic Drug Reactions
J.K. Weston and I. Uetrecht. (Pharmaceutical Science, University of Toronto, Toronto, ON, Canada)

Idiosyncratic drug reactions (IDRs) are often caused by drugs that are chemically reactive or that form reactive metabolites. While chemical reactivity represents a risk factor for the induction of IDRs, not all chemically reactive drugs or drugs that form a reactive metabolite are associated with a significant risk of causing IDRs. Most IDRs appear to be immune mediated; therefore, the ability of a reactive metabolite to induce an immune response may differentiate those reactive species that can cause IDRs from those that do not. The inflammasome, which plays a role in contact hypersensitivity reactions, appears to be activated by drugs associated with IDRs. We have developed an in vitro method for measuring a drug’s ability to activate the NLRP3 inflammasome. Methods: THP-1 human monocytes were differentiated to macrophages with phorbol myristate acetate (PMA) at 25 ng/ml for 3 days and treated with the compound of interest. Levels of secreted interleukin-1β (IL-1β) were measured by ELISA in the supernatant as an indicator of inflammasome activation. The caspase inhibitor Z-VAD-fmk was used to ensure IL-1β secretion was inflammasome mediated. Results: We found that drugs that are chemically reactive, e.g. dimethyl fumarate, or are oxidized to reactive metabolites by THP-1 cells, e.g. clozapine, and are associated with a relatively high risk of IDRs, activated inflammasomes. Furthermore, the comparators ethacrynic acid and olanzapine, respectively, that are not associated with a significant incidence of IDRs did not activate inflammasomes. Inflammasome activation may be a biomarker of IDR risk. Funded by Janssen Pharmaceutical Company.

1366 AhR-Mediated Activation of Respiratory Inflammatory Cells
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Innate lymphoid cells (ILCs) are a group of innate effector cells with transcriptional activities and phenotypic similarities to several T helper subsets. Like T helper subsets, ILCs are found at mucosal surfaces (including the lungs) and play important roles in immune responses to infection and allergens, repair after tissue damage, and lymphoid tissue formation and homeostasis; however, unlike T helpers subsets, ILCs do not respond in an antigen-specific manner. Although the aryl hydrocarbon receptor (AhR) has emerged as a master regulator of ILCs and inflammation in the gastrointestinal tract, its contributions to the fate and function of ILCs in the respiratory tract are largely unknown. The goal of the present study was to assess whether natural vs. man-made AhR ligands differentially alter the phenotype and function of naïve respiratory ILCs in vivo using C57/Bl6 and AhRd mice. Oral gavage with 150μl vehicle control (peanut oil), 0.2mg (1H-indole-3-carbonyl)-thiazole-4-carboxylic acid methyl ester (ITE), or 10μg kg-1, 2.3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD), led to AhR-mediated recruitment and activation of CD45+CD90.2+Lineage- ILCs 7 days later as measured by changes in the expression profiles of select cell surface markers, cytokines, and transcription factors. Collectively, our data suggests that systemic AhR activation may modulate the respiratory immunity via changes in the phenotype and function of ILCs. This work is supported by NIEHS/NIGMS grant R01-ES013784 (DMS) and NIEHS grant R15-Es020993 (CAB), as well as NIGMS grants P30GM103338 and P20GM105346. The contents of this publication are solely the responsibility of the authors and do not necessarily represent the official views of NIEHS, NIGMS or NIH.

1367 Shifts in Community of Mouse Gut Microbiome Exposed to TCDD
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Exposure of an environmental contaminant (2,3,7,8-tetrachlorodibenzo-p-dioxin, TCDD) known to be an aryl hydrocarbon receptor (AhR) antagonist was examined in the gut microbiomes of male mice. Mouse gut microbiomes were analyzed in two mouse populations: a control and TCDD-exposed group. Oral gavage with 150μl vehicle control (peanut oil), 0.2mg of 1H-indole-3-carbonyl)-thiazole-4-carboxylic acid methyl ester (ITE), or 10μg kg-1, 2.3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD), led to AhR-mediated recruitment and activation of CD45+CD90.2+Lineage- ILCs 7 days later as measured by changes in the expression profiles of select cell surface markers, cytokines, and transcription factors. Collectively, our data suggests that systemic AhR activation may modulate the respiratory immunity via changes in the phenotype and function of ILCs. This work is supported by NIEHS/NIGMS grant R01-ES013784 (DMS) and NIEHS grant R15-Es020993 (CAB), as well as NIGMS grants P30GM103338 and P20GM105346. The contents of this publication are solely the responsibility of the authors and do not necessarily represent the official views of NIEHS, NIGMS or NIH.

1368 Growth and Oxidative Stress Effects in Adult Male Wistar Rats Coexposed to Atrazine and Arsenic
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The wide usage of atrazine as herbicides on agricultural farmlands has resulted in its elevated levels in the environments. Since arsenic, a toxic element is a key component of atrazine, it is possible that the mixture of both contaminates the environment. The wide usage of atrazine as herbicides on agricultural farmlands has resulted in its elevated levels in the environments. Since arsenic, a toxic element is a key component of atrazine, it is possible that the mixture of both contaminates the environment.
every week, and at the end of the 30-day exposure various indices of oxidative stress such as levels of reduced glutathione (GSH) and thiobarbituric acid reactive substances (TBARS) and the activities of antioxidant enzymes like catalase and glutathione peroxidase (GPx) were determined in the blood, liver, kidney and brain. The gain in weight per week decreased significantly in all the experimental groups which did not differ from the control groups. There were indications that co-exposure to arsenic and atrazine caused oxidative stress in exposed rats; increased levels of TBARS, reduced GSH Levels and decreased activity of catalase. We did not observe a tissue-specific pattern in these indices although the changes were more pronounced in the blood especially in the kidney when both was exposed. Enzymatic activities of the contaminants. Ongoing studies are looking at the responses under a chronic exposure condition as well as the proteomic profiling that are associated with the exposure. A longer term goal will be to gain a concise insight into the mechanisms underlying the toxicity of arsenic and atrazine under a co-exposure scenario.

1369 Tissue-Specific Effects of Chronic Coexposure of Cr (VI) + B[a]P in C57Bl/6J Mice
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Exposure to a single toxic compound is uncommon in the environment. Living organisms are frequently exposed to complex mixtures of different toxicants, causing mixture-specific effects that cannot be attributed to a single mechanism. Two toxicants, with unrelated modes of action, that are often found in the environment forming complex mixtures are the heavy metal chromium VI (Cr(VI)) and the polycyclic aromatic hydrocarbon benzo[a]pyrene (B[a]P). To study the biological effects of this mixture, we exposed mice to 0.55, 550, or 5500 ppb Cr(VI) in drinking water for 2 months followed by 3 additional months of co-exposure to Cr(VI) and B[a]P, the latter included in corn oil-soaked food at doses of 0, 1.25, 12.5, or 125 mg/kg/day. A B[a]P-dependent and Cr(VI)-independent weight reduction was observed in the mice after the 5 months. Histologically, both proximal (PSI) and distal (DSI) sections of the gastrointestinal (GI) tract, showed no cumulative effects when the mice were co-exposed to Cr(VI) and B[a]P relative to the individual exposures. The lesions observed in the liver were more severe when the mice were exposed to the mixture than to Cr(VI) or B[a]P alone. In the three tissues tested, gene expression patterns were altered relative to control mice. The DSI showed more gene up-regulation, the liver more gene down-regulation and the PSI displayed both gene up- and down-regulation. Importantly, oxidative stress-related genes, like GCLC and NRF-2, showed different effects in every tissue. We conclude that the combined effect of Cr(VI) and B[a]P exposure has tissue-specific differential effects that cannot be predicted from the effects of each individual toxicant. This effect may be particularly critical in cases of extended exposure to mixtures of these agents, as may happen in the occupational setting or in areas where drinking water contains elevated levels of Cr(VI), and may contribute to the overall toxicity of the mixture. Supported by NIH grants ES010807 and R21 ES020048.

1370 Toxicity Evaluation of Binary Mixture of Benzo[a]pyrene and Cadmium in Human Hepatocellular Carcinoma Cell Line
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Polycyclic aromatic hydrocarbons (PAHs) and heavy metals are ubiquitous co-contaminants present at former gas manufactured gas plant and other industrial sites. The interaction toxicity data of PAHs and heavy metals constitute central role in human health risk assessment. The aim of this study is to determine the binary mixture toxicity of Benzo[a]pyrene (B[a]P) and Cadmium (Cd) by using human hepatocellular carcinoma cell line (HepG2). This cell line possesses a liver-like enzyme pattern including the enzymes of biotransformation. Cytotoxicity and genotoxicity induced by B[a]P and Cd individually and in combination were determined by MTS assay and micronucleus (MN) evaluation by flow cytometry respectively. To determine the cytotoxicity of B[a]P and Cd, HepG2 cells were treated with B[a]P (0-120 μM) and cadmium chloride (0-20 μM) for 24 h. Fixed ratio and factorial designs were used to study the dose response of binary mixture of these two chemicals. Joint toxicity and dose response relationship of mixture was determined by classical models of concentration addition (CA) and independent action (IA) and also by combination index (CI)- isobologram method. Genotoxicity (MN test) of individual and binary mixture was determined by flow cytometry using an in vitro Microflow kit (Liron Laboratories, USA). The chemicals were treated for 2 doubling time of cells before analysis. The results showed that cadmium is the dominant component in the mixture and the binary mixture of B[a]P and Cd showed dose additivity for the selected combinations in cytotoxicity assay. There was no significant increase in MN induction was observed for this binary mixture when compared to individual dose response of B[a]P and Cd. It is concluded that combination of HepG2 cells and interaction prediction models can be used as a valuable tool to determine the mixture toxicity of orally exposed environmental contaminants.

1371 Influence of Binary Mixtures of Aromatic Amines and Benzo[a]pyrene on CYP1A1/1B1 Activities in RT4 Cells
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At the workplace, aromatic amines and polycyclic aromatic hydrocarbons often occur concurrently, thus making risk estimations difficult. Individual substances within mixtures can influence the bioactivation of pre-carcinogens and in turn their genotoxic properties. In the present study we examined the effects of carcinogenic and non-carcinogenic arylamines and benzo[a]pyrene (B[a]P) on CYP1A1/1B1 activity. Human RT4 urinary bladder papilloma cells were exposed for 24 h to 0.1-3 μM B[a]P, 0.1-100 μM 1-naphthylamine (1-NA), 2-naphthylamine (2-NA), 3-aminoanthiphenyl (3-AP), or 4-aminoanthiphenyl (4-AP), and binary mixtures of 1 μM B[a]P + 0.1-100 μM of each arylamine. Relative enzyme activities were measured in intact cells with a luminescence-based assay. After exposure to B[a]P, a concentration-dependent increase in CYP1A1/1B1 activities up to 10-fold compared to untreated controls was measured. For 1-NA and 2-NA concentration-dependent increases in enzyme activities up to 14- (100 μM 1-NA) and 17-fold (30 μM 2-NA) were observed, while α10 μM 3-AP decreased constitutive activities down to 20% and 10% in a concentration-dependent manner. Co-exposure to 1 μM B[a]P + 1-NA or 2-NA resulted in enhanced enzyme activities compared to 1 μM B[a]P alone. Besides, enzyme activities were higher in cells co-exposed to 1 μM B[a]P + 1-NA (0.1-100 μM) or 2-NA (0.1-10 μM) compared to 1-NA or 2-NA alone. When cells were co-exposed to 1 μM B[a]P + 3-AP or 4-AP, decreased enzyme activities compared to 1 μM B[a]P alone were observed at 1 μM B[a]P + α 1.0 μM 3-AP and 3 μM 4-AP, respectively. The results show that metabolism in RT4 cells is influenced by binary mixtures of amines + B[a]P and that the effect (enhancement or inhibition) depends on the composition of the mixture. The data, however, show that enzyme induction or inhibition is mainly driven by the aromatic amines rather than B[a]P suggesting that arylamines are an important modulating factor of B[a]P-derived toxicity.

1372 Transcriptional and Biochemical Effects of Chlorpyrifos and Malathion and Their Mixtures on Neurobehavioral Function in Coho Salmon
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Exposure to organophosphate pesticides (OP) can inhibit salmon neurobehavioral function leading to loss of survival. These exposures often occur as mixtures, however, the molecular and biochemical mechanisms underlying olfactory impairment from these compounds or their mixtures are poorly understood. We hypothesized that short-term exposure to low concentrations of single OPs and binary mixtures can disrupt olfactory function independent of acetylcholinesterase (AChE) inhibition. Juvenile salmon were exposed for 24 hours in nine exposure scenarios, including 0.1, 0.5 or 2.5 ppb chlorpyrifos (CPF); 2, 10, or 50 ppb malathion (MAL); or binary mixtures of 0.1 CPF: 2 ppb MAL, 0.5 CPF: 10 ppb MAL, or 2 CPF: 10 ppb MAL. Brain AChE activity was reduced only in the highest binary mixture exposure scenario. Microarray analysis of RNA from coho olfactory rosettes revealed a number of OP-disregulated pathways involved in nervous system function and signaling, metabolic function as well as cellular responses to toxicity. Coho exposed to binary OP mixtures of 0.5 CPF: 10 ppb MAL showed a marked loss of odorant response and reduced mitochondrial respiration in the absence of effects on brain AChE. Our study provides mechanistic information underlying unanticipated neurotoxic effects of exposure to organophosphate mixtures independent of AChE inhibition that include disruption of olfactory mitochondrial function. This work was supported by the University of Washington Superfund Research Program (NEIHS P42-046096).
1373 Assessment of Blood Lead and Polychlorinated Biphenyl (PCB) Levels and Toxicity in Construction Workers in Iowa
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Lead is a prominent component of paint in houses built before 1979 and a potential source of exposure for construction workers. PCBs may also be present in many building materials such as paint, caulk and other materials in these older houses. No study to date has assessed the possible co-exposure to both toxicants in occupational settings. Our HYPOTHESIS is that construction workers are occupationally exposed to lead and PCBs from old buildings and this co-exposure has adverse health effects. After completing an interviewer-administered questionnaire addressing demographics, work history, and other possible sources of lead and PCBs exposure, blood was collected from 80 construction workers in eastern Iowa. The prevalence of blood lead level were determined using the LeadCare II analyzer. The abilities of PCBs and/or lead exposure to induce free radical mediated oxidative DNA damage was assessed by measuring 8-oxooG. Screening for DNA damage was also performed using the comet assay which measures recent induction of DNA strand breaks. Paraoxonase (PON1) activity in serum was determined spectrophotometrically using two substrates, phenyl acetate (PAPA, CMPase activity) and 4- (chloromethyl)phenyl acetate (CMPA, CMPase activity) which also provides genotypy. Preliminary results indicate that lead levels in these workers are mostly below 3 ug/dl, with only 3 of 80 workers exceeding this value. Multiple linear regression analysis of associations between PON1 activity and 8-oxo-dG, age, weight, smoking, alcohol consumption revealed that PON1 activity with both substrates was negatively associated with body weight (P<0.005). 8-oxo-dG did not show any association with age, weight, alcohol consumption or other life style habits or hobbies. Determination of exact blood lead levels by ICP/MS and levels of all 209 PCB congeners using gas chromatography/mass spectroscopy (GC/MS) are in progress. This project will add to our knowledge base of occupational lead and PCB exposure and provide essential data to facilitate the protection of workers.

1374 Effects of Taurine on Motor Activity and Anxiety in Wistar Rats Co-Exposed to Chlorpyrifos and Lead
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Pesticides and heavy metals are the most prevalent environmental pollutants due to their widespread use. Chlorpyrifos (O-O-diethyl-3,5,6-trichloro-2-pyridyl-phosphorothionate) is a widely used organophosphate pesticide. Lead (Pb) is one of the most toxic heavy metals known to man and it is persistent in the environment. Taurine (C2H7NOS) is a semi essential amino acid, antioxidant and neuroprotective biological molecule. The aim of the study was to investigate the effects of taurine on motor activity and anxiety in Wistar rats co-exposed to chlorpyrifos and lead. Fifty male Wistar rats were used for the study and they were distributed into five groups of ten rats each. The groups received the following treatments: DW group (distilled water), SO group (soya oil, 1 ml/kg), TA group [taurine, 50 mg/kg], CPF+Pb group (chlorpyrifos, 4.25 mg/kg, 1/20th LD50; lead acetate, 233.25 mg/kg, 1/20th LD50), and TA+CPF+Pb group (taurine and chlorpyrifos and lead acetate). The treatments were administered to the rats once daily by oral gavage for four weeks. Motor activity and anxiety were assessed in the rats with the open-field test at weeks 0, 5, 10 and 15. Motor activity was assessed by measuring the number of squares crossed in 2 minutes after the initial 3 minutes of habituation. Anxiety was evaluated by determining the frequency of rearing, grooming, stretch-attend posture, defaecation and urination. The CPF+Pb group exhibited a reduction in motor activity and increased anxiety. There was improved motor activity and decreased anxiety in the TA and TA+CPF+Pb groups. The adverse effects of CPF and Pb may be attributed to the disruption of the hypothalamic-pituitary-adrenal axis, GABAergic and serotonergic systems, and induction of oxidative stress. The beneficial effects of TA may be due to its anxiolytic, antioxidant and neuroprotective properties. In conclusion, TA may be useful for the enhancement of locomotion and attenuation of anxiety in animals.

1375 Single Exposures to Lead, Inorganic Mercury, and Methylmercury Do Not Correctly Predict Their Mixture Effects on the Cardiovascular System
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It is assumed that the general population is rather exposed to mixtures than to single environmental pollutants. One example mixture is exposure to lead from food and drinking water, elemental mercury from amalgam fillings and methylmercury through seafood and fish. Mixture risk assessments are often challenging because of a lack of suitable mixture data. Although Pb and Hg species are traditionally considered to be neurotoxins, an increasing number of animal and human studies associate their exposure with cardiovascular diseases. The objective of this study was to evaluate the risk estimate from single metal exposures. Male rats (n=3-6) were exposed for 28 days to either mono-methylmercury chloride (MeHg(I); 357 μg/kg-bw/d), mercury chloride (Hg(II); 357 μg/kg-bw/d) and lead acetate (Pb(II); 1607 μg/kg-bw/d) or a mixture of all three metals through the drinking water. The dose ratios of the mixtures were based on literature values for reference doses (MeHg(I);Hg(II);Pb(II) = 1:3:7 = 290:870:2030 μg/kg-bw/d) or for environmental exposure (MeHg(I);Hg(II);Pb(II) = 1:2:17 = 290:580:4930 μg/kg-bw/d). After four weeks, echocardiography, carotid artery ultrasound and intra-arterial blood pressure were performed. Systolic blood and pulse pressure were increased (p<0.05) for MeHg(I) at a dose of 357 μg/kg-bw/d, while mixtures reversed these effects. MeHg(I) or Pb(II) reduced (p<0.05) the stroke volume and cardiac output, while mixtures normalized these effects. Single metal exposures did not affect the electrical activity of the heart. In contrast, the mixture based on the reference values lengthened the QRS and QT intervals indicating an increased risk for arrhythmia and heart failure, while the environmental exposure based mixture did not have an effect. Hence, metal mixtures did not behave in an additive fashion on adverse cardiovascular outcomes.

1376 Evaluation and Modeling of the Impact of Coexposures to VOC Mixtures on Urinary Biomarkers
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Volatile organic compound (VOC) exposure assessment in biomonitoring studies is often performed through measurements of urinary biomarkers because of their non-intrusiveness but this does not consider possible toxicokinetic interactions from mixed exposures. Indeed, VOC exposures generally occur in the form of chemicals mixtures where interactions can occur, e.g., competitive inhibition between Toluene (T), ethylbenzene (E) and m-xylene (X). Other common VOCs such as chloroform (C) may also interact with TEX. The objectives of the study were therefore to i) study the impact of coexposures to CTEX on the kinetics of their respective urinary biomarkers and ii) to adapt existing PBPK mixture model of parent compounds to describe the urinary excretion of marker metabolites allowing to account for possible interactions. Five male volunteers were exposed for 6 hours to C alone and in binary and quaternary mixtures with the aromatic solvents in an inhalation chamber at 1/4 and/or 1/8 of their TLV®. Parent compounds levels were measured in exhaled air and blood samples while urinary metabolites (o-Cresols, mandelic acid and m-methylhippuric acid) were quantified in urine samples. The Vmax value of C was optimized to 3,4 mg/h/kg. Individual PBPK models of T, E, and X were adapted to describe urinary metabolite excretion and optimized when needed. No interactions were observed for any binary exposures comprising chloroform. PBPK model describing only competitive inhibition between T, E and X predicted adequately experimental data for quaternary mixtures. These results demonstrate that no other significant toxicokinetic interactions affecting the kinetics of urinary biomarkers at exposure levels used in this study are likely to occur. The present PBPK models for urinary biomarkers should prove to be useful in assessing exposure to these VOCs.

1377 Chemical Mixtures: Application of a Tiered Approach
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Evaluating potential health risks posed by exposures to chemical mixtures is challenging for toxicology research and risk assessment. Considering the current challenges in food mixtures, a hypothetical case study was conducted using a tiered screening approach, that utilized simple conservative screening assumptions in
lower tiers to focus resources on assessing chemicals of greater concern in higher tiers. Non-cancer hazards associated with chronic oral exposures were evaluated using the hazard index (HI) method and the target organ toxicity dose approach (TTD). A hypothetical new bean product is being considered to replace pinto beans in a food program. Comparison of old and new beans (NB) there is a 10% decrease in the following: cadmium (Cd), deltamethrin (De) and efluthrin. The What We Eat in America Food Commodity Intake Database (WWEIA-FCID) was used to estimate pinto bean consumption rates (PBCR) in the general U.S. population in Tier 1. This tier provided a crude filter using recent, conservative health reference values and then refined in subsequent tiers. As the HI exceeded 1, in Tier 2, we refined PBCR distributions across different age groups and assessed exposures among Mexican Americans and children <12 years of age, groups exhibiting the highest PBCRs. HI estimates and the Risk 21 model were used to prioritize refinements in Tier 3; Cd and De were the largest contributors to HI in Tier 2. In Tier 3, De and Cd exposure estimates were refined using biomonitoring data and recent Cd intake estimates from the Total Diet Study (2014). Between Tiers 2 and 3, HI estimate decreased from 35 to 0.08 in the 95th percentile youth NB consumers. In Tier 4, constituents were grouped based on specific health effects, resulting in a HI estimate of 0.01 for NB among 95th percentile youth consumers using the TTD approach. Tiered approaches are useful and resource conserving as they can be implemented to effectively screen the effects of chemical mixtures in a health protective manner. This abstract does not necessarily reflect U.S. EPA policy.

1378 M ixtures of Full and Partial Agonists: Comparison of a Pharmacologic Model with the Toxic Unit Extrapolation Method and Generalized Concentration Addition

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Concentration addition is commonly used for judging interaction of mixtures of compounds that act by similar mechanisms. But concentration addition cannot be applied at effect levels above that achievable by the least efficacious compound in the mixture, i.e., the critical effect level. As partial agonists are common, two approaches have been suggested for mixtures of full and partial agonists. Generalized concentration addition (GCA) requires that dose response curves for individual compounds be specified as mathematical functions that are invertible, yielding real numbers. The Toxic Unit method (TU) extrapolates to above the critical effect level by assuming that the dose/EDx ratio for a partial agonist is constant above some value (EDx is the dose that achieves effect level x). This model is applied in mixtures experiments with ray designs. We compare the GCA and TU approaches with a pharmacologic model for systems of ligands (full or partial agonist) and a receptor with a single binding site, followed by activation of the ligand-receptor complex. For mixtures with three components, the isoiboles of this pharmacologic model are negatively sloped planes below the critical effect level and positively sloped planes above. TU diverges from the pharmacologic model above the critical effect level. TU assumes that toxic units are non-negative, but in the pharmacologic model, the partial agonist can act as a competitive antagonist above the critical effect level and add to the negative toxic units. For binary mixtures, we show that TU will be equivalent to assuming a toxic equivalence system, i.e., isoiboles that are parallel, negatively sloped straight lines. For mixtures of three compounds, TU produces isoiboles that are negatively sloped surfaces, not necessarily planes. TU has much greater flexibility than GCA in the choice of dose-response curves it can use for individual compounds, but GCA better predicts behavior of the pharmacologic model, at least in this simple system.

1379 The Silicoat Project: In Vitro and In Vivo Toxicity Screening of Quartz Varieties from Ceramics Industry and Approaches for an Effective Quartz Surface Coating


The IARC has classified respirable crystalline silica (RCS) as human carcinogens (category 1; 1997), however, acknowledging evident differences among RCS varieties. In the traditional ceramics industry, quartz containing materials are indispensable for the manufacturing process. The SILICOAT project thus aims at increasing workers’ safety by developing cost-effective RCS coating technologies into ceramic processes to reduce quartz-specific toxic effects. As the first step 25 quartz-containing ceramic materials were screened in vitro (primary rat alveolar macrophage cell line (PMN), and in vivo (intratracheal instillation rat study) for their biological activity using highly active quartz DQ12 as positive control. Initial cytotoxicity screening processes to reduce quartz-specific toxic effects. As the first step 25 quartz-containing ceramic materials were screened in vitro (primary rat alveolar macrophage cell line (PMN), and in vivo (intratracheal instillation rat study) for their biological activity using highly active quartz DQ12 as positive control. Initial cytotoxicity screening experiments revealed no marked cyto toxic effect for all quartz-free (calcite, dolomite, alumina) and fieldspar bulk samples and almost all wet milled materials. However, bulk samples exhibited variable quartz-related cytotoxic potential. Samples with the highest quartz content were further investigated on clastogenicity (comet assay) and pro-inflammatory CXCL2 gene expression (qRT-PCR). All quartzes and kaolins, but no clay mediated DNA damage and all materials induced CXCL2 gene expression. An in-vitro validation study elicited lack of effect for clays and an initially retarded, but after 28 days progressive effect for quartzes (PMN, LDH). The most active quartz was used for promising covalent organosilane-based coating strategies in conclusion. Screening demonstrated gradually different quartz-specific or - independent biological activity of ceramic raw materials and indicated attenuation of quartz effects by wet milling with alumina balls. - Funded by the EU (FP7) under grant agreement n° 285787.0.

1380 Hist io pathological Ch agoes in the Kidney of Mouse, Mus norvegicus albinus, on Exposure to Fluoride, Aluminum, and Mixtures

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Fluoride is one of the chemical contaminants of water causing several health problems in man and animals and aluminium is the most abundant metals in the environment. Earlier studies exhibited fluorosis and aluminosis when fluoride and aluminium exposed individually. However, it is important to study two different toxicants in combination to know whether they can act as synergistic or antagonistic. In this study the toxic effects exerted by the accumulation of fluoride, aluminium and their mixtures were studied in the kidney of mice. Six different doses were provided for a period of 30 days and 60 days for their toxic effect in the kidney. Microscopic study of kidney tissue revealed interesting pathological changes in all the fluoride treated mice. Areas of cloudy degeneration, necrosis of proximal tubules and haemopoetic tissue could be resulted due to increased fluoride ions accumulation. The damage to the tissue was dose-and time dependent. The histopathological changes noticed in the kidney of mouse treated with aluminium are more or less as seen in fluoride treated mouse like vacuolation and disintegration of the epithelium of the kidney tubules, dilation in glomeruli and internal haemorrhage. In addition, aluminium specifically produced hydropic degeneration. The aluminium treated mice showed conspicuous histopathological changes in higher dose, which was more significant with the increase in period of exposure. Whereas, the histopathological changes, were not much conspicuous in lower dose groups, and also decreased with period of exposure. In the mixture of fluoride and aluminium treated mice, mild to severe changes were observed in the higher dose of fluoride with aluminium, whereas very mild and insignificant changes were observed in the mice exposed to lower dose of fluoride with aluminium. It is suggested that detoxification mechanisms could alleviate the fluoride toxicity in mice at longer period of exposure with lower doses of fluoride.

1381 An Assessment of the Impact of Concomitant Exposure and Human Variability on the Calculation of LEQ for Three Drinking Water Contaminants

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In risk assessment, “Liter equivalent” (LEQ) refers to the volume of water that needs to be ingested to absorb an equivalent dose of drinking water contaminants (DWQ) by other routes of exposure. It is typically calculated for single substances, in adults only. The objective of this study was to evaluate the impact of concomitant exposures on the calculation of LEQ for benzene (Bz), tri- and tetrachloroethylene (TCE, PERC), in adults as well as other subpopulations. A multistate probabilistic PBPK modelling approach was used to compute LEQ on the basis of absorbed dose and internal dose metrics in infants (2-6 mo), toddlers (1-3 yrs) and adults. Exposure scenarios considered a 30 min bath along with daily drinking water ingestion rates for each subpopulation. Exposure concentrations were chosen based on U.S. EPA’s 10-day drinking water guidelines: i) 200 µg/L of Bz with or without 2 mg/L of toluene (Tol); ii) 200 µg/L of TCE or 2 mg/L of PERC with or without 3 mg/L of vinyl chloride (VC). Distributions of subpopulation-specific parameters were taken from the literature and Monte Carlo simulations were performed. Mean LEQs based on respectively simulated amount metabolized (AMET) and area under the curve of blood concentration (AUC) for exposure to single substances were as follows for Bz: 1.28 and 39 in infants, 1.14 and 10.70 in toddlers, and 3.57 and 29.12 in adults; for TCE, 0.67 and 1.74 in infants, 1.12 and 6.31 in toddlers, and 6.10 and 26.56 in adults; for PERC, 0.76 and 1.22 in infants, 1.22 and 1.98 in toddlers, and 7.21 and 11.68 in adults. Also, LEQs based on AUC of TCE and PERC’s circulating metabolite, TCA, were computed and
results were similar to those based on AMET. The addition of an inhibitor had virtually no impact on LEQ values and their distributions. In conclusion, these results suggest that for the simulated concentrations, co-exposure to one other substance has no impact on the calculation of LEQ for Bz, TCE and PERC, regardless of the subpopulation considered.

1382 In Vitro Exposures to Isoprene-Derived Secondary Organic Aerosol: Assessing the Effects of SOA on Inflammation-Associated Gene Expression of BEAS-2B Using a Direct Deposition Approach

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Isoprene (2-methyl-1,3-butenadiene), the most abundant non-methane hydrocarbon emitted into Earth’s atmosphere primarily from terrestrial vegetation, has been recently recognized as a major contributor to the global secondary organic aerosol (SOA) burden. Recent work from our laboratory has shown that anthropogenic pollutants, such as nitrogen oxides and sulfur dioxide, enhance isoprene oxidation leading to SOA formation. Because SOA formation due to isoprene is a relatively new discovery, there is currently no research on the health effects of isoprene-derived SOA. Current understanding of the effects of gaseous products from oxidation of isoprene and its 1,3-butenadiene, on cytotoxicity and inflammation suggests that there may be similar effects associated with inhalation of isoprene-derived SOA. This study focused on investigating dysregulation of inflammatory gene expression associated with the inhalation of isoprene-derived SOA formed in the presence of acidic sulfate aerosol and initially high nitric oxide (NO) conditions. Outdoor smog chamber experiments were conducted to systematically generate SOA from the photochemical oxidation of isoprene and linked with a direct deposition in vivo exposure system. Human bronchial epithelial cells (BEAS-2B) were exposed to the chemical mixture generated in the chamber using an instrument called the electrostatic aerosol in vitro exposure system (EAVES). Measurements of gene expression of the inflammatory biomarkers (IL-8 and COX-2) in exposed cells together with complementary chemical measurements showed that a mass concentration as little as 20 μg/m³ of organic material, formed from isoprene photo-oxidation, leads to a significant increase in IL-8 (fold change: 2.06, p<0.0005) and COX-2 (fold change 2.31, p<0.005) mRNA levels. These data support recent work that demonstrate oxidized organic aerosols potentially induce adverse biological effects in human populations.

1383 Study of Bisphenol A and Naproxen Metabolic Interactions in Isolated Perfused Rat Liver

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Recently, the non-steroidal anti-inflammatory drug naproxen (NAP) has been shown to competitively inhibit the glucuronidation of Bisphenol A (BPA) in rat and human liver microsomes. It remains to be determined of whether this in vitro information is reflective of the in vivo situation. The objective of this study was therefore to characterize this interaction on a whole intact organ by using the isolated perfused rat liver system (IPRL). Rat livers were cannulated and perfused with a Krebs Henseleit-buffer containing BPA alone, NAP alone or in combinations at different concentrations. Using an antegrade single-pass perfusion, the rate of clearance of BPA and NAP was calculated using the measured input and output chemical concentrations in perfusate. The perfusions (n=3) were performed in a continuous flow mode for 2 hours at 37°C with a constant flow rate 20 ml/min. The analysis by nonlinear regression of Michaelis-Menten enzyme kinetics were made for the metabolism of the pollutant (Vmax = 530 nmol/min/g liver, Km = 51.6 μM) and the drug (Vmax = 178.5 nmol/min/g liver, Km = 149.2 μM) using Sigmaplot 13.0. The analysis of the combined exposures suggest that the metabolism mechanism of BPA is partially competitive inhibited in the presence of drug NAP with estimated Ki value (alpha= 2049.7, Ki = 0.3542 μM). Although both in vitro and IPRL metabolism show inhibition, the IPRL is not fully competitive as in the in vitro study. Nevertheless, the scaled in vitro Vmax and km values for BPA are very comparable to values from this IPRL study. The observed differences between both systems may result from metabolic pathways that are not accounted for in the micromass assay. Further investigations are needed to understand the origin of the in vitro-in vivo discrepancies.

1384 A Weight-of-Evidence (WOE) Approach to Include Nonchemical Factors in Chemical Mixtures Risk Assessment

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Scientific evidence links chemical and nonchemical stressors (e.g., noise, stress) to human health risk, but methods are not available to integrate such information into chemical risk assessments. Weight of evidence approaches (WOE) are often used in environmental and health sciences to derive conclusions from diverse data. The Agency for Toxic Substances and Disease Registry (ATSDR) developed a WOE approach for chemical-chemical interactions that is also now used by other governmental agencies and organizations. The ATSDR WOE approach was adapted to integrate the influence of nonchemical factors on chemical toxicity and risk assessment. As part of this process, a systematic analysis of published data was conducted. The information collected was weighted based on quality, consistency, and relevance to toxicity. The components of this proposed new approach include the direction and type of impact, strength of the adverse outcome pathway, and relevance of the available data. Analysis of benzene and toluene toxicological and epidemiologic data using this proposed approach revealed varying degrees of confidence in the outcome data. The confidence was low for the impact of toluene exposure and stress on neurodevelopmental toxicity. It was medium for two pairs of stressors on reduced birth weight (a) benzene exposure and stress impacted the outcome (b) benzene exposure and noise did not. It was high for toluene exposure and noise on hearing loss. It was also high for benzene exposure to persons with NAD(P)H:quinone oxidoreductase deficiency on bone marrow toxicity. Further analyses with additional data sets will allow the development of generic principles for integration of these factors in cumulative risk assessment, as well as formulation of testable hypotheses. When fully developed, this approach will allow determination of the influence of various nonchemical stressors, health conditions and socioeconomic factors on the toxicity of chemicals.

1385 Predicting Joint Effects of PPARgamma Ligands Using Generalized Concentration Addition

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Dose addition (or concentration addition) is a null model commonly used to test hypotheses about the effects of mixture exposures on nuclear receptor activation. Although it is applicable to modeling effects of mixtures containing multiple ligands with shared curve maxima (e.g. two full agonists), it cannot predict joint effects of full and partial (i.e. sub-maximal efficacy) agonist combinations. We previously developed the Generalized Concentration Addition (GCA) model to address this scenario with aryl hydrocarbon receptor activation. Here, we implemented a transactivation assay using a mouse peroxisome proliferator-activated response element (PPRE)-dependent luciferase reporter to determine joint effects of environmental exposures of full and partial peroxisome proliferator-activated receptor gamma (PPARγ) ligands (rosiglitazone, non-thiazolidinedione partial agonist (nTZDpa), mono-2-ethylhexyl phthalate (MEHP)). We then tested the GCA prediction, based on the individual chemical dose response curves, versus the empirical receptor activation by the mixtures. We demonstrate that GCA adequately models the joint response surface of a binary mixture activating PPARγ and highlight the limitations of the Toxic Equivalency Factor and Effect Summation approaches to full and partial agonist mixtures. Further, we show that the efficacy of a dual RXRα/PPARγ ligand (the environmental contaminant tributyltin (TBT)) for PPRE transactivation is unaltered by increasing concentrations of a PPARγ partial ligand. We extended these results to test the ability of the GCA model to predict lipid accumulation (quantified by Nile Red Fluorescence) in mouse mesenchymal stromal cells (mMSCs), a well-established downstream effect of PPARγ activation, in response to these ligand mixtures. These studies support the application of GCA modeling in predicting combinatorial toxicant effects from independent dose-response curves.

1386 Enhancing the Chemical Mixture Methodology: New Approaches Using Target Organ System Effects, Mode of Action, and Specific Target Organ Effects


The Chemical Mixture Methodology (CMM) is used by the US Department of Energy (DoE) and other governmental and private organizations for emergency response planning. The CMM provides a conservative estimate of the potential adverse health effects that may result from the exposure to airborne mixtures of
hazardous chemicals. The CMM first calculates the hazard index (HI) for each chemical in an airborne mixture. The HI is airborne concentration of the chemical at a receptor point divided by a selected health concentration limit. For most studies, the limit used is the concentration of the chemical at which an irreversible or serious health effect could impair a person’s ability to take protective actions. The HI for each chemical is then summed to provide an initial screening estimate. If the sum of the HIs is >1, protective actions may be advised and a more detailed assessment is conducted. Health code numbers (HCNs), which are similar to medical diagnostic codes, are used to identify the target organ systems (e.g., respiratory system, nervous system) that may be significantly impacted by exposure to a chemical. HIs are then summed only for chemicals in the mixture that share the same HCN category (i.e., effect the same target organ system). Health effects are evaluated using several different HCN categorization approaches that were substantially enhanced from the previous versions of the CMM. These new approaches involve Target Organ System Effects (TOSE), Mode of Action (MOA) effects, and Specific Target Organ Effects (STOE). The revised approach was thoroughly tested using 127 test mixtures evaluated using three different concentration distributions, for a total of 381 test cases. Over 23% of the test cases showed decreases in the HCN-based sum of the HI values when compared with results using the previous approaches. This reduction in over-conservatism will allow emergency planning efforts to focus on those potential release scenarios that pose significant health effects to workers and members of the public.

1387 Health Hazard Assessment Summary of Alcohol-to-Jet (ATJ) Alternative Jet Fuels

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Alcohol-to-jet (ATJ) synthetic paraffinic kerosene (SPK) fuels are produced by dehydrating and refining isobutanol; feedstocks may be non-renewable (petro-chemical) or renewable (fermented sugars from plant oils and animal fats). Toxicity testing was performed to allow recommendation of an occupational exposure level (OEL) for ATJ SPK fuels. Each toxicity test was performed with neat fuel plus a required JP-8 additive package. Four ATJ SPK fuels were tested to determine dermal irritation potential. New Zealand white rabbits were exposed to the fuels for 4 hr. ATJ SPK fuels were found to be slightly and moderately irritating under semi-occluded and occluded conditions, respectively; a petroleum derived JP-8 scored similarly. In a 90-day inhalation study, Fischer 344 rats (10/sex/concentration) were exposed to a renewable ATJ SPK fuel in an aerosol/vapor mixture (0, 200, 700 or 2000 mg/m³, 6 hr/dy, 5 dy/wk). Mild to moderate nasal respiratory epithelium lesions occurred in males and females in the 2000 mg/m³ exposure group. Minimal severity olfactory epithelium lesions occurred with low frequency (similar to control incidence) among the 700 mg/m³ exposed rats. Additional rats (5/sex/concentration) were simultaneously exposed for 2 weeks to test for micro-nucleus formation. Exposure-related trends in bone marrow cell toxicity were not observed and micronucleus formation did not increase, indicating that ATJ SPK is not clastogenic. Four ATJ SPK fuels were tested for mutagenic potential using the reverse mutation assay (Ames test), with and without metabolic activation; neither fuel was found to be mutagenic. Overall, ATJ SPK fuels were found to be similar to or less toxic than petroleum-derived JP-8. Handling of ATJ SPK fuels alone or in a 50:50 blend with petroleum JP-8 is unlikely to increase military health risks. The or less toxic than petroleum-derived JP-8. Handling of ATJ SPK fuels alone or in a 50:50 blend with petroleum JP-8 is unlikely to increase military health risks. The or less toxic than petroleum-derived JP-8. Handling of ATJ SPK fuels alone or in a 50:50 blend with petroleum JP-8 is unlikely to increase military health risks.
Trace metal ions, such as cadmium (Cd), and trace organics typified by 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD), are common co-contaminants in the environment that pose serious health risks. However, the knowledge of their joint toxic effects is largely unknown. In this study, we evaluated the combined effects of Cd\textsuperscript{2+} and TCDD on the regulation of cytochrome P450 1A1 (CYP1A1), vitellogenin (VTG), and metallothionein (MT) gene’s expression levels as revealed by using real-time PCR in zebrafish embryo, larva, and kidney and intestine of adult zebrafish. The LC50 values of Cd\textsuperscript{2+} in embryo, larva, and adult zebrafish were also determined before treatment. Then different stages of zebrafish were treated with 1 ppb of TCDD and 1%, 25% and 50% LC50 of Cd\textsuperscript{2+} for gene expression analyses. The 24 h LC50 values of Cd\textsuperscript{2+} in embryo, larva and adult zebrafish are 80 μM, 8 μM and 25 μM respectively. Our results showed that the induction of CYP1A1 gene by TCDD can be significantly decreased by Cd\textsuperscript{2+} in zebrafish larvae, larva, kidney and intestine of female zebrafish and liver of male zebrafish. Conversely, however the induction of CYP1A1 gene by TCDD can be further increased by Cd\textsuperscript{2+} in the embryo. In the kidney and intestine of male zebrafish, the inductions of CYP1A1 were not significantly affected by Cd\textsuperscript{2+}. For VTG gene, J. R. Maddox\textsuperscript{5} and J. B. Gelineau-van Waes\textsuperscript{5}. in embryo, larvae and adult zebrafish tissues. The different regulation of Cd\textsuperscript{2+} and TCDD on these three biomarker genes will help us better understand the toxic mechanisms of combined chemical contamination. The implications of our results will be discussed in our presentation.

**1392 Detecting Fumonisin-Induced Changes in Putative Sphingolipid Biomarkers in LM/Bc Mice and Humans Using Blood Spots**

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Fumonisins (FBs) are mycotoxins found in maize. They are hypothesized to be potential risk factors for neural tube defects (NTDs) in humans living in areas where maize is a dietary staple and infection with Fusarium verticilloides is likely. In LM/Bc mice, FB1-treatment of pregnant dams induces NTDs and results in increased levels of sphingoid base 1-phosphates in blood and tissues. The increased level of sphingoid base 1-phosphates in blood is a potential biomarker for FB1 inhibition of ceramide synthase in humans. Collection of blood spots on absorbent paper so as to allow quantification of the mass amount of sphingoid base 1-phosphates normalized to blood volume. To accomplish this objective, blood was collected from unexposed and FB1-exposed LM/Bc mice and urine and feces were collected at 2, 4, 8, 12 and 24 h post exposure. DON concentrations were measured using a high sensitivity competitive ELISA (Food Chem. Toxicol. 46:2826-2831). ELISA cross reactivity with the DON-3 glucuronide, the principle DON metabolite in rodents, was determined to be 100%. The rank order of DON tissue concentrations over time reported as area under the curve was observed to be: kidney > liver > plasma > spleen > heart > brain. Overall, DON organ concentrations were higher in elderly males in comparison to adult males, and higher in adult males in comparison to adult females. Urinary DON excretion occurred more rapidly in adult females than adult males. Elderly males exhibited particularly slow urinary DON excretion and at 4 hours post exposure had only eliminated 25% of the dose while adult males had eliminated 66% of the dose. These results suggest that impaired metabolism and excretion of DON corresponds to prolonged feed refusal in adult and elderly male mice in comparison to adult females. Future research exploring population susceptibility to DON-induced anorexia should consider metabolic differences as contributing factors to susceptibility.

**1393 Combined Toxic Effects of Cadmium Ions and Dioxins on Zebrafish**

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The inductions of CYP1A1 were not significantly affected by Cd\textsuperscript{2+}. For VTG gene, J. R. Maddox\textsuperscript{5} and J. B. Gelineau-van Waes\textsuperscript{5}. in embryo, larvae and adult zebrafish tissues. The different regulation of Cd\textsuperscript{2+} and TCDD on these three biomarker genes will help us better understand the toxic mechanisms of combined chemical contamination. The implications of our results will be discussed in our presentation.

**1394 Sex and Age Are Critical Factors That Impact Deoxynivalenol Clearance in Mice**

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Deoxynivalenol (DON), a trichothece mycotoxin, is a common cereal grain contaminant that is resistant to processing, leading to contamination of human and animal food. The aim of this study was to test the hypothesis that increased susceptibility to DON-induced anorexia observed in elderly and adult male mice is, in part, due to impaired organ clearance and excretion of this mycotoxin. We employed a mouse anorexia bioassay previously described by our lab (Food Chem. Toxicol. 49:1863-1869) to compare the effects of DON exposure in adult female, adult male, and elderly male mice. Mice were acutely exposed to 0 and 1 mg/kg bw DON via IP injection. For organ clearance studies, animals were euthanized at 1, 2, 4, and 12 h post exposure. For excretion studies, mice were acclimated to metabolic cages, exposed to DON, and urine and feces were collected at 2, 4, 8, 12 and 24 h post exposure. DON concentrations were measured using a high sensitivity competitive ELISA (Food Chem. Toxicol. 46:2826-2831). ELISA cross reactivity with the DON-3 glucuronide, the principle DON metabolite in rodents, was determined to be 100%. The rank order of DON tissue concentrations over time reported as area under the curve was observed to be: kidney > liver > plasma > spleen > heart > brain. Overall, DON organ concentrations were higher in elderly males in comparison to adult males, and higher in adult males in comparison to adult females. Urinary DON excretion occurred more rapidly in adult females than adult males. Elderly males exhibited particularly slow urinary DON excretion and at 4 hours post exposure had only eliminated 25% of the dose while adult males had eliminated 66% of the dose. These results suggest that impaired metabolism and excretion of DON corresponds to prolonged feed refusal in adult and elderly male mice in comparison to adult females. Future research exploring population susceptibility to DON-induced anorexia should consider metabolic differences as contributing factors to susceptibility.
prudent course of action. This may be accomplished by undertaking a review of relevant human data, including an evaluation of study quality, which is often a function of rigor and details within the study report or publication. Factors to be considered include study type, study population, number of subjects, dose, duration, endpoints, etc. Such an analysis was undertaken with melatonin (N-acetyl-5-methoxytryptamine), an increasingly popular dietary supplement ingredient used for supporting restful sleep. The use of melatonin as a dietary supplement ingredient has previously been evaluated by authoritative and regulatory bodies such as Health Canada and the Institute of Medicine, who indicated that short-term use of 10 mg or less does not pose a safety concern in adults. In the current analysis, over 40 clinical studies were reviewed that included adverse effect reporting and/or other safety related endpoints. Doses of melatonin up to 20 mg/day, for durations ranging from several days to over 3 years, were used in healthy subjects and patients with chronic diseases and conditions such as insomnia, migraines, and dementia. Several of the identified studies were of high quality, although the dataset was limited by the small number of subjects, short duration of intervention, and insufficient detail on adverse effects in some studies. The outcome of the current evaluation is consistent with previous assessments by the aforementioned authoritative and regulatory bodies. In the absence of robust data supporting longer-term and/or higher doses, consulting a healthcare practitioner may be advised.
Modulation of the Spleen Transcriptome by Aflatoxin B1 in the Turkey
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The food-borne hepato-carcinogen aflatoxin B1 (AFB1) is also a potent immunotoxin, resulting in reduced humoral and cell-mediated immunity. RNA-sequencing (RNA-seq) was used to investigate the impact of dietary AFB1 on the splenic transcriptome of turkeys, one of the most susceptible animals known to the adverse effects of this mycotoxin. We also wished to determine whether a Lactobacillus-based probiotic, showing immunomodulatory and clinical studies to be AFB1 protective, would modify expression changes. Ten-day old male turkeys were treated with probiotic (5 X 10^6 CFU/0.5 ml PBS, oral), probiotic + AFB1 (1 ppm in diet), PBS control, or AFB1 only. Birds were pretreated with probiotic or PBS for the first 10 days before starting a 10-day dietary AFB1 treatment. RNA-seq libraries were generated from 3 spleen samples per treatment group (n = 12) and were sequenced (Illumina GA Ix). More than 105 M single-end reads were produced and de novo assembled into ca. 270,000 predicted transcript fragments. 982 transcripts had significant differential expression in pair-wise comparisons. AFB1 treatment resulted in down-regulation of antimicrobial genes such as angiogenin and lysozyme G; however, other immune-related transcripts, including granulysin A, lymphotoxin, and perforin 1, were up-regulated. Increased expression of these interleukin-2 response genes could be indicative of lymphocyte activation or apoptosis. Probiotic treatment attenuated expression of some genes induced by AFB1, but the probiotic alone suppressed immune transcripts which may affect the cytotoxic potential of turkey splenocytes. One possible interpretation of these results is that probiotics alter the micro biome, altering the population of certain pathogens, and lowering expression of "maintenance" immune transcripts normally expressed in the spleen.

Pathological Changes in Acute Experimental Aflatoxicosis in Holstein Calves: Preliminary Results

Aflatoxins (AFs) are secondary metabolites that cause a detriment of productivity in farm animals. Since AFs could be in feeds; dairy cattle (DC) is exposed to AFs in a chronically, which produces subclinical changes. Aflatoxicosis in dairy cattle is difficult to diagnose because there are few clinical clinical tests. Besides, there are multiple factors involved which compound the diagnosis such as age, race, immune status, and the health of other animals in contiguity. The aim of this study is to propose certain biochemical tests as a complement to aflatoxicosis diagnosis; tests based on AF metabolism. Five day old male Holstein calves were placed into two groups: the Aflatoxin Group (AG) and the Control Group (CG). AG received AFs (3.0 mg/kg body weight) once per ore; CG did not. All calves were slaughtered after 7 days. Total Protein, Albumin, Prothrombin Time, Bilirubin, Gamma-glutamyltransferase, Alkaline Phosphatase, Alanine Aminotransferase, and Aspartate Aminotransferase were measured in plasma. The preliminary results showed that all analytes had a marked increase in the aflatoxin group compared with the control group. Moreover liver was pale and kidneys were hemorrhagic; both were friable.

The preliminary results suggest that biochemical and pathological changes were caused by AFs administration, therefore they could be used as complementary assays to diagnose aflatoxicosis in DC.

Health Hazard Assessment for Dicyaniamide and an Evaluation of Exposure via Dairy Products
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Dicyaniamide (DCD) has been applied to grazing pastures as a means to inhibit nitration that occurs in soil. It has been employed to reduce or prevent the leaching of nitrogen in soil associated with the waste products from grazing cattle into ground water and waterways. However, along with these ecosystem benefits, this environmental distribution of DCD allowed for the potential exposure to DCD by animals grazing on treated land and for the possibility of its subsequent presence in their milk. In 2012, DCD was detected at the ppb to low ppm level in milk powder and butter derived from dairy cows that grazed on pastures in New Zealand treated with this chemical. A hazard assessment for DCD exposure was first performed to evaluate the toxicity of DCD. A review of available dose-response information on the adverse health effects of DCD was conducted. DCD was found to be an acute, subacute and chronic toxix as adverse clinical effects were associated with each exposure duration. These effects include cyanosis, slowed movement, diarrhea, decreased body weight and clinical effects tied to the kidney and liver in animals. The no observable adverse effect levels for relevant critical effects were identified for each exposure duration along with related uncertainty factor(s). The tolerable daily intake (TDI) for DCD for acute, subchronic and chronic durations of exposures were determined to be 10, 12.3 and 5.4 mg DCD/kg bw/day, respectively. Next, the nature of the potential exposure to DCD associated with intake of the separate food items of milk powder and butter was evaluated. It was found that the magnitude of exposure to DCD that would occur from consumption of dry milk or butter that contained DCD at the concentration levels seen after DCD use in pastures for nitration in the New Zealand case noted above was at least 3- to 4-fold below the TDI content level associated with these dairy foods at the lowest TDI value (chronic). Hence, this analysis indicates that the consumption of the dairy products of dry milk or butter containing DCD at low ppm or lower levels would not pose a hazard to human health.
subchronic toxicity studies. The results indicate that, given the current information of benzoates and precursor compounds, additional carcinogenicity, subchronic toxicity, and developmental toxicity studies should be considered low priority.

1405 Risk Assessment of Dietary Intake of Aluminum-Containing Food Additives in China in 2009 and 2014
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Three aluminum (Al)-containing food additives were banned in China on July 1, 2014, an action triggered by a 2009 report in which dietary intake of Al in Hong Kong was noted to be approaching the provisionally tolerable weekly intake (PTWI). Since the 2009 report, JECFA doubled the PTWI from 1 mg/kg bw/w to 2 mg/kg bw/w (as of 2011) and Al intake in China was predicted to decrease by up to 86% as a result of the ban. The risks associated with remaining dietary Al intake in China from other ingredients were evaluated by (1) reviewing Al intake estimates from 2009 onward; (2) characterizing the relevant toxicological effects of Al; and (3) comparing exposures from use of Al-containing food additives in China. In 2009, mean dietary Al exposure in Hong Kong was estimated to be 0.6 mg/kg bw/w. More recently, the mean reported intakes range from 1.3 (in Shenzhen, 2014) to 1.5 mg/kg bw/w (in Guangdong, 2013). High consumption estimates were reported to be 4.7 (P95 in Shenzhen) to 11 mg/kg bw/w (P97.5 in Guangdong, 2013). The Al PTWI was doubled to 2 mg/kg bw/w in light of bioavailability data and a new NOAEL of 30 mg/kg bw/d. The oral bioavailability of Al is poor (~0.1% from foods) and Al is readily eliminated renally. Adverse effects observed in humans have been limited to cases of parental exposures (IV) and in those with compromised renal function and were not considered relevant in assessing risk of Al use as a food additive. Furthermore, neurotoxic and reproductive effects are not observed in studies with occupationally exposed workers. No studies correlating any increase in morbidity with Al-containing food additives in China were identified. In conclusion, the mean intakes of Al in China since 2009 fall below the current PTWI. Although high intake estimates exceed the PTWI, no causative relationship between Al intake from food additives and any adverse outcomes have been established and the recent ban on 3 Al-containing additives is predicted to greatly lower intakes. Thus, the remaining uses of Al-containing food additives in China are unlikely to pose a significant health risk.

1406 Accumulation of Lolitrem B Residues in the Fat of Cattle Fed Perennial Ryegrass Straw
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Farmers across the country struggle with insect damage to their pastures and forage crops which cause major losses in agricultural revenue. In some cases, this can be avoided by the use of grasses that are infected with endophytic fungi which act as insect-feeding deterrents due to the toxins they produce. However, these compounds also cause disease sequelae in livestock that ingest them, including "ryegrass staggers," a neurological syndrome in livestock caused by ingestion of Neotyphodium lolii-infected perennial ryegrass with high levels of the mycotoxin lolitrem B (LB). Due to its lipophilic nature, concerns exist for the potential public health hazard lolitrem B presents as a residue in animal products containing fat. To study the deposition of lolitrem B in the fat of animals consuming it, we conducted a two month feeding trial in 24 steers that were continuously fed endophyte-infected perennial ryegrass straw containing four levels of lolitrem B as follows (n=6/group): group I (2356 μg/kg LB); group II (1554 μg/kg LB); group III (1011 μg/kg LB); and group IV (control, 246 μg/kg LB). Tail fat biopsies were taken on day -2 and either day 37 or 65 for each steer (n=3/group for each day) and analyzed for lolitrem B via LC-MS/MS. All animals had LB below the detection limit (BLD) on day -2 (<10 ng/g). On day 37, the control group had LB levels BDL and groups I-III had levels of 25, 46 and 32 ng/g, respectively. By the end of the study on day 65, the groups had diverged in a dose response manner with LB fat concentrations of 203, 148 and 57 mg/g (the control group remained BDL). This study showed that, when chronically fed, cattle accumulate LB residues in fat tissue. However, the levels of LB detected are similar to previous studies which have stated that it likely poses little to no risk to human consumers.

1407 Acute Renal Toxicity Induced by Oral Exposure to Diglycolic Acid

Diglycolic acid (DGA) is the nephrotoxic metabolite of diethylene glycol and is an impurity produced during the synthesis of carboxymethyl starches. Carboxymethyl starches are used in both the food science and pharmaceutical industries as viscosity modifiers, emulsion stabilizers, and suspending agents/tablet binders. Acute DGA exposure has been associated with acute renal failure due to proximal tubule necrosis, cortical tubule degeneration and centrolobular hepatic necrosis. Since DGA cannot be completely removed from carboxymethyl starches the potential exists for unintended human exposure. Acute in vivo dose-response, sub chronic, or chronic studies have not been conducted with pure DGA to establish a complete dose-response assessment of this compound. To assess the acute oral toxicity of pure DGA on the kidney two studies were conducted: 1) Acute Oral Toxicity Up Down Study Procedure (OECD Test Guideline 425) and 2) a 28-day repeat oral dose toxicity study in rats (n=8 rats/group) using 7 dose groups (300.0, 100.0, 30.0, 10.0, 3.0, 1.0, 0.3 mg DGA/kg body weight) and a vehicle control. Acute oral toxicity, as determined by the up down study design, was 463 mg/kg bw. In the 28 day study, dose related effects were only observed in the 300 mg/kg bw dose group and included a marked increase in kidney weight and a decrease in feed/ fluid consumption, body weight gain and changes in blood chemistry parameters in comparison to the control and other dose groups.

1408 Dietary Glycotoxins Increase Advanced Glycation End Products, but Do Not Accelerate Atherosclerotic Plaque Formation in Diabetic Apolipoprotein E-Deficient Mice

Advanced glycation end products (AGEs) are formed both in the body and in foods during heat treatment. Endogenous AGE levels are increased in the case of hyperglycemia, and these compounds play an important part in the development of diabetic atherosclerosis (AS). It has been implicated that dietary AGEs can also accelerate diabetic AS. In addition, foods contain reactive dicarbonyls, so-called glycotoxins, which are precursors of AGEs. The role of glycotoxins in the development of AS is unknown. The aim of the study was to test the atherogenic potential of the dietary glycotoxins glyoxal (GOX) and methylglyoxal (MGO). Standard and streptozotocin-induced diabetic apoE/- males (10-12 weeks old), which spontaneously develop atherosclerotic plaques, were randomized into three different treatment groups (n=9); tap water (control), tap water with 0.01% glycotoxins, or tap water with 0.1% glycotoxins. After 10 weeks of treatment, the mice were sacrificed for blood and aortic tissue collection. The AS lesions were quantified by two different methods; enface analysis of the aortic arch and ascending aorta, and paraffin cross-sections of the aortic root. Carboxymethyllysine (CML), a major AGE, was measured in serum, liver and kidney by GC-MS. Analysis by GC-MS showed a treatment-related increase of CML in serum, kidney and liver of diabetic mice, reaching significance in serum (p=0.045) and kidney (p=0.021). Surprisingly, unexposed diabetic mice did not have a higher CML level compared with unexposed non-diabetic mice. Diabetic mice had a greater area (%) of atherosclerotic plaques compared to non-diabetic mice (p=0.001). However, there was no detectable effect of glycotoxin treatment on plaque formation in either group. In the present study, a mixture of dietary glycotoxins did not contribute to the development of diabetic AS, although an increase in both tissue-bond and circulating CML was observed.

1409 Lack of Toxicity of the Omega-3 Fatty Acids, EPA and DHA
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There is general consensus among authoritative and regulatory bodies that intake of the n-3 long-chain polyunsaturated fatty acids, Eicosapentaenoic Acid (EPA) and Docosahexaenoic Acid (DHA), is associated with potential health benefits. However, there is inconsistent or missing guidance on tolerable upper intake levels (ULs), which the Codex Alimentarius Guidelines on Nutrition Labelling indicates
should be taken into account when establishing Nutrient Reference Values (NRVs) for the general population. Thus far, there is neither a no observed adverse effect level (NOAEL) nor a lowest observed adverse effect level (LOAEL) for EPA or DHA, alone or combined. Since the establishment of an UL is dependent upon the existence of a NOAEL or LOAEL, an UL has not been set. In fact, for the last 25 years, every known comprehensive safety evaluation has concluded that there is insufficient evidence to establish an UL for EPA and DHA, alone or combined. In 2005, a workshop convened by the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) was held with the purpose of discussing a model for nutrient risk assessment. Of particular interest were nutrients without reported adverse health effects. For such nutrients, the highest observed intake (HOI) level was introduced as a strategy to provide guidance to risk managers. “The HOI is derived only when no adverse health effects have been identified. It is the highest level of intake observed or administered as reported within the (a) study(ies) of acceptable quality.” While there is a desire to establish a NRV for EPA+DHA, it will require an acceptance of utilizing HOI. Recent safety evaluations concluding an absence of sufficient evidence to establish an UL for EPA and DHA, alone or combined, provided levels at which there existed no safety concerns. The European Food Safety Authority (EFSA) and the Norwegian Scientific Committee for Food Safety (VFRM) indicated 5 g/day and 6.9 g/day respectively – levels at least 20X higher than the FAO minimum recommended intake of 250 mg/day.

**1410 Toxicity Studies of Icacinia trichantha Tuber Extract and Fractions**

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Icacinia trichantha Oliv. (Icacinaceae) is a traditional herbal medicine used in Nigeria and West Africa, locally known as “Urmunha” or “Etiajoba” among the Ibois of Nigeria, or “Gbenge” by the Yorubas of Western Nigeria. The tuber is prescribed by the herbalists to treat food poisoning, constipation, and malaria. The plant drug is also a household medicine for emergency uses; hence, virtually every household in Nigeria would keep a supply of the macerated tuber in alcohol as a first-aid medicine. Recent studies on I. trichantha have revealed hypoglycemic effect in alloxan-induced diabetic rats, antimicrobial, sedative and antiinflammatory effects in mice, and protection from death due to leptazol-induced convulsions. The study was carried to evaluate the toxicity effects of chronic administration of I. trichantha tuber extract in rats and also to evaluate the in vitro toxicity of its fractions. Graded doses (0 g/kg, 0.25 g/kg, 0.5 g/kg and 1 g/kg) of Icacinia trichantha tuber extract in feed were administered to rats for 90 days and the effects on body weight, organ weight, clinical signs, gross pathology, hematology, histology and serum biochemical parameters were evaluated. In vitro toxicity study of the crude extract, petroleum ether-soluble, ethyl acetate-soluble, n-butanol-soluble, and water-soluble fractions was also evaluated using the MDA-MB-435 human melanoma cells, HT-29 human colon cancers cells and 3T3-L1 cell lines. Chronic administration of Icacinia trichantha tuber extract in feed did not show any significant toxicity changes in any of the parameters evaluated nor were there any marked histopathological lesions. However, the crude extract and the ethyl acetate-soluble fractions displayed remarkable Cytotoxicity on MDA-MB-435 human melanoma cells, HT-29 human colon cancers cells and 3T3-L1 cell lines.

**1411 Polyphenolics from Mango (Mangifera indica L.) Suppress Breast Cancer Ductal Carcinoma by Targeting the mTOR Pathway In Vivo**

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It is estimated that 1 in 8 women will be diagnosed with breast cancer in their lifetime, and this incidence is the highest among cancers affecting women. Therefore, it is critical to develop botanical-based alternative treatments and preventive regimens. Overall, polyphenols are botanical bioactives that have demonstrated tumor-cytotoxic and preventive properties that in some cases also synergize with conventional chemotherapy, therefore reducing the required dose and reducing deleterious side effects. In this study, a galacto-rich extract from mango (Mangifera indica) and a major intestinal metabolite pyrogallate were used as treatments. Anti-proliferative activities and underlying mechanisms including the mTOR pathway was investigated in 6-8 week female athymic nude mice, which were xenografted with ductal carcinoma cells MCF10DCIS.COM into the mammary pads. Mice were orally gavaged with either a mango extract or pyrogallol at 0.8 mg/day and 0.2 mg/day of control, respectively for five weeks. Tumor volume was assessed weekly. Biomarkers for proliferation, cell death and inflammation were investigated by real-time PCR, Western Blotting, and immuno-histochemistry. Mango extract or pyrogallate significantly reduced tumor volume compared to the control. Gene expression and protein-activity along the IGFR-1-AKT-mTOR axis was significantly reduced by both treatments. AKT expression was significantly reduced by more than 50% in tumors treated with either mango or pyrogallol. IGF-R1 was also significantly reduced by 60% and 80% by mango and pyrogallate, respectively. mTOR total protein along with phosphorylated protein were also significantly reduced. Data demonstrates that mango polyphenols and their major intestinal metabolite pyrogalloyl are effective in reducing tumor size in xenografted nude mice via regulation of the IGFR-1-AKT-mTOR axis.

**1412 Aflatoxin B1-Induced Gut Microbiome Changes in Male F344 Rats**

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Aflatoxins are a group of mycotoxins commonly found in maize and groundnuts. The most potent and abundant aflatoxin congener, aflatoxin B1 (AFB1), is classified as a group-1 human carcinogen by IARC due to its role in hepatocellular carcinoma carcinogenesis. Multiple mechanisms have been suggested to contribute to the acute and chronic toxicity of AFB1, including genotoxicity and immunotoxicity. Gut microbiota has long been suggested to play an important role in human physiology and pathogenesis. High-throughput sequencing technologies now make it possible to determine the response of gut microbiota after exposure to carcinogens. We conducted a preliminary dosing experiment to explore whether AFB1 would modify the gut microbiota in a rat model. Male F344 rats were randomly allocated into three dosing groups: control, low-, and high-AFB1 (5 per treatment group). Rats were gavaged daily with control vehicle (DMSO) or AFB1 in DMSO (25 or 75 μg AFB1 / kg body weight) 5 days per week for 4 weeks, and were sacrificed at 24 h after the last dose. Microbiome DNA was extracted from faeces collected during the experiment. The V4 region of 16s rRNA was amplified and sequenced using the illumina MiSeq platform. The community biodiversity of the microbiome from each sample was analyzed based on sequencing data. Significant differences on microbiome biodiversity were found in AFB1-treated groups as compared to the control animals. Among control samples, the phylum Firmicutes are the major bacteria phylum, followed by the phyla Bacteroidetes, Verrucomicrobiaceae, Proteobacteria, with other phyla being less common. Among AFB1-dosed samples, the relative abundance of the phylum Bacteroidetes increased, whereas Firmicutes decreased. The frequency change was dose-dependent. Further work focuses on confirmation of these observations and their correlations with AFB1 toxicity using quantitative methods.

**1413 “Substantially Equivalent”: The Intersection of Toxicology and Policy in Characterizing Genetically Modified Foods (GMFs)**

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Global policy varies regarding the concept of “substantial equivalence” (SE) and genetically modified foods (GMFs). A logic model provides an illustration of an underlying conceptual framework and the logical connection within and between systems. A logic model was utilized to present the SE approach to characterizing GMFs. An underlying assumption of the analysis of GMF policy is that regulatory decisions are essential to public health and food safety. Inputs included stakeholders and resources impacted by the policy to either accept or reject the SE concept. The logic model also considers positive and negative outcomes of acceptance or rejection, including a comparative analysis of policies in the United States, the European Union, and select developing countries. Short and long-term outcomes include consideration of acute and long-term toxicity of GMFs, utilization of resources, and economic and environmental costs of GMF policies. Impacts of food safety and availability of food sources, globally, are significant factors which intersect science and society, with implications for the health of some of the world’s most vulnerable populations.


1414 Effect of Exposure of Diacetyl in Proteomics and Metabolomics Approach in Mice Model

The diacetyl (an important flavoring widely used in the food industry) react with peroxinitrite and produce acetyl radicals which attack the alpha carbon. These radicals are able to acetylate proteins and nitrogenous bases in vitro. The exposure to diacetyl is associated to several illnesses, mostly lung diseases. The non controlled radical acetylation may cause changes in protein and biomolecular functions and genetic failure which implies in diseases and mutation and possible change in metabolism function. The purpose of this work is to verify the radical acetylation caused by peroxinitrite/diacetyl reaction in vitro and the influence of these changes in the proteomic and metabolomic expression in mice exposure of diacetyl. The answers to these questions may contribute to understanding the origin of the several illnesses and it will drive the need to control the use of diacetyl in food. Twelve weeks old C57 black mouse were treated by oral administration of diacetyl in water, they were divided in seven groups: Male and Females controls; female that received 540 mg/kg/day, male that received 100, 200, 300, 400 and 500 mg/kg/day of diacetyl in water. The tissue samples were frozen in liquid nitrogen immediately after sacrifice, proteins were extracted, lung and liver samples were submitted to in-solution trypsinization and analyzed by mass spectrometry using MALDI TOF/ MS/MS and LC-MS/MS. Plasma samples were subjected to metabolomics analysis using the Biocrates Absolte ID Kit analyzed with UPLC-MS/MS. MS analyses of lung and liver samples by control and treated groups revealed differences in protein expression in liver and lung tissues, that are acetylated in the treated group but are unlabeled in the control groups: CPV1; P2Y12; LYZ1; NRIP2; PRIMA; SUZ12; MMAB; SP011. CHD9 and TOP3A. The other groups were submitted to metabolomics assay, and we found differences in metabolic profile, amino acids were increased, biogenic amines were mostly decreased compared to control group.

1415 Avian Reproductive Toxicology Studies: Historical Data– Coturnix coturnix japonica

The avian reprotoxicity study is the only routine regulatory, specific test for reproductive performance of non-mammalian terrestrial vertebrates. The study is a standard part of testing of agrochemical products such as seed coatings or granules which may be consumed by wild birds following application by farmers on agricultural land. Other chemical products that may trigger a requirement for avian reprotoxicity testing based on exposure scenarios and/or physicochemical characteristics include Biocides, REACH chemicals and Pharmaceutical products (under environmental risk assessment). The test is also a constituent of the testing package for endocrine disruptors, which may increase demand for this type of testing in the future. In this presentation we have compiled reference data from avian reprotoxicity tests performed in our facility. The study design involves exposure of birds, usually Japanese Quail (Coturnix coturnix japonica) in the diet or via drinking water. Birds are housed in cages of one male and one or two females. Suitable environmental conditions apply, in line with recent EU animal welfare regulations requiring a cage area of 1 m² for each pair of birds. After an acclimatization period, birds are usually subject to a short day-length for approximately 8 weeks during which time females become mature but egg laying is not fully initiated; day-length is increased and for approximately 9 weeks, birds are in full egg production. Eggs are counted and collected daily and incubated to hatching. Measurements made include effects on the parental birds, egg production, egg shell thickness, hatch-ability and chick growth to day 14. Historic data on Japanese Quail in this facility include mean values of: Eggs per hen/week=5.82 ; Egg weight=12.53 g ; Eggs/Shell thickness=0.24 mm ; Viability=81.3% ; D0 Chick weight=8.7 g ; D14 Chick weight=41.7 g. The total number of eggs evaluated is well over 2000. Reference: OECD Test Guideline 206, Paris 1984.

1416 The Effects of Long-Lasting Hypoglycemia on Male Reproductive Organs in Rats

Glucose has an important role in spermatogenesis. Nevertheless there are few reports in which effects of long-lasting hypoglycemia on male reproductive organs were evaluated. To evaluate the effects, insulin at some dose levels was administered subcutaneously to male rats twice a day for one month. By the dosing regimen, plasma glucose levels rapidly decreased after dosing and the low glucose levels lasted for several hours through the dosing period. During the dosing period, no abnormalities were observed in clinical signs and body weight. No statistically significant differences were noted in the organ weights of testes, epididymides, prostate, seminal vesicles, and pittitary glands. In the histopathological examination; degeneration of seminiferous tubules in testes and ephidymal ducts, germ cell lumps in lumen of epididymides as a secondary change of the testicular lesions were observed in the insulin-treated animals. The incidence rose with an increase in the dose level. The sperm examination of the highest dosage group showed the average sperm concentration with a tendency toward decrease and the average incidence of sperm malformations with a tendency toward increase. The possibility that the testicular lesions were caused by disruption of sex hormones was considered to be low because there were no changes in the weights of prostates and seminal vesicles which were well known to be highly sensitive to changes of circulating concentrations of the hormones. Because germ cells in testes rely on glucose and lactate which are converted from glucose, an inadequate nutritional status seems to affect spermatogenesis. Our result suggests that long-lasting hypoglycemia affect male reproductive organs in rats.

1417 Nonhuman Primate Pre/Postnatal Studies: Does Measurement of Monoclonal Antibodies in Breast Milk Provide Useful Information?

More than 40 therapeutic monoclonal antibodies (mAbs) have been approved or are in review in the European Union or United States, and it is estimated that the number of approved biopharmaceuticals will double in the next 7–10 years. Many mAbs are pharmacologically active only in nonhuman primates (NHPs) and therefore, the use of NHPs in nonclinical safety assessment, including developmental and reproductive toxicology (DART), has increased in recent years due to the growing number of biotechnology-derived drugs. For several mAbs (golimumab, natalizumab, omalizumab, ustekinumab, rituximab), low levels of drug in NHP breast milk have been reported in the literature. These nonclinical data typically appear in the pregnancy sections of the package inserts of the approved drugs. Most mAbs are constructed on an IgG scaffold, and IgG antibodies represent only a small component of the total antibodies in breast milk (IgG predominates), are not absorbed across the infant gut in humans and NHPs, and are susceptible to proteolytic degradation. IgG antibodies in breast milk are therefore unlikely to contribute substantially to infant systemic exposure in humans or NHPs. In order to better understand the toxicological significance of low levels of mAbs in NHP breast milk, concentration values from enhanced Prenatal and Postnatal studies (pPND) studies conducted at Charles River were evaluated along with data reported in the literature. These data indicate that: 1) the success rate of milk sample collection in NHPs is generally <50 percent; 2) the excretion of mAbs in NHP breast milk is highly variable; 3) concentrations of mAbs in NHP breast milk are generally very low compared to serum levels; and 4) any minimal contamination of the milk (e.g., with blood, which cannot be visually identified) can substantially contribute to the observed concentrations. Considering these factors, the measurement of mAbs in the milk in NHP pPND studies may have limited value in safety assessment.
caudal arches, which showed a high incidence at all dose levels, including control. There were also no effects on fetal body weight or on other ossification sites; therefore the increased incidence of unossified caudal arches with carbenzil present was not considered exposure-related or adverse. There were no exposure-related external or visceral findings or malformations. This study confirmed a low hazard of reproductive or developmental toxicity from carbenzil exposure in the diet. This study was sponsored by the TM/MBC Task Force’s MBC Technical Committee.

1419 Extended One-Generation Reproductive Toxicity Test in Rats Exposed to 3-Nitro-1, 2, 4-Triazol-5-One (NTO)

NTO, an insensitive, energetic material used in explosive formulations, induced testicular toxicity and oligospermia in repeated-dose oral toxicity tests. To evaluate whether the testicular toxicity is indicative of further reproductive/developmental effects, a modified extended one-generation reproductive toxicity test was conducted. Rats were given ad libitum access to NTO in drinking water at four concentrations (0, 144, 720, or 3600 mg/l NTO). Treatment of the P generation began two (females) to four (males) weeks pre-mating and continued until weaning of the litters. Direct dosing of F1 animals occurred from weaning through puberty. Number and sex of pups, stillbirths, live births, and the presence of gross anomalies in each litter were determined on post-natal day (PND) 0/1. Ano-genital distance (AGD) was measured on PND 4 and males were examined for the presence of nipples on PND 13. F1 offspring were examined daily for markers of puberty (vaginal opening (VO) and preputial separation (PS)) in females and males, respectively. At termination, blood samples were collected for clinical chemistry, hematology, and thyroid hormone analyses and a gross necropsy was conducted. The mating index, pre-coital interval, gestation index, litter size, number of live and stillborn pups, and sex ratio did not differ among control and NTO treated groups. The fertility index was slightly reduced in the 3600 mg/l (88%) compared to the control (96%). Nipple retention was increased in NTO exposed males compared to the control group (p=0.028). Age at PS was increased in the 3600 mg/l exposed males by 2.6 days relative to the control (p=0.001). Body mass at PS was not affected by NTO treatment. Age and body mass at VO were not affected by treatment with NTO. Combined with the previously documented testicular toxicity, the effects on male reproductive development suggest either an endocrine disrupting effect of NTO or a cascade of effects following testicular toxicity.

1420 Ethynylestradiol (ETU): An Extended One-Generation Reproductive Toxicity Study (EOGRTS) in Rats
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ETOX toxicity was assessed at multiple life stages in an EOGRTS. Groups of 27 male (M) and 27 female (F) rats were fed diets containing 0, 2.8/3.8, 28/38, and 140/190 ppm ETU (0.02, 2, and 10 mg/kg bw/day) for 4 weeks prior to and continuing through breeding (6-2 weeks). After breeding, P1 rats were given test diets for another 5-7 weeks (10-12 weeks total). At weaning, F1 offspring were divided into 4 groups: Cohort 1A to evaluate reproductive, endocrine, and systemic toxicity up to postnatal day (PND) 90, Cohort 1B to examine endocrine or equivocal responses up to PND 120, Cohort 2A for developmental neurotoxicity (DNT) assessments up to PND 78 and Cohort 2B for neuropathology on PND 22. High-dose (HD) P1 and F1 females and F1 males had decreased feed consumption and bodyweights. Thyroid was confirmed as the most sensitive target organ for ETU toxicity; effects included changes in thyroid hormones, thyroid weights and follicular cell hyperplasia at ≥28/38 ppm in both M and F at multiple life stages. At 2.8/3.8 ppm, non-adaptive thyroid responses (follicular cell hyperplasia) were evident. Slight hypertherpy also was seen in some pituitary cells, a normal response to hypothalamic-pituitary-thyroid axis stimulation to re-establish thyroid hormone homeostasis. At ≥28 ppm, decreased thymin weights and/or lymphoid tissue atrophy were seen in P1 and F1. There was no evidence of treatment-related reproductive toxicity or effects on the estrogen- and androgen-signalling pathways at any dose. For DNT, HD effects on overall brain size were seen in Cohort 2A, but lower dose animals and Cohort 2B rats were unaffected. These small changes in brain size were seen in adults but not weanlings; thus, these effects may be due to greater-than-nominal ETU exposures during the post-weaning period. There were no treatment-related effects on neuropathology or neurobehavioral endpoints at any dose. This study established a NOAEL based on thyroid toxicity, of 2.8 ppm (0.2 mg/kg bw/day) in M and F rats across all life stages.
EHMC is a UV blocker commonly found in sunscreens. EHMC is absorbed through the skin and is detected in human urine. EHMC was nominated to the NTP due to widespread exposure and reported estrogenic and reproductive effects; it is used in cosmetics, pharmaceuticals, and personal care products. It is a UV blocker commonly found in sunscreens and is absorbed through the skin.

**1424 Effect of Orally Administered Encenicline (EVP-6124), a Selective α7 Nicotinic Acetylcholine Receptor Partial Agonist, on Pre- and Postnatal Development of Rats**

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Encenicline (EVP-6124) is a selective α7 nicotinic acetylcholine receptor (nAChR) partial agonist being evaluated for the treatment of cognition impairment in schizophrenia and Alzheimer's disease. The α7 nAChR is present throughout the CNS, emerging early in embryonic brain development of both rodents and humans with relatively lower expression reported in the placenta and several reproductive tissues (prostate, uterus, ovary, and fallopian tubes). The role of the α7 nAChR in reproduction and development is not fully understood since α7 nAChR knockout mice are fertile and produce viable offspring. Potential effects of encenicline were assessed in a pre- and postnatal development (PND) study where timed mated Sprague Dawley rats were dosed from gestation day (GD) 6 through lactation day (LDD) 20 at 20, 60, or 180 mg/kg/day (p.o.). Offspring were evaluated on growth, viability, development, and subjected to measurements of puberty onset and estrous cyclicity. The results suggest that encenicline, at the exposure levels examined, is associated with minimal findings in offspring.

**1425 Intramuscular Administration of CpG7909 in Rats and Rabbits to Assess Fertility, Embryo-Fetal Development, and Postnatal Potential Toxicity**


CpG7909, a synthetic oligodeoxynucleotide containing CpG motifs, is an immunostimulant included in the AS15 Adjuvant System used in combination with cancer antigens in immunotherapeutics. Its potential effects on female fertility, embryo-fetal development (EFD), and pre- and postnatal developmental (PPN) was evaluated in Cr:CD(SD) rats and New Zealand White rabbits in studies designed reflecting the planned intramuscular (IM) intermittent clinical use of AS15. Intermittent IM injections of CpG7909 were administered to rats or rabbits 28 and 14 days prior to pairing, on gestation days 3, 8, 11, 15, and 24 (rabbits only) and on day 7 of lactation. Doses given to rats (as 2 injections of 100 µL/occasion) were 840, 4200 µg/kg, while doses given to rabbits (as 2 injections of 500µL/occasion) were 420, 840 or 4200 µg/kg. Viability, abortions (rabbit only), number of resorptions, fetal body weight, morphology, pre-weaning development and growth, as well as survival incidence and the attainment of developmental landmarks, were evaluated as the main end-points in both species. In rats, the no observed adverse effect level (NOAEL) of CpG7909 on female fertility, EFD, and PPN is the highest dose of 4200 µg/kg, corresponding to 500-fold the human dose (HD) (assuming 50 kg human body weight). In rabbits, 840 µg/kg was considered to be the NOAEL for maternal toxicity, based on lower mean food consumption associated with lower body weight gain (between G9 and G16/G20) in the 4200 µg/kg group, corresponding to 100-fold the HD. 4200 µg/kg was considered to be the NOAEL in the offspring, corresponding to 500-fold the HD. These data support the safety of intermittent intramuscular injections of CpG7909 at a dose of 420 µg as a component of AS15 to be used in human immunotherapeutic clinical trials. (This work was sponsored by GlaxoSmithKline Biologic S.A.)

**1426 Prenatal Exposure to Di-(2-Ethylhexyl) Phthalate May Have Two-Generation Effects on Female Reproductive Outcomes**

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Di-(2-ethylhexyl) phthalate (DEHP) is a plasticizer used in vinyl flooring, medical devices, baby toys, food containers, and automotive parts. DEHP can leach from products and expose consumers through ingestion, inhalation, and dermal contact. Prenatal DEHP exposure adversely affects the development and function of the male reproduction system, but relatively little is known about the effects of DEHP on the female reproductive system. Further, little is known about whether prenatal DEHP exposure has two-generational effects on female reproduction. Thus, the objective of this study was to test the hypothesis that prenatal DEHP exposure affects female reproductive outcomes in the F2 generation of mice. To test this hypothesis, pregnant CD-1 mice (7-10 dams per treatment group) were orally dosed daily with tocopheryl-stripped corn oil (vehicle control) or DEHP (20 µg/kg/day, 200 µg/kg/day, 500 µg/kg/day, or 750 µg/kg/day) from gestation day 10 until birth of the pups. The subsequent generation (F1) offspring were then bred to produce the F2 generation, without further DEHP treatment. On postnatal days (PND) 8, 21, and 60 of the F2 offspring, at least one female pup from each litter was euthanized, the ovaries and uterus were collected and weighed, and then subjected to histological evaluation. At PND21-60, the pups were weaned and subjected to measurements of puberty onset and estrous cyclicity. The results show that prenatal exposure to DEHP (20 µg/kg) significantly decreases uterine weight on PND21 and ovarian weight on PND60 compared to controls. Further, prenatal DEHP exposure (750 µg/kg) significantly alters estrous cyclicity so that prenatally DEHP exposed mice spend less time in proestrus compared to controls. In contrast, prenatal DEHP exposure does not affect ovarian follicle numbers or the timing of the onset of puberty compared to controls. These data suggest that prenatal DEHP exposure may have two-generation effects on some female reproductive outcomes. Supported by NIH P01 ES022848 and EPA RD-83459301.
1427 Comparative Assessment of the Effects of Prenatal Exposures to Bisphenol A (BPA) and Di (2-Ethylhexyl) Phthalate on Testicular Development in Male Rats
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The industrial chemicals bisphenol A (BPA) and di (ethylhexyl) phthalate (DEHP) are widely used in the manufacture of polycarbonate plastics and other consumer products. BPA and DEHP are known to possess hormonal activity, thereby raising concerns that exposure of the population to them may lead to adverse effects on reproductive health. The present study was designed to investigate the effects of prenatal exposures to BPA and DEHP on testicular development. Timed pregnant Long-Evans dams were gavaged with BPA at 2.5 or 5 mg and DEHP at 5 or 50 μg/kg body weight from gestational day 12 to parturition. Male weanling rats were assessed at 21, 35 and 90 days post-partum. There were no effects on mating or fertility. All animals mated within a similar number of days and all females were pregnant (except one low dose animal). There were no effects on the duration of gestation, number of post-implantation losses, number of offspring or viability index. There was no evidence of an effect of treatment with L-ergothioneine on litter size, clinical signs, body weight and sex ratio. The unexpected results of this study are that the testis is a major target for endocrine disruptors such as BPA and DEHP.

1428 Enhanced Reproductive and Behavioral Deficits Induced by Maternal Exposure to a Mixture of Low-Dose Endocrine-Disrupting Chemicals

Understanding the toxicity of low dose, endocrine disrupting chemical (EDC) mixtures is of paramount importance given recent studies demonstrating that mixtures deferentially-acting on androgens can produce additive male reproductive dysfunction and little is known about CNS effects. To assess how low dose developmental EDC exposure alters reproductive physiology and behavior, pregnant mice were exposed to relatively low doses of four EDCs. Atrazine (ATR: 10mg/kg), Perfluorooctanoic acid (PFOA: 0.1mg/kg), Bisphenol-A (BPA: 50μg/kg), 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD: 0.25μg/kg) and their mixture (MIX) or vehicle, from gestational day 7 until weaning. Anogenital distances (AGD) and reproductive organ weights were measured. Adult offspring were exposed to 3 behavioral tests to assess activity, sociality, and temporal discrimination learning: locomotor activity, conditioned place preference (CPP) and fixed interval (FI) reinforcement schedule performance. During CPP, a unique deficit in social preference was observed in MIX exposed males. MIX mice preferentially returned to a solitary site, compared to VEH males that returned to the social site, where time was spent with a cage mate. A replicated enhanced effect of increased FI response rates was found in MIX males during the acquisition of FI behavior, suggesting an inability to inhibit early responding. Increased response rates were not seen in any other group until more than 25 sessions occurred, after which BPA and TCDD male FI rates also increased. MIX exposed females did not show increased FI response rates, however, TCDD females increased rates dramatically. Deficits did not correspond to locomotor activity. An enhanced effect of the MIX was also seen on male reproductive physiology, with MIX males having longer AGDs and greater penile weights. ATR males had significantly lower testicle weights. Taken together, reproductive and behavioral deficits occurred after exposure to relatively low maternal doses of EDCs with enhanced effects seen in males.
but the erythrocyte count, packed cell volume, haemoglobin concentration and haematometric indices, were significantly decreased (p<0.05) at 1600 mg/kg-1 dose. Markers of hepatic damage (Aspartate aminotransferase and alanine aminotransferase) and renal damage (urea and creatinine) were significantly elevated (p<0.05) at 800 and 1600 mg/kg-1. The bioactivity (reproductive) study revealed significant increases (p<0.05) in testicular weight, spermatozoa count and motility, and serum testosterone levels, at the 200 and 400 mg/kg-1. The study concludes that the extract of Alchornea cordifolia leaves has toxic potential at 800 mg/kg-1 and 1600 mg/kg-1 doses, but is safe and has beneficial effects on male reproduction when used at doses equal to or lower than 400 mg/kg-1.

**1433 Enhanced Reproductive Toxicological Testing of Prochloraz at Environmentally Relevant Concentrations**

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Prochloraz is a imidazole-fungicide, registered as a pesticide under 1107/2009. In order to better understand different sensitivities of endocrine-related endpoints, we performed a pre-/post-natal reproductive toxicity study (GD 6 – PND 83) of three doses of prochloraz (effect dose, expected NOAEL, ADI) investigating all classical regulatory and additional sensitive endocrine endpoints (e.g. different subsets, extended histopathology, areaolu/nipple retention, hormones). The male and female offspring were investigated in three subsets: at PND 21, at puberty and at PND 83. Results: At the top dose of prochloraz, increased gestation lengths and lower pup viabilities were seen at maternally toxic doses. The male offspring showed slight “anti-androgenic” effects at this dose: Male offspring showed delayed sexual maturation at the top dose. However, the offspring male body weights were considerably lower at this dose. A transient increase in male offspring nipple/areola retention on PND 12, but not on PND 20, without changes in ano-genital distance on PND 1 is considered non-adverse. At the same dose, decreased male reproduction organ weights were observed in the young adult animals in the PND 83 subset only. There was no indication for changes in circulating blood hormone levels in males. No effects on fertility, or organ development were seen. Also the sperm parameters were not affected. Female animals at the top dose group had significantly increased ano-genital distances at PND 1. Females showed increased testosterone levels in blood at PND 83. No effects were noted at the lowest dose, as expected for the ADI. Conclusion: Prochloraz caused no severe adverse endocrine-related effects after perinatal dosing to rats. A NOAEL of 5 mg/kg bw was found in this study. No effects were seen at doses below regulatory NOAELs derived by EFSA. There is no evidence for a non-monotonic dose response or for any effects at an environment-relevant dose.

**1434 Reproductive Toxicity and Meiotic Dysfunction following Exposure to the Pesticides Maneb, Diazinon, and Fenamidol**

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The comprehensive identification and mechanistic analysis of reproductive toxicants constitutes one of the major hurdles in the toxicological assessment of chemicals originating from the large number of chemicals to be tested and the difficulty in examining germ cells at various stages of their development. We previously described the development of an assay in the roundworm Caenorhabditis elegans that allows the detection of chemicals bearing aneugenic activity and that could be used for the detection of germine toxicity. We present here new evidence for the reproductive toxicity of three pesticides Maneb, Diazinon and Fenamidol, three pesticides identified as part of our germine toxicity assay. We show that all three pesticides cause an acute germ cell loss in the germines of exposed nematodes in a dose-dependent fashion. The loss of germ cells coincides with the meiotic stage of pachyteny during Prophase I and is dependent on the germine apopotic machinery suggesting activation of a meiotic checkpoint. Further investigation revealed a profound dysregulation of the meiotic program revealed by (1) an alteration of the kinetics of double strand repair, (2) the disruption of the process of chromosome morphogenesis at the end of Prophase I and (3) the reorganization of the meiotic differentiation gradient inherent to the C. elegans germline following exposure to Maneb and Diazinon. These defects correlate with a significant increase in embryonic lethality and a corresponding decrease in the number of progeny. These results therefore provide strong evidence for the reproductive toxicity of Maneb, Diazinon and Fenamidol rooted in the alteration of early steps of germ cell differentiation.

**1435 Validation of an ELISA for Analysis of Inhibin B in Rat Serum**


Inhibins are dimeric polypeptide hormones which belong to the transforming growth factor β super family. The primary endocrine role of Inhibin B (IhbB) appears to be the regulation of gametogenesis via a negative feedback mechanism on the production of FSH, by the pituitary gland. In females, IhbB is a useful marker for assessment of ovarian reserve, oocyte quality, and granulosa cell tumors, and in males is a marker for spermatogenesis and testicular function. Currently, the only commercially available ELISA kits to measure InhB levels, in serum or plasma, are manufactured for the detection of human Inhibin B. We have validated an ELISA for detection of IhbB in rat serum using kits purchased from ANSH Labs (Webster, TX). The LLOQ was determined to be 24.4 pg/ml. Recovery of IhbB was determined over 6 assays. The mean slope of the regression lines was 0.9242 (R = 0.9981), and the coefficient of variation of the slope of the regression lines was 13.05%. Parallelism was determined by assaying rat serum spiked with IhbB, as well as different volumes of an incurred rat serum sample, and comparing the slopes of the resulting regression lines to the slope of the standard curve. The slope of the regression line for the High QC sample (% binding vs. volume added) was identical to the slope for the standard. The correlation coefficient (R) was 0.998. The slope of the regression line for the incurred sample vs. the slope of the regression line for the standard was not significantly different (p>0.05). The correlation coefficient (R) was 0.997. Reproducibility of the assay was established by measuring concentrations of IhbB in incurred sample in 2 assays. The inter-assay CV was 9.53%. Stability of IhbB in rat serum was measured following 3 freeze/thaw cycles, and following 6 hour storage in ice-water. The average differences in concentrations of analyte measured was -15.7% and 7.40% respectively. In conclusion, we have validated an ELISA for the measurement of IhbB concentration in rat serum. This assay should prove valuable in the measurement of IhbB as a marker for the assessment of gonadotoxicity in the pre-clinical setting.

**1436 Ethylene Glycol Monomethyl Ether-Induced Testicular Toxicity in Cynomolgus Monkeys**


Objectives: To establish the testicular toxicity model in cynomolgus monkeys, a testicular toxicant, ethylene glycol monomethyl ether (EGME) which has been reported to induce testicular toxicity in several mammalian species such as mice, rats and humans was administered to monkeys. Furthermore, circulating and testicular microRNA (miRNA) profiles involved in EGME-induced testicular toxicity were investigated by using miRNA microarray and real-time quantitative RT-PCR (qPCR) methods. Material and Method: EGME at 300 mg/kg or vehicle was orally administered to mature male cynomolgus monkeys for consecutive 4 days (n = 3/group). Plasma samples were obtained at several time points for miRNA analysis by Taqman® micro RNA assays. Histopathology was performed on Day 5. 3D-Gene® miRNA microarray analysis in plasma and the testicular samples were also performed on Day 5. Results and Conclusion: EGME induced testicular toxicity characterized by decreased number of pachyteny spermatocytes in all monkeys. In microarray analysis of plasma samples, 326 down-regulated miRNA were identified, whereas no up-regulated miRNA was noted compared with control group. In the testis, 347 up-regulated and 16 down-regulated miRNA were detected. Among these miRNA, 186 miRNA including miR-663a and miR-4741 which showed increases in the tests were decreased in plasma; however, time-course qPCR analysis revealed that decreased plasma levels of both miRNA were within individual variations. Interestingly, miR-449a and miR-34b-5p which were reported to be enriched in meiosis cells like pachyteny spermatocytes were decreased in the testis, suggesting pachyteny spermatocytes damage by EGME treatment. In conclusion, EGME-induced testicular toxicity model in cynomolgus monkeys was established, and this model would be useful for investigating mechanism of testicular toxicity. Additionally, testicular miR-449a and miR-34b-5p were suggested to be involved in pachyteny spermatocytes damage.
Comparative Mammary Gland Development in Male and Female Harlan Sprague-Dawley Rats From Bud Development to Adulthood

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Previous studies have shown that the male rat mammary gland (MG) is sensitive to ethinyl estradiol and genistein, yet little else is known about the effects of endocrine disrupting chemicals (EDCs) in male MGs. To enable future EDC testing, we compared the normal development of the MG in male and female Harlan Sprague-Dawley (HSD) rats, which has not been previously documented. Between gestational days (GD) 15.5-21.5, cross sections of male and female fetuses were collected to capture the progression of mammary bud development. On post natal days (PND) 1, 4, 8, 12, 15, 21, 33, 46 and 70, the 4th and 5th MGs were collected for H&E staining, MGs whole mount evaluation, and staining for the estrogen (ER) receptor. We show normal mammary bud development between males and females are similar from GD15.5 through GD 18.5. At GD 18.5, the skin above the female MG bud begins to invaginate, and is unchanged in the males. By GD 21.5 both sexes have branching ducts in the fat pads of the MGs with the beginning of nipple formation in the female. We confirmed that the female MG undergoes similar developmental time points to other rat strains, the ducts and alveoli have a distinct lumina lined by a single layer of cuboidal epithelial cells with a scant cytoplasm. The male MG is structurally and morphologically similar to the females before the onset of puberty. After puberty, the male MGs develop large vacuolated lobules with a pseudostriatified or stratified epithelium and no visible lumens, similar to other sexually mature male rat strains. ERα is more highly expressed in the developing female MGs than the males and AR is more highly expressed in the males than the females. ER is present only in female MGs. Because of the early life similarities in MG development between sexes, male MG development may serve as suitable marker of endocrine disruption. These studies will serve in understanding and interpreting effects of EDCs on male and female MGs in the HSD strain.

Derivation of a Maximum Allowable Dose Level for Methyl Chloride

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Methyl chloride (MeCl, CAS #74-87-3) is included on the State of California’s list of chemicals known to the State to cause cancer or reproductive toxicity (i.e., Proposition 65), due to its identification by the U.S. EPA as a male reproductive toxicant. Products containing Proposition 65 substances are subject to warning requirements, unless exposure is below California’s “safe harbor” level. For reproductive/ developmental toxicants, the safe harbor level is termed the Maximum Allowable Dose Level. As no MADL for MeCl has been established by California, the goal of this work was to derive an MADL according to CalEPA guidelines. MeCl has been used as an industrial intermediate, solvent, and propellant, and is a CNS depressant and neurotoxicant that produces peripheral nerve degeneration and cerebellar degeneration and hepatic lesions in animal studies. Renal lesions, including tumors, associated with exposure to MeCl have been attributed to unique metabolism in the kidneys of male mice. In rats and mice, degeneration and atrophy of the seminiferous tubules, epididymal sperm granulomas, sperm abnormalities, and reduced male fertility occur following short-term and/or longer-term inhalation of this substance. Following California’s procedure for MADL development, we conducted a concentration-response evaluation of the available inhalation-route toxicity studies for MeCl, and identified a 2-generation study in rats as the most appropriate study of sufficient quality upon which to base the MADL calculation. The NOAEL associated with the critical male reproductive effect was subject to conventional duration, dosimetric, and body weight adjustments and was ultimately divided by uncertainty factors totaling 1,000 to achieve the MADL of 4.4 mg/day. Available pharmacokinetic data prompted a further adjustment to 2.2 mg/day for absorbed MeCl. These values may serve as the basis through which manufacturers can assess consumer exposures to this substance and, ultimately, demonstrate compliance with Proposition 65 requirements to achieve “safe harbor”.

A Data-Based Proposal for Derivation and Validation of a Specific Concentration Limit (SCL) Threshold for the Reproductive Toxicity of Tetrapropenyl Phenol (TPP)


Current ECHA guidance provides that a specific concentration limit (SCL) for reproductive toxicity may be set when adequate, reliable, and conclusive scientific information is available to indicate that the hazard of a substance is not evident at a level above the concentrations set in Part 2 of Annex 1 or above the generic concentration limits (GCL). The guidance published begins with calculation of the ED10 value(s) followed by placement of the ED10 within a potency group after including consideration of modifying factors. Potency groups may be a useful tool for data limited substances, but such groupings may result in inappropriate classification of substances that would not, if tested, identify a reproductive hazard. We evaluated reproductive toxicity test data of tetrupropenyl phenol (TPP), an intermediate in the manufacture of engine lubricant additives. TPP is a substance with robust data set for both the test substance and as a manufacturing impurity contained in other substances. Based upon findings from two TPP reproductive toxicity studies in rats (one-generation oral gavage, two-generation dietary), the most sensitive reliable endpoint was reduction of ovary weight. The NOAEL and the lower confidence limit of the BMD10 were calculated for this parameter in both studies. There was high concordance between the lowest lower confidence limit of the BMD10 value, 18.6 mg/kg/day, and the NOAEL, 15 mg/kg/day. Using the lower value of 15 mg/kg/day, which is equivalent to an SCL of 1.5 w/w% (1.5% of the OECD 416 limit dose of 1000 mg/kg/day), we then validated this SCL against the results of four existing reproductive toxicity studies (OECD 416 or similar) for substances that contain TPP as a manufacturing impurity. In these studies, ovary weight was unaffected at dose levels in the range of the SCL. The results indicate that both the BMD10 and NOAEL approaches can confidently be used to derive an SCL that will appropriately classify substances for reproductive toxicity.

A Data-Based Proposal for Derivation and Validation of a Specific Concentration Limit (SCL) Threshold for the Reproductive Toxicity of Tetrapropenyl Phenol (TPP)

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Mono-(2-ethylhexyl) phthalate (MEHP) is the active metabolite of the chemical plasticizer, di(2-ethylhexyl) phthalate, a known Sertoli cell toxicant that induces germ cell apoptosis. The infiltration of macrophages (CD11b+ cells) into the testicular interstitial space of peripubertal (postnatal day; PND 28) Fischer rats exposed to varying doses (1.0, 0.75, and 0.5 g/kg, p.o.) of MEHP was characterized by flow cytometry. Significant increases of CD11b+ cells are evident at 12h and remain elevated at 48h after exposure to 1.0 and 0.75 g/kg MEHP. The increase in infiltrating cells was further characterized by immunohistochemistry to be pro-inflammatory CD68+ macrophages. Additionally, the effect of MEHP (1.0 g/kg, p.o.) on the production of cytokines was assessed in testicular interstitial fluid of peripubertal rats at selected time points (3, 6, 12, 24, and 48h). Due to the low volume of interstitial fluid recovered, corresponding treatments and time points were pooled. Cytokines (IL-10, IL-1Î², TNF-Î±) and the chemokine MCP-1 were analyzed using a Multiplex Rat Cytokine and Chemokine assay on a Luminex200 System. MEHP-induced Sertoli cell-injury disrupted cytokine secretion in the testicular fluid. Initially (3h), the anti-inflammatory cytokine, IL-10 and the pro-inflammatory cytokine, TNF-Î± were increased. However, at peak infiltration of macrophages (12h), IL-10 and TNF-Î± returned to control levels, while IL-1Î² and MCP-1 (pro-inflammatory signal) increased by 3- and 24-fold, respectively signifying a shift in the normal testicular immune microenvironment. Immunofluorescence staining revealed that peritubular myoid cells are the main cellular source of MCP-1 in the testis. Taken together, these results indicated that MEHP-induced Sertoli cell-injury stimulates testicular inflammation and secretion of pro-inflammatory cytokines, possibly disrupting spermatogenesis.
Simvastatin Reduces Fetal Testosterone Production and Permanently Alters Reproductive Tract Development in the Male Crl:CD(SD) Rat


Androgen signaling by fetal Leydig cells is critical in the proper development of the male reproductive tract. As cholesterol is a precursor for hormone biosynthesis, inhibition of the cholesterol pathway during sex differentiation may reduce testosterone (T). We hypothesized that simvastatin (SMV), a cholesterol-lowering drug, would reduce fetal T, ultimately resulting in reproductive tract malformations in the male rat. In a prenatal assessment study, dams were given 0, 15.6, 31.25, or 62.5 mg SMV/kg/day via oral gavage from gestational day (GD) 14-18, which includes the critical period of sex differentiation in the rat. Fetal testicular T production and plasma lipid concentrations were measured at GD 18. In a postnatal assessment study, dams received the same doses of SMV from GD 8-18 (covering organogenesis and sex differentiation) or 62.5 mg SMV/kg/day from GD 14-18.

Anogenital distance (AGD) and nipple retention were measured on postnatal day (PND) 2 and 13, respectively, and onset of puberty (measured by preputial separation, PPS) was monitored from PND 37 until complete. At necropsy, F1 adult males were examined for reproductive tract malformations. SMV exposure resulted in decreased fetal lipids and T production, with T levels reduced to 76.0 ± 3.9% of control in the 31.25 and 62.5 groups, respectively (p < 0.001 vs control for each). In the postnatal assessment, there was a 92% mortality rate in the 62.5 (GD 8-18) group at birth. F1 males exposed to 31.25 mg SMV/kg/day had decreased AGD, delayed puberty (onset of PPS), and a 14.3% incidence (p < 0.05 vs control) of testicular malformations. In the 62.5 (GD 14-18) group, F1 males had decreased androgen-dependent tissue weights (seminal vesicle and LABC, p < 0.05 vs controls) and retained nipples. Together, these studies suggest that in utero exposure to SMV reduces fetal T production and permanently alters reproductive tract development in the male rat. Abstract does not reflect U.S. EPA policy.

Di(2-ethylhexyl) phthalate (DEHP) is a ubiquitous environmental toxicant used as a plasticizer in consumer, medical, and building products. Humans are exposed to DEHP on a daily basis via oral ingestion, inhalation, and dermal contact. Little is known about the effects of DEHP on normal ovarian function; however, our lab has shown that DEHP exposure for 96 hr decreases estradiol levels in cultured mouse antral follicles. This is of concern because ovarian-derived estradiol is necessary for normal reproductive and non-reproductive health. However, the mechanism by which DEHP decreases estradiol levels is unclear. Thus, we tested the hypothesis that DEHP decreases the levels of precursor hormones following 48 hr of exposure, which precedes the decrease in estradiol levels following 96 hr of exposure. To test this hypothesis, isolated antral follicles from adult CD-1 mice were cultured with vehicle control (dimethylsulfoxide (DMSO)) or DEHP (1-100μg/ml) for 48 hr (n=3-5 separate experiments). Following culture, media were utilized to identify and characterize immune cells in the testis of 28 day-old Fischer rats after exposure to MEHP (1g/kg). The total number of macrophages (CD11b positive cells) in the tests increased 12 hours after acute MEHP exposure and peaks 24 hours post-exposure. Testicular macrophage levels begin to decrease but are still significantly raised 48 hours after MEHP exposure compared to untreated controls. Macrophages are functionally heterogeneous, existing in both pro-inflammatory (M1) and anti-inflammatory (M2) states. Immunofluorescence was performed to further characterize the subpopulations of macrophages present in the testes by identifying the presence of the specific M1 or M2 markers, ED1 (CD68) and ED2 (CD163), respectively. The number of ED1 positive macrophages increased in the tests 12 and 24 hours after MEHP exposure, and began to decline after 48 hours. In contrast, the number of ED2 positive macrophages continually increased, reaching significance at 24 and 48 hours post-exposure. In order to assess the direct action of MEHP on macrophage functionality, in vitro immunofluorescence and qPCR experiments were performed using cultured macrophages collected from the bone marrow and tests. Based on the current findings, it is hypothesized that MEHP alters the polarization of the testicular macrophages which ultimately may exacerbate germ cell loss.

Di(2-ethylhexyl) phthalate (DEHP) is a ubiquitous environmental toxicant used as a plasticizer in consumer, medical, and building products. Humans are exposed to DEHP on a daily basis via oral ingestion, inhalation, and dermal contact. Little is known about the effects of DEHP on normal ovarian function; however, our lab has shown that DEHP exposure for 96 hr decreases estradiol levels in cultured mouse antral follicles. This is of concern because ovarian-derived estradiol is necessary for normal reproductive and non-reproductive health. However, the mechanism by which DEHP decreases estradiol levels is unclear. Thus, we tested the hypothesis that DEHP decreases the levels of precursor hormones following 48 hr of exposure, which precedes the decrease in estradiol levels following 96 hr of exposure. To test this hypothesis, isolated antral follicles from adult CD-1 mice were cultured with vehicle control (dimethylsulfoxide (DMSO)) or DEHP (1-100μg/ml) for 48 hr (n=3-5 separate experiments). Following culture, media were utilized to identify and characterize immune cells in the testis of 28 day-old Fischer rats after exposure to MEHP (1g/kg). The total number of macrophages (CD11b positive cells) in the tests increased 12 hours after acute MEHP exposure and peaks 24 hours post-exposure. Testicular macrophage levels begin to decrease but are still significantly raised 48 hours after MEHP exposure compared to untreated controls. Macrophages are functionally heterogeneous, existing in both pro-inflammatory (M1) and anti-inflammatory (M2) states. Immunofluorescence was performed to further characterize the subpopulations of macrophages present in the testes by identifying the presence of the specific M1 or M2 markers, ED1 (CD68) and ED2 (CD163), respectively. The number of ED1 positive macrophages increased in the tests 12 and 24 hours after MEHP exposure, and began to decrease after 48 hours. In contrast, the number of ED2 positive macrophages continually increased, reaching significance at 24 and 48 hours post-exposure. In order to assess the direct action of MEHP on macrophage functionality, in vitro immunofluorescence and qPCR experiments were performed using cultured macrophages collected from the bone marrow and tests. Based on the current findings, it is hypothesized that MEHP alters the polarization of the testicular macrophages which ultimately may exacerbate germ cell loss.
Phthalate Mixture Exposure Affects Folliculogenesis and Induces Oxidative Stress in Neonatal Mouse Ovaries

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Phthalates are used as plasticizers in a large variety of products, such as medical devices and cosmetics. Most previous studies on phthalates have focused on single phthalates and not mixtures. It is important to study mixtures because humans are exposed to phthalate mixtures on a daily basis. Thus, we tested the hypothesis that exposure to an environmentally relevant phthalate mixture alters folliculogenesis and induces oxidative stress in the ovary. To test this hypothesis, ovaries from neonatal CD-1 mice (postnatal day 2) were collected and cultured with dimethylsulfoxide, DMSO (vehicle control), or phthalate mixture (1-500 μg/mL; n=5-15/group) for 6 days. The phthalate mixture was based on the composition of phthalates detected in urine samples from pregnant women. The mixture included 35% diethyl phthalate, 21% di(2-ethylhexyl) phthalate, 15% dibutyl phthalate, 15% diisononyl phthalate, 8% diisobutyl phthalate, and 5% benzylbutyl phthalate. After culture, ovaries were subjected to histological evaluation of germ cell and follicle numbers or to quantitative polymerase chain reaction (qPCR) to measure the expression of various key antioxidant enzymes: Cu/Zn superoxide dismutase (Sod1), catalase (Cat), glutathione peroxidase (Gpx), and glutathione reductase (Grx). The results indicate that the highest dose of phthalate mixture (500 μg/mL) significantly decreases the number of germ cells, primordial follicles, and primary follicles compared to control (p≤0.01). The results also indicate that exposure to the phthalate mixture (500 μg/mL) significantly decreases expression of Sod1 (p<0.01) and increases the expression of Cat (p<0.05), but does not affect Gpx and Grx expression compared to control. These data suggest that exposure to a phthalate mixture significantly affects early folliculogenesis and interferes with the expression of key antioxidant enzymes. Supported by NIH P01 ES022848 (JAF), EPA RD.

Di(2-ethylhexyl) phthalate (DEHP), a plasticizer used in plastics, has been shown to impair fertility by acting as an endocrine disruptor. Since exposure to high doses of DEHP reduces cleavage rate and the proportion of embryos developing to the blastocyst stage in an in vitro system, this study evaluated whether in vivo exposure to an environmentally relevant dose of DEHP alters cleavage rate to the 4-cell stage. Female CD1 mice (n = 6 per group) were exposed orally to DEHP (2000 mg/kg/day) or vehicle every 24 h for 3 estrous cycles. Following treatments, mice on estrus received equine chorionic gonadotropin hormone (eCG; 5 IU) ip, 48 h post-hCG mice were either euthanized to collect oocytes or bred with a fertile male mouse to collect zygotes. Oocytes were incubated with hyaluronidase to remove cumulus cells and then subjected to an in vitro fertilization assay using a standard protocol. Zygotes recovered from the oviduct and those obtained after in vitro fertilization were stained with Hoechst 33342 and then rated for cleavage. Viability in cumulus free oocytes was examined to determine cytotoxicity after staining with propidium iodide/Hoechst 33342. No significant differences in viability were observed in DEHP-treated mice compared to control indicating that DEHP was not toxic to the oocyte. Significant increased 1-cell zygotes and significant decreased 4-cell zygotes were observed in DEHP-treated mice following an in vitro fertilization, but no statistical differences in cleavage rate to the 4-cell stage were observed in control and DEHP-treated mice following an in vitro fertilization. We suggest that an environmentally relevant dose of DEHP decreases cleavage rate to the 4-cell stage possibly mediated by oviductal secreted proteins. Conacyt-Mexico CB-167678.

Effects of Tamoxifen on the Signaling Pathway of Ovary Maintenance and Activation of Sex Reversal in Adult Female Mice

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The aim of the study is to evaluate the effects of Tamoxifen (TAM) on the key genes in maintaining sex stability in adult mice. The expression pattern and levels of key genes and proteins were analyzed by quantitative-PCR, western blot and immunohistochemistry. Besides, hormone production was also analyzed by RIA. Quantitative-PCR and western blotting analysis showed that TAM exposure induced abnormal up-regulation of P450 aromatase expression, with significant decreases of aromatase, 32% di(2-ethylhexyl) phthalate, 21% di(2-ethylhexyl) phthalate, 15% dibutyl phthalate, 15% diisononyl phthalate, 8% diisobutyl phthalate, and 5% benzylbutyl phthalate. After culture, ovaries were subjected to histological evaluation of germ cell and follicle numbers or to quantitative polymerase chain reaction (qPCR) to measure the expression of various key antioxidant enzymes: Cu/Zn superoxide dismutase (Sod1), catalase (Cat), glutathione peroxidase (Gpx), and glutathione reductase (Grx). The results indicate that the highest dose of phthalate mixture (500 μg/mL) significantly decreases the number of germ cells, primordial follicles, and primary follicles compared to control (p≤0.01). The results also indicate that exposure to the phthalate mixture (500 μg/mL) significantly decreases expression of Sod1 (p<0.01) and increases the expression of Cat (p<0.05), but does not affect Gpx and Grx expression compared to control. These data suggest that exposure to a phthalate mixture significantly affects early folliculogenesis and interferes with the expression of key antioxidant enzymes. Supported by NIH P01 ES022848 (JAF), EPA RD.

Outcome in Zygote Development after In Vivo Exposure to Di(2-ethylhexyl) Phthalate May Depend on the Fertilization System

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Di(2-ethylhexyl) phthalate (DEHP) is a plasticizer in plastics, and it has been shown to impair fertility by acting as an endocrine disruptor. Since exposure to high doses of DEHP reduces cleavage rate and the proportion of embryos developing to the blastocyst stage in an in vitro system, this study evaluated whether in vivo exposure to an environmentally relevant dose of DEHP alters cleavage rate to the 4-cell stage. Female CD1 mice (n = 6 per group) were exposed orally to DEHP (2000 mg/kg/day) or vehicle every 24 h for 3 estrous cycles. Following treatments, mice on estrus received equine chorionic gonadotropin hormone (eCG; 5 IU) ip, 48 h later mice received hormone human chorionic gonadotropin (hCG; 5 IU) and 16 h post-hCG mice were either euthanized to collect oocytes or bred with a fertile male mouse to collect zygotes. Oocytes were incubated with hyaluronidase to remove cumulus cells and then subjected to an in vitro fertilization assay using a standard protocol. Zygotes recovered from the oviduct and those obtained after in vitro fertilization were stained with Hoechst 33342 and then rated for cleavage. Viability in cumulus free oocytes was examined to determine cytotoxicity after staining with propidium iodide/Hoechst 33342. No significant differences in viability were observed in DEHP-treated mice compared to control indicating that DEHP was not toxic to the oocyte. Significant increased 1-cell zygotes and significant decreased 4-cell zygotes were observed in DEHP-treated mice following an in vitro fertilization, but no statistical differences in cleavage rate to the 4-cell stage were observed in control and DEHP-treated mice following an in vitro fertilization. We suggest that an environmentally relevant dose of DEHP decreases cleavage rate to the 4-cell stage possibly mediated by oviductal secreted proteins. Conacyt-Mexico CB-167678.

Genistein May Alter Steroidogenesis by Decreasing Steroidogenic Enzyme Expression in Mouse Ovaries

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Genistein is a naturally occurring isoflavone phytoestrogen commonly found in plant products such as soybeans, lentils, chickpeas, and sunflower seeds. Genistein, like other phytoestrogens, has the potential to mimic, impair, or enhance the estradiol biosynthesis pathway, potentially altering ovarian follicle growth. Previous studies indicate that at 96 hours (h) of culture, genistein significantly inhibits the growth of cultured mouse antral follicles. Additionally, exposure to genistein significantly decreases estradiol levels, while increasing progesterone levels at 96h. In the estradiol biosynthesis pathway, a number of steroidogenic enzymes participate in the conversion of cholesterol to estradiol. For the purpose of this experiment, we focused on two steroidogenic enzymes from the cytochrome P450 family: CYP17A1 and CYP19. This study was designed to test the hypothesis that genistein exposure (6.0 and 36μM) decreases the expression of Cyp17a1 and Cyp19. To test this hypothesis, antral follicles were mechanically isolated from adult CD-1 mice and cultured with either vehicle control (dimethylsulfoxide; DMSO) or genistein (6.0 and 36μM) in supplemented α-minimum essential media for 96h. Individual follicle diameters were measured every 24h to monitor follicle growth. At the end of the culture period, the follicles were subjected to real-time PCR for analysis of the steroidogenic enzyme genes Cyp17a1 and Cyp19. From this data, we conclude that genistein exposure decreases Cyp17a1 expression, which in turn may lead to the observed increase in progesterone and decrease in estradiol levels. This hormonal imbalance may result in the overall inhibition of follicle growth. Support: NIH R01ES019178.

Equol Inhibits Growth and Estradiol Production in Mouse Antral Follicles In Vitro

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Equol is a non-steroidal estrogen metabolite exclusively produced by microbial conversion of the ingested soy isoflavone daidzein in the gut of some humans and many animal species. Daidzein is one of the major isoflavone phytoestrogens found in soybeans. A few studies show that phytoestrogens can affect the endogenous estradiol biosynthesis pathway by mimicking, potentiating, or impairing different components of this pathway, and this in turn, could alter ovarian follicle growth. However, no studies have examined the effects of equol exposure on intact antral
folicles. Thus, we tested the hypothesis that equol, (6, 36, and 100 μM) inhibits follicle growth and estradiol production in mouse antral follicles. To test this hypothesis, antral follicles were manually isolated with forceps from ovaries of cycling CD-1 mice at postnatal days 33-35, and cultured with vehicle control (dimethyl sulfoxide; DMSO) or equol, (6, 36, and 100 μM) in supplemented alpha minimum essential medium for 96 hours (h). Individual follicle diameters were measured every 24 h, and at completion of culture, media were collected and subjected to enzyme-linked immunosorbent assays for measurement of estradiol levels. The results indicate that equol at the highest concentration tested (100 μM) significantly inhibited follicle growth at 72 h (DMSO: 127.3 ± 4.03 percent change; Equol 100 μM: 109.2 ± 1.08 percent change, n=4, p<0.05) and 96 h (DMSO: 146.8 ± 5.35 percent change; Equol 100 μM: 120.1 ± 2.72 percent change, n=4, p<0.05) compared to control. Additionally, equol at 100 μM significantly inhibited estradiol levels measured at 96 h compared to control (DMSO: 4135.08 ± 592.32 pg/ml; Equol 100 μM: 75.98 pg/ml; n=3, p<0.05). Exposure to equol doses of 16 and 36 μM did not significantly affect follicle growth or estradiol levels when compared to control. Collectively, these data suggest that equol inhibits growth and estradiol production in cultured mouse antral follicles. Support: NIH RO1ES019178

1450 Quercetin Attenuates Oxidative Testicular Damage in STZ-Induced Diabetic Rats

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Diabetes mellitus (DM) with the consequent oxidative stress represents a major risk factor for testicular dysfunction. Antioxidants showed beneficial effects on spermatogenesis and testicular oxidative damage during DM. Quercetin (QR), a naturally occurring antioxidant, has been shown to reduce oxidative stress in the long-term treatment of streptozotocin (STZ)-induced DM in animal models. The present study aims to examine the influence of QR on STZ-induced oxidative stress in rat’s testicular cells. Male Wistar rats (n=36) were allocated into 6 groups: (1) control, (2) QR (25 mg/kg), (3) QR (50 mg/kg), (4) STZ (65 mg/kg), (5) STZ+QR (25 mg/kg) and (6) STZ+QR (50 mg/kg). After 5 weeks of QR treatment, animals were sacrificed and testes were dissected immediately. A cross-section from each group was prepared in 10% formalin for histopathology. In testicular tissues, thioharbituric acid reactive substances (TBARS), total glutathione (T-GSH), and non-protein sulfhydryl groups (NP-SH) levels were estimated. Superoxide dismutase (SOD), catalase (CAT) and glutathione-S-transferase (GST) activities were also determined. In STZ group, TBARS levels were significantly increased while T-GSH and NP-SH (CAT) and glutathione-S-transferase (GST) activities were also determined. In STZ group, TBARS levels were significantly increased while T-GSH and NP-SH levels were markedly reduced as compared to control rats. QR treatment to diabetic rats showed significant inhibition in increased TBARS levels and decreased T-GSH and NP-SH levels when compared to untreated diabetic rats. Enzymatic activities of SOD, CAT and GST were significantly inhibited in diabetic rats. QR treatment enhanced these activities compared to untreated diabetic rats. Histopathological evaluation revealed damage in testicular cells of diabetic rats and the treatment with QR showed protection. These findings suggest QR as a potentially beneficial factor for testicular dysfunction. Antioxidants showed beneficial effects on spermatogenesis and testicular oxidative damage during DM.

1451 Prior Attenuation of KiSS1 mRNA Expression in LH-Surge Center Is a Trigger for the Delayed Effect Induced by Neonatal Exposure to Estrogens in Rats

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Delayed effects, a late-occurring adverse effects induced by neonatal exposure to various estrogenic compounds, is a growing concern to cause the persistent reproductive impairment in wide range of species. We previously reported that neonatal exposure to ethynyl estradiol (EE) cause the delayed effects characterized by the early onset of age-matched abnormal estrous cycling after sex maturation in rats. KiSS1/GPR54 signaling is a crucial site-specific regulator of estrous cycles in the anteroventral periventricular nucleus (AVPV) and of folliculogenesis in the arcuate nucleus (ARC). To clarify the involvement of KiSS1/GPR54 in the delayed effect, we investigated the LH surge and KiSS1 mRNA expression in the AVPV and ARC of cycling rats neonatally exposed to EE at delayed effect-inducing doses. The results were also compared to those in middle-aged rats with or without cyclicity. KiSS1 mRNA expression in the anterior hypothalamus decreased in both EE 20μg/kg exposed and middle-aged rats with cyclicity. Corresponding to this finding, the number of KiSS1 mRNA-positive cells detected by in situ hybridization was also decreased in the AVPV. KiSS1 mRNA expression in the ARC was not changed. In addition, the peak level and area of LH dose-dependently decreased in EE 20 and 0.2μg/kg exposed rats, and the reduction was more evident in the middle-aged rats. These results indicate that the prior attenuation of KiSS1 mRNA expression and consequent depression of the LH surge occurred before the appearance of abnormal estrous cycling, thus the alteration in kispeptin neuron might be an important trigger for the delayed effect.

1452 Individual and Mixed Endocrine Activity of BPS and BPC Using In Vivo Estrogenic/Androgenic Transcriptional Activation Assays and the In Vitro Uterotrophic Assay


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Bisphenol A (BPA) is gradually being phased out of many consumer products and processes leading to potential increases in human and environmental exposures to relatively understood replacement compounds, including Bisphenol S (BPS) and Bisphenol C (BPC). Research from our lab has shown that BPA and Bisphenol AF (BPAF) display nearly identical anti-androgenicity and similar estrogenicity (BPAF ~10-fold more potent) in vitro, whereas BPAF stimulated uterine weight gain with oral administration in vivo (50 mg kg −1) but BPA had virtually no effect. Here we ask, do BPA and BPC behave similarly to either BPA or BPAF in vitro and/or in vivo estrogenicity/anti-androgenicity assays? We conducted estrogen receptor (ER) transcription assays utilizing T4/T5-KBlue cells and androgen receptor (AR) antagonism assays utilizing transient transduction of CV-1 cells with chimp AR. We found that BPS was less potent than BPA at both inducing ER gene expression (∼6-fold greater Ec50 and AR antagonism (∼100-fold greater Ec50) in vitro. In contrast, BPC was remarkably potent at both inducing ER activation and AR antagonism as compared to BPA (∼100-fold lower Ec50 for both). Since BPA and BPS are currently in use, exposures to mixtures of BPA, BPS, BPC, other replacement compounds, and associated metabolites are possible. In order to determine if a mixture of BPA and BPS acts in an additive, antagonistic or synergistic manner, we conducted ER transcription assays with binary mixtures of BPA and BPS (8x8 factorial design) and found that these compounds conformed to dose addition model predictions for ER agonism in vitro. In addition, we are examining the effects of BPS, BPC, and mixtures with other chemicals in vivo using oral exposures to determine the estrogenicity of these chemicals when administered via a relevant route of exposure. Abstract does not reflect U.S. EPA policy.
be a more sensitive target for these chemicals. Because early BPA exposures are thought to contribute to the onset of adult diseases, later time points may need to be assessed.

**1454** The Effect of Bisphenol A on Cumulus Cells Expansion and Oocyte Viability in an In Vitro System

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Ovulation is a process in the ovary that allows the release of a fertilizable oocyte from a mature follicle. Before ovulation, luteinizing hormone (LH) stimulates the cumulus cells-oocyte complex (COC) to trigger oocyte maturation, COC expansion and oocyte meiotic resumption (from prophase I to metaphase II). It is known that high concentrations of bisphenol A (BPA; 100 μM), a monomer used to manufacture polycarbonate plastics that can contaminate food and beverages, interfere with COC expansion. However, whether low doses of BPA exert the same effect is unknown. This study evaluated whether BPA exposure at environmentally relevant doses of BPA alters cumulus cells expansion. Further, this study evaluated the oocyte viability as a cytotoxic standard parameter. Mouse COC primary cultures were exposed to 2.2, 22, 220 and 2200 nM BPA (2 h/37°C), and then incubated with phorbol myristate acetate (PMA) to promote oocyte maturation during 16 h. Control and BPA-treated COCs were subjected to assessment of cumulus cells expansion after in vitro maturation using Image Pro Premier® software. Oocyte viability was evaluated by Hoechst 33342 and propidium iodine staining. The data show that the exposure to 220 and 2200 nM BPA significantly reduced COC expansion compared to COC controls. Viability was above 90% in oocytes from either control or BPA-treated COCs, indicating that the BPA concentrations used in this study were not cytotoxic to the female gamete. These results suggest that environmentally relevant doses of BPA inhibit COC expansion. Further studies are being conducted to evaluate whether these effects of BPA on cumulus cells expansion relates with effects on the oocyte meiotic resumption. This study was supported by CONACyT-México CB-167678.

**1455** Chronic Low Levels of Bisphenol A Exposure Impairs Uterine Functions during Early Pregnancy by Disrupting Progesterone Receptor-Mediated Signaling

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Bisphenol A (BPA), a chemical widely used in polycarbonate plastics and epoxy resins, has received much attention due to its widespread toxic effects and chronic exposure in humans. While BPA exposure has been linked to infertility and recurrent miscarriage in women, the impact of its exposure on uterine function during early pregnancy remains unclear. In this study, we exposed female mice to low levels of BPA, via repetitive feedings, to mimic the exposure situation in humans, while BPA exposure has been linked to infertility and recurrent miscarriage in women. We further postulate that toxicant-induced dysregulation of reparative autophagy is a common novel pathway central to impaired fertility and premature menopause in women who smoke compared to non-smokers. The data suggests that CS exposure is hazardous to ovarian function. We previously demonstrated that CS exposure induces autophagy in ovarian granulosa cells in preference to apoptosis and is a potential novel alternative cell death pathway. However the mechanisms regulating autophagy in ovarian granulosa cells are poorly defined. Therefore, the objective of this study was to investigate smoke-induced changes in autophagy signalling. Briefly, adult female mice were exposed to mainstream CS twice daily for 8 weeks; equivalent to a pack of cigarettes a day, using a whole body exposure system. One ovary/mouse was harvested for gene and protein analysis. Using a gene array, we found that CS induced a greater than 2-fold significant increase in the expression of pro-autophagy genes Cdkn1b, Maplc4a, Bad and SquenIP16/2. Whilst expression of Cts and Pek3g were attenuated by more than half the expression of the control group. Q-PCR revealed that CS induced a significant increase in the expression of Abp (p=0.0001), Bad (p=0.0001), Gapdh (p=0.0019), Eif4ebp1 (p=0.0078) and Tnfa (p=0.0039) and a non-significant decrease of Akt1 and Ccrl4 (p>0.05) genes. Western blot analysis revealed a significant up-regulation of AMPK α1/2 (p=0.0119) and ATGP7 (p=0.0006) and a down-regulation of Akt1 (p=0.0021), p-AKT (p=0.05), CCR4 (p=0.005), mTOR (p=0.0005) and CTKN1/p27 (p=0.0001) proteins. In summary our data suggest that CS-exposure induces ovarian follicle loss via induction of the autophagic cascade through AMPK activation in response to elevated levels of reactive oxygen species. We further postulate that toxicant-induced dysregulation of reparative autophagy is a common novel pathway central to impaired fertility and subfertility.
Polychlorinated biphenyls (PCBs) are used in several fields in industry. Aroclor 1254 is a widely used derivative of Aroclor, which consists of an important group of PCBs. Studies on laboratory animals have reported that Aroclor 1254 has developmental and reproductive toxic effects. Although the evidences are limited, it seems reasonable that Aroclor 1254 may have a potential for similar adverse effects in humans. Selenium is a trace element that is crucial for male reproduction. By taking into account the high prevalence of inadequate selenium intake, essentiality of selenium in testicular structure and function, this study will be designed to investigate the effect of Aroclor 1254 on sperm parameters in selenium-deficient male rats and selenium-supplemented rats. Selenium deficiency was generated by feeding 3-week old Sprague-Dawley rats with <0.05 Se mg/kg diet for 5 weeks. Supplementation group was on 1 mg Se/kg diet. Aroclor 1254-treated groups received 10 mg/kg dose by gavage during the last 15 days of feeding period. Testis weight and relative testis weight, sperm count and sperm motility were evaluated. Both tests (29%) and relative testis weights (20%) decreased significantly in Aroclor 1254-exposed rats compared to control group. Besides, sperm count (50%) and sperm motility (40%) were also markedly lower in Aroclor 1254 group vs. control. The effects of DEHP were much more pronounced in Se-deficient rats, whereas Se supplementation was found to be protective.

A cocktail of pesticides called “Double dose”: Iprodione 25% + Carbendazim 25% WP is commercialized. We have shown that low doses of Carbendazim (CBZ) (50 nM and 500 nM) have an endocrine disrupting effect on the pubertal rat seminiferous epithelium. Iprodione (IPR) is known to have antiandrogenic activity in vivo. We used our validated rat seminiferous tube culture model (Bio-AlteR®) developed by Durand’s team for testing IPR 50 nM, CBZ 50 nM and the cocktail CBZ 50 nM + IPR 50 nM (Cktl). Cultured seminiferous tubules from 20-22 day old rats were treated with CBZ, IPR or Cktl. The integrity of the blood-testis barrier (Trans-Epithelial Electrical Resistance (TEER), immunocytochemistry of Connexin 43 (Cx43) and of Claudin 11), the different cell populations (flow cytometric analyses and quantification of mRNAs specific of the different cell populations) and steroid hormone receptor mRNAs were studied. The TEER was slightly increased by CBZ from day 16 onward, but not modified by IPR or Cktl. The expression of Cx43 was decreased by CBZ on day 16 only, but both on day 7 and day 16 by Cktl. The expression of Claudin 11 was increased by CBZ on day 16 but decreased on day 7 and day 16 by Cktl. CBZ increased the number of somatic cells, while IPR and Cktl did not modified them. IPR alone and Cktl, unlike CBZ, decreased the numbers of spermatogonia and secondary spermatocytes on day 21. Round spermatids were decreased from day 7 onward by CBZ, IPR and Cktl. IPR and Cktl, unlike CBZ, did not increase round spermatid specific mRNAs (TP1, TP2). Cktl increased the expression of ERα and ERβ on day 21 while only ERβ was increased by IPR alone. Hence, this study highlights the anti-androgenic effect of IPR ex vivo. CBZ alone or IPR alone induced alteration of spermatogenesis. Cktl possessed similar effects as CBZ on germ cell populations. Moreover Cktl cancelled the CBZ increase of secondary spermatocytes and exacerbated the effect of IPR alone and CBZ alone on the blood-testis barrier.

Introduction: 1-bromopropane (1-BP), an alternative to ozone-depleting solvents, exhibits reproductive toxicity in rats and mice. The present study investigated how 1-amino-benzotriazole (ABT), a cytochrome P450 inhibitor modulates the adverse effects of 1-BP on male reproductive parameters in mice. Methods: 36 male C57BL/6Jcl mice were randomly divided into 6 groups of 6 each. 3 groups of mice injected with 50mg/kg, ABT twice per day were exposed to 1-BP at 0, 50 and 250 and 1200 ppm 8hr per day for 28 days. 3 groups of mice injected with saline were exposed to 1-BP at 0, 50 and 250 ppm. After exposure, all mice were dissected under anesthesia. Results: Sperm count and motility in the epididymis decreased and morphologically abnormal sperm increased depending on 1-BP level in the mice treated with saline, but did not change with 1-BP level in the mice treated with ABT. On the other hand, weight of seminal vesicle plus prostate decreased significantly at 250 ppm of 1-BP compared to 0 ppm groups both in ABT-treated and saline-treated groups. Weight of testis and epididymis did not change significantly with exposure level of 1-BP both in ABT-treated and saline-treated groups. Discussion: ABT reduced adverse effects of 1-BP on number, motility and motility.

In vitro models of male reproductive toxicity are in demand due to the high cost and number of animals used for in vivo testing. We developed an organotypic in vitro model of male reproductive development using neonatal rat testes (3D- TCS). This model is a co-culture of testes cells within a three dimensional matrix, creating an in vitro-like environment. To characterize toxicity signals captured by the 3D-TCS, we tested 71 diverse compounds for cytotoxicity, including therapeutic compounds and environmental contaminants. Thirty-one of the 71 compounds caused >10% increase in cytotoxicity, measured using a lactate dehydrogenase assay. For some compounds (crizotinib), cytotoxicity was a sensitive indicator of reproductive toxicity at concentrations near relevant therapeutic levels. For other compounds (vinclozolin) cytotoxicity was not a good predictor of reproductive toxicity, indicating the need for further analysis. Eight compounds (arsenic, crizotinib, dibutyl phthalate, diethyl phthalate, diethylhexyl phthalate, nicotine, valproic acid and vinorelbine) were selected for testing with additional end points. We measured levels of testosterone and several macrophage produced cytokines (IL-6, TNF-α, KC/GRO) in cell media. Western blots indicated the presence of macrophages in the 3D-TCS, suggesting that macrophage mediated responses could be used to detect compounds that cause inflammation in the testes. Significant decreases in testosterone and/or increases in cytokine levels were observed for all chemical treatments, except valproic acid, at lower doses or earlier time points than those associated with cytotoxicity. These results indicate that these functional end points will be informative of mechanisms of toxicity in the 3D-TCS and suggest a potential role for resident macrophages. Supported by: FDA(U10FD004242), EPA(RD8317090, RD83451401), NIEHS(P01 ES009001).
phology of epididymal sperm, but had no effect 1-BP mediated changes in weight of seminal vesicle plus prostate, which is known to reflect blood androgen level. Oxidative metabolites of 1-BP may contribute to toxicity of 1-BP to sperm, but might have less impact on endocrine effects of 1-BP.

1463 SO2 and Fluoride Changed the miRNAs Expression Profiling in Mice Testis

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MicroRNAs (miRNAs) are believed to play important roles in the development of mammalian spermatogenesis. Fluoride and sulfur dioxide are two well known environment pollutants, which present a serious threat to male reproduction. To compare the global miRNA expression profiling and regulation pattern in mouse testes between normal and F or SO2-treat mice by using miRNA microarray technology. Mouse model were treated with 150 mg NaF/L in their drinking water, SO2 in ambient air (26.2 mg/m3 SO2, 3hr/day), or were exposed to both together for 15 weeks. The results exhibited that MiR-34a, and other miRNAs have changed significantly in testes of mice. Further investigation indicates that predicted pathways according to significant miRNA are primary related to cell cycle, apoptosis, and MAPK signal pathway. Furthermore, the development of testis and reproductive capacity of male mouse have been weakened by F or SO2, especially, by F and SO2 together treatment. FCM analysis show that the percents of apoptosis cells were increased significantly and DNA contents distributions have been disturbed in testis by F or SO2. Expression of p53 and bcl-2 mRNA increased, and bax was increased by QRT-PCR. However, both F or SO2 and the combination heighten the ratios of p53/bcl-2 and bax/bcl-2, all these changes lead to a common result of the spermatogenic cell apoptosis. In conclusion, changed miRNAs expression regulation should be look at as the main mechanism of depressed male reproduction function of mouse induced by F or SO2 or their combination. This is the first miRNA microarray study to focus on evaluating altered miRNA expression profiles in testis of mice exposure to F or SO2.

1464 Mechanisms Underlying the Testicular Toxicity of Atrazine in Rats and Sertoli-Germ Cell Coculture

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Atrazine (2-chloro-4-ethy lamino-6-isopropylamino-striazine) (ATZ) is a chloro-s-triazine herbicide employed extensively in the US and world wide for over 40 years for the control of broadleaf and grassy weeds in the cultivation of corn, sorghum and sugar cane is now recognized to have disrupting effects on the reproductive systems of mammals. Because of its persistence in the environment (soil and water), human and wildlife are at risk of exposure. Using sertoli-germ cell co-culture, we evaluated the effects of ATZ on the transcritps levels of genes critical for steroidogenesis. Western blot analysis of proteins involves in cell death and survival was monitored to show the mechanism of Sertoli-germ cell death-induced by ATZ. ATZ up-regulated the mRNA expression of the transcription factor, GATA-4, stem cell factor (SCF), androgen receptor (AR) and NF-kB and down regulated the expression of COX-2 mRNA. Furthermore ATZ up-regulated the expression of StAR and P450sc mRNA. Western blot analysis revealed that ATZ induces the expression of Bax, and c-Jun and down regulates the expression of, NF-kB, and c-Fos. ATZ administered to rats at varying doses orally for 7 and 16 days impaired reproductive function and elicited a depletion of the antioxidant defense system in the testis and epididymis, indicating the induction of oxidative stress. Overall, our data suggest that the toxicity of ATZ in the testis of rats and testicular cells is mediated by mechanisms involving apoptosis, oxidative stress and dysregulation in the expressions of several genes specific for the steroidogenic pathway. The data have implication for the toxicological risk evaluation of atrazine.

1465 A New Oxidative Mechanism of Methyl Parathion Reproductive Toxicity: Disruption of the Blood-Testis Barrier

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Methyl-parathion (Me-Pa) is a extremely toxic organophosphorous pesticide, and it is associated with reproductive effects, including poor semen quality, reduced fertilization capacity, sperm DNA damage, and protein phosphorylation. Me-Pa is bio-activated via the cytochrome P450 pathway generating oxidative stress, and it is able to alter proteins, lipids and DNA through three mechanisms: oxidation, phosphorylation and alkylation. The blood-testis barrier (BTB), which regulates the spermatogenesis consists of tight junction proteins between Sertoli cells, such as ZO-2, ZO-1, occludin (occ1), claudin 11 (cl11) and claudin 3 (cl3) that may be targets of toxicants. We studied the effect of repeated doses of Me-Pa (6 mg/Kg/day/5 days, i.p.) on the function of BTB of adult ICR-CD1 mice evaluated by biotin infiltration. Data showed biotin infiltration through the paracelluar pathway between Sertoli cells, indicating the opening of the BTB by the exposure to Me-Pa. In addition, we evaluated the localization of ZO-2, ZO-1, occ1, cl11 and cl3 by immunohistochemistry; however no changes were observed in the localization of these proteins that could suggest the opening of the BTB. On the other hand, we observed the carbonylation of proteins in testis homogenate, indicating that Me-Pa has an oxidizing effect on proteins. The co-administration of Me-Pa with the antioxidant α-tocopherol (50 mg/Kg/day/5 days, i.g) was performed, and interestingly, no biotin infiltration was observed in the co-administered group, and the carbonylation of proteins was significantly reduced. These results showed that Me-Pa exposure alters the BTB by an oxidative mechanism that could interfere with the function of tight junction proteins, since these effects were prevented by the co-administration with the antioxidant. Finally, the BTB opening may contribute to the impaired spermatogenesis and reduced male fertility caused by Me-Pa.

1466 Modulation of Nrf2 and OGG1 Expression in Germ Testicular Cells by Methyl Parathion Exposure: An Epigenetic Mechanism


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Methyl-parathion (Me-Pa) is a high toxic organophosphate pesticide (OP) widely used in several countries, which is activated by cytochrome P450 generating oxidative stress. Me-Pa reproductive toxicity includes reduced semen quality and fertilization capacity, and oxidative and genetic damage in spermatozoa. Me-Pa alters proteins and DNA through oxidation, phosphorylation or alkylation (methylation). Some studies reported the alkylating capacity of Me-Pa, and it is known that DNA methylation (5-methylcytosine; 5-mC) in gene promoters represses their expression. The transcription factor Nrf2 regulates the expression of the 8-oxoguanine DNA glycosidase (OGG1) that removes DNA oxidative lesions, and its own expression through the antioxidant response element (ARE). Me-Pa caused an oxidative damage at single doses but did not induce the expression of Nrf2 or OGG1. The aim of the study was to evaluate the effect of repeated doses of Me-Pa (6 mg/Kg/day/5 days, i.p.) on the global and specific DNA methylation in AREs of Nrf2 and OGG1 promoters as modulators of their expression in mice testicular germ cells. Global DNA methylation was determined by an ELISA technique (%5-mC), the specific methylation by pyrosequencing, and the gene expression by qRT-PCR. Data showed that Me-Pa exposure did not induce mRNA levels of OGG1, and slightly decreased the Nrf2 expression. There was not a significant difference in the global DNA methylation by Me-Pa exposure. However, Me-Pa significantly increased the methylation in CpG sites located in OGG1 and Nrf2 promoters. Additionally, there was an increase in the oxidation (carbonylation) of total proteins in germ cells of animals exposed to Me-Pa. In summary, our results suggest that Me-Pa modulates the expression of both the antioxidant gene Nrf2 and the DNA-repair gene OGG1 through an epigenetic mechanism. This could involve both, the capacity of Me-Pa to methylate and to oxidize biomolecules, which regulated the gene expression and/or impairment of antioxidant and DNA-repair protein functions.
The activity of many nuclear receptors (NRs) can be readily manipulated by various endogenous and exogenous ligands, making NRs of particular interest to drug developers and toxicologists. Also, many exogenous NR ligands are shown to bind and regulate more than a single NR. Thus, comprehensive evaluation of environmental compounds as potential NR ligands is important for assessing their bioactivity. To assess the effects of environmental compounds on multiple NRs we developed a multiplexed reporter assay (trans-FACtORIAL) that enables a quantitative assessment of a compound's impact on multiple NRs in a single assay well. Here we profiled the activities of 1,800 of ToxCast (EPA) compounds against 24 human nuclear receptors in HepG2 cells. We found that the compound profiling by trans-FACtORIAL assay produced reproducible NR response patterns (NR signatures). The most frequently activated NRs (>2 fold) were as follows: PXR > ERα > PPARγ > RXRα > PPARα. Importantly, the NR signatures of ToxCast compounds formed distinct clusters, thus suggesting predictive capabilities of the NR signatures. For example, the NR profiling of structurally-related organotins produced very similar NR signatures with the similarity scores above 0.85. Hierarchical clustering revealed that the tight cluster of organotins had an adjacent cluster of structurally related compounds including 2,4,6-Tris(tert-butyl)phenol that exhibited organotin-like NR signatures, suggesting that bioactivities of these compounds may be similar to those of the rexinoid-like group of endocrine disruptors. This study demonstrated that the trans-FACtORIAL assay is well suited for high-content assessment of putative environmental NR ligands and indicated the utility of the multi-endpoint NR signatures for the classification of the compounds according to their impact on the NR superfamily. This abstract does not necessarily reflect US EPA policy.

Isoloflavones are uniquely-rich dietary sources of isoflavones classified as both phytoestrogens and selective estrogen receptor modulators (SERMs). The impact of isoflavone intake on breast cancer has been the subject of intense debate. Exposure to isoflavones in childhood and/or adolescence is hypothesized to make breast cells more resistant to transformation into cancer cells. However, concern exists that the phytoestrogenic properties of isoflavones may worsen the prognosis of breast cancer patients and increase the likelihood that high-risk women will develop breast cancer. No clinical trials have evaluated the impact of isoflavone exposure on breast cancer recurrence or mortality. Several have evaluated effects on breast cell proliferation and/or apoptosis. Immunohistochemical increases in proliferation as measured by changes in Ki67 and/or decreases in apoptosis are viewed as adversely affecting breast cancer prognosis. Six studies were identified (based on a PubMed search using key words: clinical, isoflavones, soy, breast cancer; and references within relevant papers and that came to the authors' attention) in which proliferation and/or apoptosis were measured in response to isoflavone exposure. The intervention period ranged from approximately 2 weeks to one year, participant number from 18 to 104 and isoflavone dose (expressed in aglycone equivalent weight) from 36 to 235 mg/d. One study involved healthy women, one involved high-risk women, three involved breast cancer patients and one involved healthy women and breast cancer patients. None of the six trials found that at study termination proliferation differed between the placebo and isoflavone groups nor was apoptosis affected in the four trials in which this endpoint was assessed. In conclusion, although high-dose exposure may exert subtle estrogen-like effects on breast tissue, there is no change in proliferation and apoptosis. Therefore, the published clinical data are supportive of the safety of isoflavone exposure.
existing epidemiology, animal, and in vitro toxicity data for 27 BPA analogues of emerging interest to research and regulatory communities. 4,840 literature records have been screened at the Title and Abstract level for relevance and initial categorization by chemical, health outcome category, and evidence stream (human, animal, in vitro). Studies were assessed for inclusion by full text screening, and data extraction is in progress. Preliminary inventorying indicates there is human biomonitoring data on 8 analogues (tetrabromo-BPA, tetrachlor-BPA, benzylparaben, bisphenol F, S, B, E, and AF) and epidemiological evidence on 5 analogues (tetrabromo-BPA, tetrachloro-BPA, benzylparaben, bisphenol F and S). For 12 analogues, there are no human biomonitoring, epidemiological, animal, or in vitro reports. Structural and biological similarity profiling compared 13 BPA analogues that were tested in the National Toxicology Program’s Tox21 and/or U.S. Environmental Protection Agency’s ToxCast high throughput screening platforms to BPA or ethinyl estradiol (EE2). In general, BPA analogues were more structurally similar to each other than to EE2. Ten BPA analogues had estrogen receptor (ER) agonist activity similar to BPA. Bisphenol B was the most bioequivalently similar to BPA whereas bisphenols C and AF were the most similar to EE2 when assessed across all of the Tox21 or ToxCast assays. Bis(3-allyl-4-hydroxyphenyl) sulfone (TGSa) was the most unique, having significant aryl hydrocarbon receptor activity and very little other receptor activity. Results of these analyses are being used to guide further in vivo and in vitro testing.

1472 Estrogen Receptor Isoforms ER Alpha 66, 46, and 36 Exhibit Distinct Signalng Interactions during Estrogen-Mediated Proliferative Events

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Traditional testing paradigms for endocrine disruptor screening are being challenged as the future of risk assessment moves towards fit-for-purpose in vivo assays to predict adverse outcomes. To replace current in vivo assays, an understanding of how the key events in estrogenic signaling must be achieved. To this end, human endometrial Ishikawa (IK) cells, previously shown to demonstrate phenotypic responses to estrogens, were used to examine the role of estrogen receptor (ER) isoforms in estrogen-mediated proliferation. IK cells constitutively express all five of the known ERs: ERα66, ERα46, ERα36, ERβ, and GPER (measured by PCR and western). We used selective ER agonists to evaluate the role of ER isoforms in proliferation. Agonists for ERβ (DNP) and GPER (G1) did not induce proliferation. However, ERα-specific agonist (PPT) induced robust proliferation, indicating Erα as the main receptor mediating these events. Since PPT may bind all three ERα isoforms, we next asked if the isoforms had unique contributions to uterine cell proliferation. We designed overexpression systems by inserting lentiviral constructs encoding ERα-FP fusion proteins specific for each isoform in IK cells. High-content imaging confirmed expression of the proteins. Preliminary analyses indicate overexpression of ERα66 or ERα36 increases baseline proliferation in IK cells and ERα66 further increased both sensitivity and proliferative response to ethinyl estradiol (EE). ERα46 overexpression, however, correlated with a significant reduction in EE-induced proliferative pathways. These data suggest that while ERα66 and ERα36 initiate proliferative signaling, ERα46 may antagonize EE-induced proliferative pathways. Furthermore these studies demonstrate the importance of non-canonical ERα signaling in proliferation and highlight the need for screening assays that incorporate key biological processes driving chemical induced estrogenic responses rather than simply focusing on ERα-mediated transactivation.

1473 Direct Measurement of Estrogenic and Androgenic Compounds in Human Plasma Using Cell-Based Reporter Gene Assays

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Over 230,000 cases of breast cancer are diagnosed annually in US women and approximately 40,000 die of the disease each year. Progress has been made in identifying risk factors strongly related to breast cancer risk, including plasma steroid hormone (estradiol and testosterone) levels. However, additional risk factors likely exist, such as environmental chemicals that act on estrogenic and androgenic pathways. To identify novel chemicals with endocrine disrupting activity, we have refined methods for the assessment of total activity against estrogen receptors (ER) and androgen receptors (AR) in human plasma. Bioassays using T47D-kb luc and MDA-kb2 cell lines were optimized to measure ER and AR activation, respectively, in archived plasma samples of 90 Mexican American women from the San Francisco Bay Area Breast Cancer Study (SFBCS). As expected, we found a statistically significant difference in ER activity levels between pre-menopausal (mean=498pM) and post-menopausal (mean=125pM) women (p<0.001). There was no statistically significant difference in post-menopausal women. There was also no statistically significant difference (p=0.12) between breast cancer cases (n=15) and controls (n=75) in part due to our small sample size. Identifying subjects with widely differing levels of ER and AR activity (after subtraction of the natural steroid hormone component) is possible by measuring protein levels and metabolite levels simultaneously. This should provide a rapid, agnostic, sensitive and cost effective method for identifying potential environmental compounds with estrogenic and androgenic potential.

1474 Enabling a More Predictive Assessment of Effects on Stereoidogenesis (OECD TG 456) by Applying an Improved LC-MS/MS Method

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Endocrine disruption is a topic currently under scientific and political debate. In this context, much effort has been done to establish in vitro methodologies that could give reliable insights into the mechanisms of endocrine effects of compounds without the use of animals. Amongst those methods, the steroidogenesis assay a regulatory accepted method (OECD TG 456 and OPPTS 890.1550) aims to identify compounds that interfere with the steroid hormone synthesis. It uses the human adreno-carcinoma cell line H295R, which harbors the genes encoding key enzymes for steroidogenesis. The assay determines changes in estradiol (E2) and testosterone (T) levels measured in the supernatant of treated cells. Analytical methods such as LC-MS/MS offer the possibility to determine several hormones in parallel and largely avoid cross-reactivity of the test substance, as it could occur with the classical immunoassays. Herein, we present a newly developed method that not only determines E2 and T in a single run, but also 19 additional steroids involved in the sex-hormones biosynthesis: androstenedione, dihydrotestosterone, 11-deoxy cortisol, 18-hydroxy-dihydrocortisol, 18-hydroxy-cortisosterone, 18-hydroxy cortisol, corticosterone, cortisol, 17a-hydroxy-cortisol, androstenedione, aldosterone, cortisone, 17a-hydroxyprogesterone, etiocholanolone, DHEA, aldosterone, 17a-hydroxyprogrenolone, estrone, estradiol, pregnenolone and progesterone. This improved method not only meets all quality criteria required under the OECD TG 456 with high efficiency, but it opens the possibility to better understand the mode of action of test substances and identify specific steroidogenesis targets.

1475 Determination of Steroidal Compounds in Plasma from Individual Zebrafish Using 5-Minute-Capillary Electrophoresis Separations

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Zebrafish are an important in vivo model to evaluate endocrine disruption because the number of viable eggs produced is readily quantified. They are easy to maintain and reproductively active at 16 weeks. Chemical signatures from circulating steroids are a direct measure of endocrine system function, but measurement of multiple steroids is difficult because of the limited plasma volumes generated by small model fish. As a result, steroids are measured in pooled samples or only a single steroid can be assessed in each sample. New technology is described to address this using 5 minute capillary electrophoresis separations that incorporate secondary equilibria via sodium dodecyl sulfate and cyclodextrin in order rapidly quantify a set of six steroidal compounds. The UV-visible absorbance detection coupled to the separation generated detection limits ranging from 0.2 - 1 pg/µL by taking advantage of pH-mediated stacking of negatively-charged cyclodextrin carrier molecules at a discrete pH interface between the reconstituted sample and the separation electrolyte. The effectiveness of the method for measurements of multiple steroids in volume-limited samples is demonstrated in individual fish with total circulating blood volumes of approximately 5 µL. The method was applied to reproducibly active zebrafish and changes were detected in the levels of circulating steroids as a result of exposure to different solvents and to endocrine disrupting compounds. For example, estrone was only detected in animals exposed to acetone and butachlor. Decrease in 17a,20β-dihydroprog-4-en-3-one and increase in 11-ketotestosterone were observed in females following exposure to 17α-ethynyl estradiol. This technology provides a means to assess steroid concentrations directly in individual fish. Research reported in this abstract was supported by the National Institute of Environmental Health Sciences under award number 5R21ES023575.
Interssex, induction of vitellogenin (Vtg) and zona radiata protein (Zrp) in male and juvenile fish by environmental estrogens in vertebrates has been proposed as an effective and sensitive biomarker of endocrine disruption. Despite the universal use of these biomarkers, their application as reproductive alteration endpoints for tropical species and for developing countries, such as Nigeria, is limited and almost non-existent. Herein, a total of 101 tilapias (Tilapia zillii, T. guineensis, Sarotherodon melanotheron, S. galileica and Oreochromis niloticus) were collected from Eleyele Lake receiving sewage and industrial effluents from point sources. Vtg and Zrp gene expressions were analysed using RT-qPCR. The gonads were histologically and morphologically examined and GSI evaluated. Plasma levels of E2, 11-KT, FSH and LH were measured using ELISA. We detected Vtg and Zrp mRNA expressions, with significantly higher expressions in males compared to females. While no significant sex-related alterations in plasma hormone were observed, the GSI of females were significantly higher than males. We are currently analyzing biota and sediment contaminants to discern cause and effects relationships between the measured androgen (AR) or glucocorticoid (GR) receptor-mediated activity using triadof estrogenic activity in the post-treatment extracts was not quantifiable in 13 out of the 16 extracts and reduced to less than 0.1 ng/L in the remaining 3. The differences in the results of the source water extracts between the ER-TA and the analytical chemistry can likely be explained by the fact that the ER-TA assay detects total estrogenic activity of all estrogenic compounds, not just estrogen, and the assay has a lower limit of detection greater than the analytical chemistry. In conclusion, using both assays allows one to detect very low levels of estrogenic activity and also to identify at least some of the chemical(s) contributing to the total activity. Importantly, this study indicates that the treatments used by the drinking water plants are effective at reducing and/or removing estrogenic activity from source water. This abstract does not necessarily reflect U.S. EPA policy.

In hormone-dependent breast cancer, CYP19 expression is increased via activation of promoters (PII, 1.3, 1.4, 1.7). CYP19 biosynthesizes estrogens, which stimulate cell proliferation. Estrogens are also responsible for proper development of the placenta, where CYP19 is regulated by promoter L1. Exposures to certain pesticides, such as atrazine, are associated with increased CYP19 expression, but little is known about the endocrine disrupting potential of neonicotinoids. Our objective was to develop cell-based assays to identify chemicals that alter the promoter-specific expression of CYP19 and its catalytic activity. H295R human adrenocortical carcinoma and HUVEC human primary umbilical vein endothelial cells were exposed to atrazine or neonicotinoids (imidacloprid, thiaildiazem, thiamethoxam) (0.1-30μM) for 24h. CYP19 expression was measured by RT-qPCR and catalytic activity by titrated water-release assay. In H295R cells, atrazine concentration-dependently increased PII- and L3-mediated CYP19 gene expression (7-fold), and aromatase activity (2-fold). Thiacloprid (0.3, 3 and 10μM) induced PII and L3-mediated CYP19 expression (3-14 fold) and aromatase activity. At 0.1μM, thiamethoxam induced PII- and L3-mediated CYP19 expression (14-fold) and aromatase activity (2-fold). At 3μM, imidacloprid slightly inhibited PII and L3-mediated CYP19 expression, but had no effect on activity. In HUVEC cells, atrazine concentration-dependently increased CYP19 expression and activity, but no effects were observed on L1-promoter. PII and L3 were not detected. Thiacloprid (0.3-10μM) induced L1-mediated CYP19 expression and aromatase activity. Thiamethoxam (0.1-10μM) had no effect on CYP19 expression, but activity was induced by 3-fold at 5 and 10μM. Imidacloprid did not affect on L1-mediated expression or aromatase activity. These novel screening tools will be helpful in assessing the risk chemicals may pose to exposed women by altering the tissue- and disease-specific promoters involved in CYP19 regulation.

In this study, scientists from the U.S. Environmental Protection Agency (EPA) and the U.S. Geological Survey (USGS) studied source and treated samples collected from drinking water treatment plants (DWTP) across the U.S. For this study, extracts of source and treated water samples from 24 plants were assessed for both estrogenic activity using T4/3/4-mediated transcription activation (ER-TA) assay, and LC/MS/MS analytical chemistry for about 12 estrogenic analytes. Estriol was the only estrogenic analyte identified above the minimum reporting limit for the chemistry, which was present in only 4 source water samples. In the ER-TA assay, however, 16 of the 24 source water extracts had low but detectable estrogenic activity. Importantly, ER-TA estrogenic activity in the post-treatment extracts was not quantifiable in 13 out of the 16 extracts and reduced to less than 0.1 ng/L in the remaining 3. The differences in the results of the source water extracts between the ER-TA and the analytical chemistry can likely be explained by the fact that the ER-TA assay detects total estrogenic activity of all estrogenic compounds, not just estriol, and the assay has a lower limit of detection greater than the analytical chemistry. In conclusion, using both assays allows one to detect very low levels of estrogenic activity and also to identify at least some of the chemical(s) contributing to the total activity. Importantly, this study indicates that the treatments used by the drinking water plants are effective at reducing and/or removing estrogenic activity from source water. This abstract does not necessarily reflect U.S. EPA policy.
Screening of a small chemical library of 130 different xenobiotics for their ability to stimulate/inhibit luciferase expression from the pL4 aromatase gene promoter identified genistein as an activator of the aromatase pL4 promoter. This novel cell line is being optimized for large-scale screening. Although no stable cell lines containing luciferase under control of aromatase gene promoters were isolated, screening of the small chemical library using SKBR3 cells transiently transfected with these aromatase gene promoter-luciferase was carried out and identified several activators of reporter gene expression. The availability of recombinant cell lines for breast cancer specific aromatase gene promoters analysis could identify novel chemicals that can stimulate/inhibit breast cancer growth by affecting estrogen production. (CBCRP17UB-8703)

1481 The Impact of Estrogens on Epithelial to Mesenchymal Transition in Lung Cells

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Idiopathic pulmonary fibrosis (IPF), a fatal disease without effective treatment and unknown etiology, is a result of chronic and progressive lung tissue scarring (fibrosis) and subsequent stiffening and loss of function. Epidemiological studies suggest sex-specific trends where the prevalence of IPF is higher in males while females have better survival rates. This suggests that sex hormones may contribute to IPF pathogenesis. A process pivotal to IPF is epithelial to mesenchymal transition (EMT) which is characterized by a loss of alveolar epithelial cell polarity and transition to a mesenchymal phenotype which actively secretes extracellular matrix components. While the molecular mechanisms driving EMT have been extensively studied, few investigations have probed the involvement of hormones, namely estrogens, in this process. Modulation by estrogens highlights EMT as a target potentially susceptible to environmental estrogens. To begin to study the role of estrogens in EMT we focused on a lung cell model. Using alveolar epithelial cells (BEAS-2B) we are able to induce EMT after a 5 day exposure to 5 ng/ml transforming growth factor beta 1 (TGF-B1) by measuring a decrease in expression of the epithelial cell marker E-cadherin and an increase in expression of the myofibroblast and mesenchymal markers α-smooth muscle actin and vimentin. Connective tissue growth factor (CTGF), a pro-fibrotic protein was also highly induced. Preliminary experiments in which cells were co-exposed to TGF-B1 and 10 nM 17β-estradiol (E2) surprisingly indicate that E2 may exacerbate TGF-B1-mediated CTGF expression. These data highlight a potential and novel role for E2 in IPF. Studies are currently under-way to investigate the involvement of select estrogen receptors and modulation by environmental estrogens in potentiating EMT. Results from this work will increase the understanding of IPF pathogenesis, particularly sex-specific differences and identify targets for modulation by environmental estrogens.

1482 Androgen Receptor Binding Affinity Prediction of Chemicals Using Pharmacophore and 3D-QSAR Models


Concerns have been raised about chemicals with endocrine disrupting (ED) activities. Consequently, European Authorities have taken actions and banned some of these from consumer goods (e.g.: bisphenol A), which may be present within pharmaceuticals, plasticizers, pesticides or consumer products. The context of the European Regulations (REACH, Cosmetic Directive) implies, in the development of new cosmetic ingredients, as an important and early step, to identify a potential endocrine modulation activity of the molecules in the course of their discovery process, during the ingredient design phase. Thus, as part of the selection process for new compounds, pharmacophore and 3D-QSAR models were built to predict their affinity to the androgen receptor (AR). To build these models, we used the structural information available in the Protein Data Bank (PDB) for the androgen receptor and the public data (binding of chemicals on the AR) available on the web-site of the US-EPA. The first step of this work aimed at designing and selecting complementary pharmacophore models from the structures of the AR available in the PDB (approx. 50). Four pharmacophore models were selected. Accordingly, a 3D-QSAR model was built to quantify the affinity to the AR, using the training set of molecules (from the US-EPA) after pharmacophore alignment in the receptor active site. This model estimates the logarithm of the Relative Binding Affinity (RBA) of the molecule with respect of the affinity of dihydro-testosterone (DHT). The prediction (RBA) allows determining the activity class (e.g.: inactive or active). The overall performance of these models (pharmacophore alignments followed by 3D-QSAR predictions) is suitable, in comparison with commercial dedicated software, offering a sensitivity of 0.90 and a specificity of 0.74 with respect of the log(RBA). Thus, our model allows selecting planned cosmetic ingredients, which probably will not interact with the AR, prior their synthesis, with a low rate of both false positive and false negative results.

1483 Establishment of Sensitive, Quantitative, and Real-Time Cellular Assays for Assessment of Modulators for Human Androgen Receptor Signaling Pathways


Androgen receptor (AR) plays critical role modulating androgen function for the development and maintenance of male sexual characteristics, and the carcinogenesis and metastasis of certain prostate cancer. The signaling pathways are further complicated by frequent mutations in AR genes. Using real time impedance technology, cell based kinetic assays for detection of AR modulators and understanding the AR signaling pathways were developed. Two androgen responsive human prostate cancer cell lines 22Rv1 and LNCaP were used for the study. Stimulation of these cells with androgen agonists such as R1881 and DHT, lead to alterations in cell number and cell adhesion, which can be detected by gold microelectrodes embedded in the bottom of the well of specialized microelectrode plates. The time-dependent cellular kinetic response profiles were different in 22Rv1 and LNCaP cells, indicating distinctive endogenous androgen signaling pathways in these two cell lines. Both cell types exhibited EC50 values in the picomolar range, indicating high sensitivity to androgen receptor stimulation. The specificity of the assay for AR activity was established using pure AR antagonists, such as bicalutamide and nilutamide, and chemicals known with anti-androgen side effect, such as vinclozolin. More interestingly, when LNCaP cells were starved, the kinetic response profile to AR agonist was changed, reflecting altered native androgen response pathways in response to changes in growth condition. In addition, under this condition, nilutamide and vinclozolin displayed AR agonist rather than AR antagonist effects in the real time cellular assay, consistent with reported effect of the T877A mutation in LNCaP AR. The data suggests that the impedance based real time cellular assay system has the capacity to sensitively, selectively and quantitatively detect endogenous AR responses. The information can be used to understand endogenous AR signaling pathways, and to develop new AR modulators for therapeutic applications.

1484 Cadmium Uregulates Transcription of the Steroidogenic Acute Regulatory Protein (StAR) Gene through Phosphorylated CREB Rather Than SF-1 in K28 Cells

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Cadmium is a widely used heavy metal in industry and affects the male reproductive system of animals, including humans, as a result of occupational and environmental exposures. However, the molecular mechanism underlying its effect on steroidogenesis in gonads remains unclear. In this study, we demonstrated that exposure of K28 mouse testicular Leydig tumor cells to cadmium led to a significant increase in the mRNA level, promoter activity and protein level of the steroidogenic acute regulatory protein (StAR), an essential factor for steroid biosynthesis. It has been well documented that StAR gene transcription is regulated by CREB family members and SF-1. Cadmium treatment caused an increase in StAR gene transcription. However, the level of both false positive and false negative results. Consequently, European Authorities have taken actions and banned some of these from consumer goods (e.g.: bisphenol A), which may be present within pharmaceuticals, plasticizers, pesticides or consumer products. The context of the European Regulations (REACH, Cosmetic Directive) implies, in the development of new cosmetic ingredients, as an important and early step, to identify a potential endocrine modulation activity of the molecules in the course of their discovery process, during the ingredient design phase. Thus, as part of the selection process for new compounds, pharmacophore and 3D-QSAR models were built to predict their affinity to the androgen receptor (AR). To build these models, we used the structural information available in the Protein Data Bank (PDB) for the androgen receptor and the public data (binding of chemicals on the AR) available on the web-site of the US-EPA. The first step of this work aimed at designing and selecting complementary pharmacophore models from the structures of the AR available in the PDB (approx. 50). Four pharmacophore models were selected. Accordingly, a 3D-QSAR model was built to quantify the affinity to the AR, using the training set of molecules (from the US-EPA) after pharmacophore alignment in the receptor active site. This model estimates the logarithm of the Relative Binding Affinity (RBA) of the molecule with respect of the affinity of dihydro-testosterone (DHT). The prediction (RBA) allows determining the activity class (e.g.: inactive or active). The overall performance of these models (pharmacophore alignments followed by 3D-QSAR predictions) is suitable, in comparison with commercial dedicated software, offering a sensitivity of 0.90 and a specificity of 0.74 with respect of the log(RBA). Thus, our model allows selecting planned cosmetic ingredients, which probably will not interact with the AR, prior their synthesis, with a low rate of both false positive and false negative results.

1485 Vitamin E and Quercetin Attenuate Nigerian Bonny-Light Crude Oil-Induced Neuronal and Testicular Toxicity in Wistar Rats

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There is mounting experimental evidence accentuating the testicular and neuronal toxicity of environmental/industrial chemicals in experimental animals via mechanism involving oxidative damage. Nigerian Bonny Light Crude Oil (BLCO) has been reported to exhibit reproductive and neuronal toxicity in male rats. Studies
have shown that vitamin E and quercetin protects rat neuronal and testicular cells from environmental/chemical-induced oxidative damage. We investigated the possible protective role of quercetin and vitamin E in BLCO induced-neuronal and testicular toxicity. Male rats were administered BLCO at doses of 400 and 800 mg/kg body wt/day-p.o. 3 times/week for 6 weeks. Other groups were co-administered BLCO (400 and 800 mg/kg body wt/day-p.o.) with/without vitamin E (E) (50 mg/kg body wt/day-p.o.) or quercetin (Q) (10mg/kg body wt/day-p.o.) 3 times/week for 6 weeks respectively. Semen quality deteriorated; testosterone and luteinizing hormone (LH) levels were significantly decreased while follicle stimulating hormone (FSH) increased following BLCO-treatment. There was a significant decline in the activities of superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), and glutathione –S-transferase (GST) with concomitant increased levels of lipid peroxidation and activities of Xanthine oxidase (XO) in a dose dependent manner, in testes and brain of rats. Co-administration with vitamin E or quercetin reversed BLCO-induced neuronal and testicular toxicity by preventing the oxidative stress, improving sperm quality and restoring hormonal levels relative to control. Quercetin and vitamin E showed possible chemo-protection against BLCO induced reproductive and neuronal toxicity.

### 1486 Evaluation of the Androgen Antagonist Potential of Quinoline (CAS: 91-22-5) in Surgically Castrated Peripubertal Male Rats

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The potential effects of the Quinoline as androgen antagonist was investigated in the Herbergher Bioassay using castrated male Wistar rats. A total of 30 rats were divided into 5 groups comprised of 6 rats. Negative control [Testosterone propionate (TP) – 0.4 mg/kg; subcutaneous], positive control [Flutamide (FLU) – 3 mg/kg; oral + TP-0.4 mg/kg; subcutaneous] and three groups of Quinoline (50, 100 & 200 mg/kg; oral + TP – 0.4 mg/kg; subcutaneous) were treated for 10 consecutive days. All animals were sacrificed approximately 24 hours following the last dose. No treatment related mortality was observed during the study. Weakness and lethargy were observed in 200 mg/kg Quinoline group. Body weight, body weight gain and feed consumption of the positive control group were comparable with the negative control group. Terminal body weight of the 100 and 200 mg/kg Quinoline groups were statistically significant decreased compared to the negative control group. Body weight, body weight gain and feed consumption of the positive control group were comparable with the negative control group. Absolute and relative organ weights of androgen dependent organs (glans penis, LABC, coxer’s gland, ventral prostate and seminal vesicle) of Quinoline treated groups were comparable to the negative control group. Statistically significant decreases in absolute and relative organs of androgen dependent organs were observed in the positive control group as compared to the negative control group. Based on the result of study Quinoline showed no evidence of androgen antagonist activity.

### 1487 Obesogenic Effects of Endocrine Disrupting Chemicals in Tilapia Species from Ogun River, Nigeria

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The environmental obesogen hypothesis has proposed the role of EDGs in the development of obesity through the activation of PPARα, a key regulator of adipogenesis and medically implicated in obesity. In addition, the induction of cytochrome P450 by environmental pollutants (PAHs, PCBs, dioxin and furans) in organisms through the AhR is generally used as a sensitive and reliable biomarker of environmental pollution. To evaluate the obesogen hypothesis and biomarker responses, 1074 samples of Tilapia species were collected from three sampling points, reflecting different degrees of anthropogenic contamination and a putative control site along Ogun River, Nigeria. Hepatic mRNA was analyzed using RT-qPCR and fish condition factor (k-factor) (a physiological index of fish health and growth) were also evaluated. A significant increase in mRNA expression of PPARα, β and γ were observed in fish from polluted sites of the river compared with control site. In addition, a significant increase in mRNA expression of cyp1a, cyp1b and cyp1c were observed in male and female fish collected from polluted sites receiving industrial effluents from point sources. Both male and female fish also displayed considerable EDC biomarker expression of Vtg and Zrp. The condition factor for fish from the polluted sites were >1 (around 2) indicating good health and growth status. We are currently analyzing biota and sediment contaminant levels to discern cause and effects relationships between the measured responses and chemical burden. Overall, our findings demonstrate the ability of EDGs in Ogun River to elicit an increase in fish growth by activating PPARα.

### 1488 PCB126-Induced Activation of Aryl Hydrocarbon Receptor Inhibits Adipogenesis

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Emerging evidence indicates that persistent organic pollutants (POPs), including polychlorinated biphenyls, are involved in the development of diabetes. PCBs are lipophilic and target the adipose tissue. Dysfunctional adipocytes play a significant role in initiating insulin resistance. Preadipocytes make up a large portion of adipose tissue and are necessary for the generation of functionally mature adipocytes to maintain metabolic homeostasis. PCB126 is a dioxin-like PCB and a potent aryl hydrocarbon receptor (AhR) agonist that has been associated with metabolic syndrome. We hypothesized that PCB126 may be involved in the development of metabolic syndrome through inhibition of adipogenesis. Using a newly developed immortal human preadipocyte cell line, we found that pre-exposure of preadipocytes to PCB126 resulted in significant reduction of their ability to subsequently differentiate into adipocytes, more so than when the cells were exposed to PCB126 during differentiation. Reduction in differentiation by PCB126 was associated with down regulation of transcript levels of a key adipocyte transcription factor, PPARγ, and adipocyte marker genes, adiponectin and fatty acid binding protein 4. Decreasing the activity of AhR through stable knockdown of AhR using shRNA or an antagonist CH223191 mitigated the metabolic toxicity of PCB126 on lipid accumulation in preadipocytes. These studies indicate the need to understand the functional role of adipose tissue in the overt toxicity of PCB126 that also leads to hepatic steatosis. Our initial studies on rats injected with PCB126 have shown significant changes in the levels of cholesterol and triglycerides in the serum. However, the specific effects of PCB126 on the function of adipose tissue in vivo need to be further investigated. These studies indicate that human preadipocytes are particularly sensitive to AhR activation by PCB126 that may lead to metabolic dysfunction of adipocytes to cause metabolic disruption.

### 1489 Mechanisms of Doxorubicin Toxicity in Pancreatic Beta Cells

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Exposure to chemotherapeutic agents has been linked to an increased risk of type 2 diabetes, a disease characterized by both peripheral tissue insulin resistance and impaired insulin secretion. Recent interest in endocrine-disrupting chemicals (EDCs) - environmental chemicals that interact with estrogenic and androgenic systems - have shown significant changes in the levels of cholesterol and triglycerides in the serum. Doxorubicin (adriamycin) is a common drug used to treat various cancers. Doxorubicin caused a dose-dependent impairment of glucose-stimulated insulin secretion (GSIS) from INS-1 832/13 cells, within 6 h of exposure. Toxicity (measured as leakage of an intracellular protocase) and apoptosis (measured as caspase 3/7 enzymatic activity) were both significantly increased after 6 h of doxorubicin exposure. Doxorubicin toxicity has previously been shown to be mediated in part via redox cycling and DNA damage in other tissues. However, oxidative stress did not play a major role in the induction of toxicity and apoptosis in β-cells, since doxorubicin did not redox cycle in live INS-1 832/13 cells or with cellular lysates. A decrease in the total (NADH+NAD+) pool was observed following 6 h of doxorubicin treatment, consistent with the activation of the poly-ADP ribose polymerase (PARP) pathway, which generates a polymer from NAD+ at the DNA damage site to recruit DNA repair enzymes. Doxorubicin-mediated toxicity and depletion of the (NADH+NAD+) were temporarily attenuated by the addition of MK-8427, a potent, selective PARP inhibitor. Together, these data suggest that PARP activation, not oxidative stress due to redox cycling, is the primary mechanism of toxicity in pancreatic β-cells.
1490  Effect of In Vitro DDE Exposure on Pancreatic Beta Cell Markers Related to Beta Cell Dysfunction and Type 2 Diabetes Mellitus
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Epidemiological evidence in humans suggest that higher levels of some organochlorine compounds (OC), including the DDT metabolite DDE, is associated with type 2 diabetes mellitus (T2D) and may disrupt physiological functions that contribute to T2D such as beta cell dysfunction. Beta cell dysfunction is characterized by altered insulin secretion, insulin transcription, and oxidative stress levels. The in vitro effect of DDE exposure on pancreatic beta cell function was investigated by measuring insulin accumulation/secretion, reactive oxygen species (ROS) activity, and the insulin transcription factor pancreatic and duodenal homeobox factor-1 (PDX-1) levels in murine Beta-Tumor Cell-6 (B-TC-6) pancreatic cells. Cells were exposed to 0, 5, 10, or 15 mM glucose for 2 hours to measure glucose-responsive insulin secretion. Cells were pretreated with 10 uM DDE alone for 24 hours, or pretreated with 10 uM DDE for 24 hours and exposed to 5 mM glucose for an additional 2 hours before immunoblot assay to measure insulin secretion. ROS activity was measured by immunofluorescence from cells exposed to 0, 5, or 10 mM glucose for 3 hours and from cells exposed to 5 mM glucose simultaneously with 10 uM DDE for 3 hours. PDX-1 protein levels were measured by immunohistochemistry from cells exposed to 10 uM DDE simultaneously with 5 mM glucose for 24 hours. DDE exposure significantly increased intracellular insulin accumulation and secretion suggesting a role in the regulation of insulin synthesis, transport, and/or exocytosis. ROS levels were significantly increased for DDE-exposed cells indicating oxidative stress as a potential mechanism. PDX-1 protein levels were increased in DDE-exposed cells suggesting a possible effect on insulin gene transcription. DDE exposure may provide a causative link to the increased prevalence of T2D by its effect on beta cell function.

1491  Comparison of Responses of Primary Adrenocortical Cells from Rat and Dog to Stauroporine and the Adrenal Toxicant Lyso ptrone
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Dog primary adrenocortical cells were isolated, characterized and compared to similar primary rat cultures for the use of investigative toxicity studies. Cell numbers and viability were similar with adrenals shipped overnight or when processed within 2 hr after resection. Collagenase digestion provided more viable cells than trypsin digestion. This is consistent with the fact that collagenase digestion is more specific than trypsin digestion. Aldosterone production decreased rapidly overnight, then decreased further with time, whereas cortisol production decreased more gradually. Both adrenocorticotrophic hormone and forskolin increased and prolonged steroidogenesis and steroidogenic enzyme gene expression. Primary rat adrenal cells were similar to dog cells, although rat cell plating and survival were better than for dog cells. Responses to known toxicants were also compared, when testing for metabolic activity (Celltiter blue, CTB), lactate dehydrogenase leakage (LDH), and caspase 3/7 activation in cells exposed to stauroporine (mitocontacts, drug for Cushing’s and adrenal tumors) or stauroporine (inhibitor of apoptosis). Lysoptorone decreased LTB (50%) and slightly increased LDH in dog cells. Caspase activation was slightly increased at 6 hr, but not 24 hr, after exposure. Lysoptorone in primary rat cells reduced CTB (40%) of controls, but did not significantly affect LDH and caspase. Stauroporine significantly increased caspase activation, especially after 6 hr post-exposure, and reduced CTB, but had no effect on LDH in either model. Aldosterone and cortisol (dog) or corticoste rone (rat) production was decreased with both compounds, but only at cytotoxic concentrations. These results demonstrate functionality of primary adrenocortical cells for both species, but suggest only acute effects should be examined in adosterone-producing cells.

1492  Calcium Signaling Disruption As a Mechanism for Altered Hormone Secretion

Non-coplanar polychlorinated biphenyls (PCBs) and polybrominated biphe nyl ethers (PBDEs) alter thyroid hormone (TH) signaling in exposed animals. Regulating TH homeostasis is a complex set of negative and positive feedback loops which comprises the Hypothalamic-Pituitary-Thyroidal (HPT) axis. On a basic level, the hypothalamus secretes thyrotropin releasing hormone (TRH) which regulates anterior pituitary secretion of thyroid stimulating hormone (TSH) which stimulates thyroxine synthesis in the thyroid gland. Studies aimed at understanding chemical induced effects on TH signaling have focused on mechanisms involving TH receptor-mediated impacts or altered TH transport and metabolisms. However, there remain questions regarding the role that these mechanisms play in inducing thyroid hormone dysregulation. Interestingly, chemicals known to alter circulating TH levels also alter important aspects of Ca2+ signaling in excitable cells. In adrenocortical cells, Ca2+ signaling plays a crucial role in endocrine hormone secretion yet this has not been investigated as a mechanism leading to pollutant induced TH alterations. Utilizing a novel thryostere cell line, ToT1, together with Ca2+ sensitive fluorescent dyes, we monitored chemical induced changes in Ca2+ signaling as a mechanism leading to altered secretion of TSH. We have demonstrated that 10uM PCB 95 and PBDE 49 significantly alter Ca2+ signaling behavior in 85% of acutely exposed ToT1 cells, respectively. These changes were not seen in cells exposed to coplanar PCB 77 or mono-ortho PCB 66. Findings also suggest that sub-acute exposure to lower concentrations of PCB95, but not PBDE 49, leads to altered response to TRH known to regulate TSH secretion. We investigated the role of various Ca2+ channels in chemically induced Ca2+ signaling disruption in ToT1 and how changes in Ca2+ relate to altered TSH secretion after sub-acute exposures. Findings suggest that Ca2+ signaling may represent a novel mechanism of endocrine disruption with important implications on the health of exposed organisms. Supported by 5P42 ES04699, 2P01ES011269, 1R01 ES014901 and T32 HL086350.

1493  Development of a Tiered Screening Strategy for a Molecular-Initiating Event: Thyroid Peroxidase Inhibition
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Adverse outcome pathway (AOP) analyses illustrate that some molecular-initiating events (MIEs) for thyroid disruption, including thyroid peroxidase (TPO) inhibition, are not evaluated by current ToxCast/Tox21 high-throughput screening (HTS) assays. A novel HTS assay for TPO inhibition was developed by adaptation of the guaiacol oxidation assay with a fluorescent peroxidase substrate (Amplex UltraRed, AUR, LifeTech) in a rat thyroid microsome-based assay optimized to 384-well format. Initial testing of the AUR-TPO assay was conducted using a 21-chemical training set that included a reference chemical, methimazole (MMI), known TPO inhibitors, and negative controls. The AUR-TPO assay signal was stable (30-120 min), the dynamic range and Z’ score with MMI were 11-fold and 0.93, and the IC50 for MMI was 0.025 μM (compared to 2.20 μM in a guaiacol-based 96-well assay). The ToxCast Phase I & II libraries (1060 chemicals) were tested with the AUR-TPO assay using an initial screen of one high concentration to identify candidate inhibitors. Chemicals that exceeded 20% inhibition were then screened in concentration-response (6 or 8 concentrations for Phase I & II). In addition, 2 parallel screens were conducted: ATP availability in HEK293T cells (24 hr exposure) to indicate cytotoxicity; and, luciferase inhibition to identify nonspecific protein inhibitors. Results demonstrated known and novel chemicals that inhibited TPO activity in the ToxCast assay that did not inhibit AUR-TPO, AOP-based analyses for thyroid screening enabled development of a new HTS assay that allowed screening of ToxCast chemicals for TPO inhibition. This assay provides an additional data stream to support prioritization of chemicals within the Endocrine Disruptor Screening Program. This abstract does not necessarily reflect the policy of the US EPA.

1494  The Tadpole Visual System As a Model for Assessing the Effects of Thyroid Hormone Disruption on Brain Development

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Thyroid hormone plays an important role in brain development, and evidence shows that some compounds used in the production of industrial and consumer products can disrupt thyroid hormone signaling, with severe consequences for behavior. How thyroid hormone disruptors impact neural circuit development is still unclear, however, Tadpoles are useful models for better understanding endocrine disruption because they are acutely sensitive to changes in thyroid hormone signaling. In addition, their external development allows for manipulation and observation of the early stages of brain development which are relatively inaccessible.
for study in mammalian systems. Here we show that visual system development in *Xenopus laevis* tadpoles is affected by changes in thyroid hormone signaling. Our data show that thyroid hormone acts directly on the brain and the retina to significantly increase the rate of proliferation. In another experiment, we sought to examine the effects of thyroid hormone on axonization of visual system neurites. We treated tadpole brains with thyroid hormone that had been electroporated with GFP-overexpressing plasmids seven days prior and imaged these neurons with a 2-photon microscope each day for four days. We found that thyroid hormone treatment significantly increased dendrite arbor length and branch tip number by 66% and 73%, respectively. Last, we treated tadpole brains with thyroid hormone and seven days later electroporated the brains with plasmids that selectively overexpress GFP in neural progenitors and examined division and differentiation of neural progenitor cells with a 2-photon microscope. We found that thyroid hormone increased the rate of neuronal differentiation by more than 300%. These experiments establish a baseline for the sensitivity of tadpole brain development to changes in thyroid hormone signaling against which we will compare the effects of thyroid hormone disruptors.

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**1495 Xenopus laevis Müllerian Ducts Are Sensitive Indicators of Estrogenic or Androgenic Chemical Exposure In Vivo**


The Larval Amphibian Growth and Development Assay (LAGDA) is one of a series of Tier 2 test guidelines being developed by the US EPA under the Endocrine Disruptor Screening Program. The LAGDA was designed to evaluate effects of lower-dose, longer-term chemical exposure on (a) amphibian metamorphosis mediated by the hypothalamic-pituitary-thyroid (HPT) axis, and (b) reproductive development mediated by the hypothalamic-pituitary-gonadal (HPG) axis. In the development phase of the assay, chemicals with known modes of action were chosen to determine the assay’s performance. Twelve chemicals were chosen to target the HPG axis, a weak estrogen receptor agonist, 4-tert-octylphenol, and an androgen receptor agonist, 17β-Trenbolone. *Xenopus laevis* embryos were constantly exposed (flow-through conditions) to various doses of either 4-tert-octylphenol (6.25, 12.5, 25, 50 μg/L) or 17β-Trenbolone (12.5, 25, 50, 100 ng/L) and clean water controls until 8 weeks post-metamorphosis, at which time growth measurements were taken and histopathology assessments were made on gonads, reproductive ducts, liver, and kidneys. There were no effects on growth in both studies and only minimal pathologies found in the liver, kidneys and gonads of frogs in the high treatments. However, Müllerian duct development was significantly affected following exposure to both chemicals, as maturation (oviduct formation) and Müllerian duct regression are estrogen and androgen-dependent processes respectively. 4-tert-octylphenol exposure caused dose-dependent formation and maturation of oviducts in both male and female frogs, whereas 17β-Trenbolone exposure caused accelerated regression in males and complete regression in >50% of the females in the 100ng/L treatment making them ostensibly unable to reproduce. Based on these results, it appears that the Müllerian ducts are more sensitive to estrogenic and androgenic influence than are the gonads or other reproductive tissues within the *Xenopus* HPG axis.

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**1496 Inhibitory Effects of Metallothionein-III on Production of Amyloid-Beta and Induced Cytotoxicity in Neuronal Cells**

B. Park, H. Kim and H. Jeong.

The aggregation and cytotoxicity of amyloid-beta (Aβ) with redox-active metals in neuronal cells have been implicated in the progression of Alzheimer disease (AD). Metallothionein-III (MT-III) is a metal-binding protein with established antioxidant capabilities. The expression and induction of MT-III has been associated with protection against the Aβ aggregation and induced toxicity. This study investigated that MT-III is involved in generation of Aβ and induced toxicity in neuronal cells. MT-III increased soluble APPα levels and reduced Aβ peptide levels. MT-III increased the activity of a disintegrin and metalloproteinase 10 (ADAM10) through the increase of proprotein convertase7 (PC7) and furin, and PKCζ. Reduced Aβ production recovered by specific inhibitor of furin and PC7, and PKCζ. MT-III reduced the activation of caspase-3 in cells. In addition, MT-III increased Bel-2 levels and reduced Bax levels. These results suggest that MT-III inhibited Aβ production by active ADAM10. Also, MT-III can protect neuronal cells against Aβ-induced cytotoxicity.

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**1497 Sulforaphane Alleviates Scopolamine-Induced Memory Impairment in Mice**

S. Lee, J. Kim, S. Seo, B. Cho, J. Han, K. Lee and J. Kim.

Sulforaphane, an organosulfur compound present in cruciferous vegetables, has been shown to exert neuroprotective effects in experimental *in vitro* and *in vivo* models of neurodegeneration. To determine whether sulforaphane can preserve cognitive function, we examined its effects on scopolamine-induced memory impairment in mice using the Morris water maze test. Sulforaphane (10 or 50 mg/kg) was administered to C57BL/6 mice by oral gavage for 14 days (days 1–14), and memory impairment was induced by intraperitoneal injection of scopolamine (1 mg/kg) for 7 days (days 8–14). Mice that received scopolamine alone showed impaired learning and memory retention and considerably decreased cholinergic system reactivity in the hippocampus and frontal cortex, as indicated by a decreased acetylcholine (ACh) level and an increased acetylcholinesterase (AChE) activity. Sulforaphane significantly attenuated the scopolamine-induced memory impairment and improved cholinergic system reactivity, as indicated by an increased ACh level, decreased AChE activity, and increased choline acetyltransferase (ChAT) expression in the hippocampus and frontal cortex. These effects of sulforaphane on cholinergic system reactivity were confirmed *in vitro*. Sulforaphane (10 or 20 μM) increased the ACh level, decreased the AChE activity, and increased ChAT expression in scopolamine-treated primary cortical neurons. These observations suggest that sulforaphane might exert a significant neuroprotective effect on cholinergic deficit and cognitive impairment.

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**1498 Hyperphosphorylation of Tau in Brain of DAB-Treated Mice Is Associated with the Decreased Hippocampal Neurogenesis**

M. Kim, Baek and S. Kang.

Organic solvent exposure has been suspected as one of culprits of neurodegenerative diseases. 1,2-Diacyetylbenzene (DAB), a neurotoxic metabolite of 1,2-diethylbenzene, causes central and peripheral neuropathies that lead to motor neuronal deficits. The phosphorylation status of the GSK3β (pGSK3β) influences tau hyperphosphorylation which is related to protein aggregation such as neurofibrillary tangle in Alzheimer’s disease. Typical two phosphorylation sites of GSK3β were chosen for our research. One is active form (Tyr216) and another one is inactive form (Ser9) of GSK3β. Active pGSK3β (Tyr216) phosphorylates tau protein and leads to disturbed microtubule stability and protein aggregation. Previously we reported that DAB increased oxidative stress and protein adduct along with impaired hippocampal neurogenesis in mice. In the present study, we examined whether impaired hippocampal neurogenesis is associated with the hyperphosphorylation of tau protein. Six-week-old male C57BL/6 mice were treated with 1 or 5 mg/kg DAB for 2 weeks. pTau and pGSK3β (Tyr216) expression in hippocampal brain homogenate were increased both 1 and 5mg/kg DAB. These results suggest that decreased hippocampal neurogenesis by DAB is related to Tau hyperphosphorylation.

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**1499 Mass Spectrometry Imaging: Linking Neurodegeneration with Environmental Exposure**

J. Laskin and C. Timchalk.

Neurodegenerative disease (ND) has been linked with chemical exposures; however, the exact role of environmental agents and the establishment of disease markers remain elusive. For many NDS, oxidative stress and mitochondrial dysfunction are known contributing factors and the lipid rich regions of the brain are sensitive targets. Correlating toxicant localization with alterations in lipid chemistry offers important information on the functional impact of dosimetry. Nanospray desorption electrosprary ionization mass spectrometry imaging (nano-DESI MSI) relies on localized liquid extraction of molecules from tissue sections followed by gentle ionization and analysis. Nano-DESI MSI enables quantification and spatial localization of molecules providing important information on sub-regional brain dosimetry and lipid chemistry. Quantification is performed by adding an isotopically labeled standard to the nano-DESI solvent, which then compensates for matrix effects and ion suppression that are detrimental to accurate molecular imaging. The analyte image is obtained by normalizing the analyte signal to the signal of the standard in each pixel. Naïve or nicotine exposed (ip 10 mg/kg) rats
were humanely euthanized and brains cryosectioned for nano-DESI MSI. Minute amounts of nicotine (0.6 fmol/pixels) were quantified in rat brain sections by adding d3-nicotine to the working solvent. Lipid quantification in brain was performed using a shotgun-like approach, in which phospholipid standards are added to the nano-DESI solvent and all endogenous phospholipids from the same class are quantified simultaneously. Overall quantitative data were obtained for ~30 high- and low-abundance phospholipids in 7 brain regions. The results show significant regional variations in nicotine and lipid concentrations in rat brain tissue. Nano-DESI has the potential to significantly impact characterization of the spatial localization of toxicants, lipids, and metabolites in heterogeneous tissue to advance the molecular level understanding of the effect of environmental chemical exposure on ND. Funding from NIEHS R21ES024229.

**1500** Tacrine Neurotoxicology and Proteome Pathway Analysis in Neuro-2a Cell Culture Model

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Tacrine is a potent inhibitor of acetylcholinesterase (AChE) that has been used over decades as a therapeutic for Alzheimer’s and other neurodegenerative diseases. However, the exact neurodegenerative function of tacrine remains relatively unclear. Our objective was to elucidate the complex molecular mechanism of action for tacrine within a neuroblastoma Neuro-2a cell culture model. Neuro-2a cells were exposed for 24h to increasing doses of tacrine (10 to 100 microM). Samples were analyzed by quantitative Tandem Mass Tags (TMT) liquid chromatography-mass spectrometry (LC-MS/MS) in combination with proteome pathway analysis. TMT LC-MS/MS quantitative proteomics detected 535 proteins of which 363 were converted by the BioMart search engine into GeneWiki identifiers. Cytoscape 3.1.1 pathway analysis using Reactome F.4.1, Ibeta and ClueGo 2.1.2 applications correlated gene ontology (GO) terms for glutamate cytotoxicity, calcium overload, energy loss, and formation of excessive reactive oxygen species (ROS) with a tacrine dose-dependent increase in cell death. In addition, we also found a tacrine-dependent increase in GO-terms for DNA damage related chromatin remodeling, modulation of the heat shock protein response and targeted protein degradation through ubiquitination in parallel with a reduction in inflammatory mediators. The gained information will aid in understanding the complex cellular signaling pathways activated during tacrine-dependent neuronal mechanisms that might be relevant for the development of novel therapeutics targeted towards neurodegenerative diseases.

**1501** Characterization of the Alzheimer’s Disease Risk Gene SORL1 in the Zebrafish: Assessment of Expression Differences by Sex, Age, and Influences of an Embryonic Lead (Pb) Exposure

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The zebrafish has emerged as a complementary model system to *in vitro* mammalian organisms for studying neurodegenerative diseases including Alzheimer’s disease (AD). Sortilin-related receptor (SORL1) is a recently identified AD-risk gene which has limited characterization in the zebrafish model system. To further characterize SORL1 in zebrafish, we first performed in-depth protein sequence comparison between zebrafish and humans. As a result, we confirmed that the zebrafish SORL1 demonstrates almost complete conservation of domain and domain sequence with overall 64% amino acid identity with the human protein. Secondly, we carried out *in situ* hybridization to identify spatial expression of SORL1 in zebrafish larvae and observed a diffuse pattern of expression throughout the brain. Thirdly, we assessed the potential impacts of sex and age on expression of SORL1 in adult zebrafish. Quantitative polymerase chain reaction (qPCR) analyses were conducted to compare levels of SORL1 expression between young (3 months of age) and aged (12 months of age) zebrafish by sex. qPCR analysis revealed a significant increase in SORL1 expression in aged females compared to young female zebrafish, but not in males. Our results indicate that sex-specific alteration of SORL1 expression occurs during the aging process. The zebrafish was then utilized to investigate the impacts of an embryonic lead (Pb) exposure as an environmental risk factor for altering expression of SORL1. Zebrafish were exposed to Pb during embryogenesis and then sex-specific qPCR analysis was completed on zebrafish (aged 3 months or 12 months) to compare those developmentally exposed to Pb or a control treatment. No significant difference in SORL1 expression was observed in any Pb treated groups analyzed. Further characterization of other genes associated with AD will facilitate the use of this fish model for understanding the impact of various environmental factors on neurodegenerative disease pathogenesis.
5005 Neurotoxic Effects of Ultraviolet Particular Matter Found in Ambient Air Pollution on Alzheimer’s Disease Model Cells

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Alzheimer’s disease (AD) is an age-related dementia that not only destroys a person’s memory, but also destroys their ability to learn, reason, and execute daily activities. The disease is characterized by a widespread loss of neurons and synaptic connectivity, which is driven by accumulation of toxic β-amyloid peptide. As life expectancy continues to grow, the amount of Americans diagnosed with Alzheimer’s dementia is also expected to grow, tripling in number by 2050. Despite these facts, we still do not have a full understanding of what causes the disease and there continues to be no effective treatments. While it has been well documented that exposure to ambient air pollution can cause respiratory and cardiovascular disease, much less attention has been paid to its effects on neurodegenerative diseases such as Alzheimer’s. Our previous work assessed the neurotoxic effect of ultrafine particles (PM0.1) from traffic in ambient air pollution. These cells have never been used for such a purpose and may be a good alternative to current animal models for studying AD. AD and wildtype (WT) iPSC were treated with H2O control, 2ug/mL UFP or 20ug/mL UFP collected from a high traffic area of Los Angeles. After 4 and 24 hours of treatment the cells were tested for changes in reactive oxygen species (ROS) production, mitochondrial function, cell viability and cell proliferation. From these studies we found that AD iPSC display an increase in ROS formation upon UFP exposure, which may cause the neuroinflammation that leads to Alzheimer Disease progression. We believe that the use of this cell model system may lead to a greater understanding of the underlying molecular events governing AD, which will ultimately lead to a more effective treatment.

5006 Dual Actions of 3-Hydroxykynurenine in the Rat Striatum

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3-Hydroxykynurenine (3-HK), an intermediate metabolite of the kynurenine pathway, has been largely hypothesized as a neurotoxic molecule contributing to neurodegeneration in several experimental conditions. This work was designed to investigate whether or not 3-HK is toxic to cells. In order to evaluate these effects, 3-HK was administered in vitro to isolated striatal slices, and in vivo to the striatum of rats. In striatal slices, 3-HK exerted a concentration- and time-dependent effect on lipid peroxidation, inducing both pro-oxidant actions at low (5-20 μM) concentrations, and antioxidant activity at a higher concentration (100 μM). Interestingly, while 3-HK was unable to induce mitochondrial dysfunction in slices, at the same range of concentrations it presented the deleterious effects exerted by the neurotoxin and related metabolite quinolinic acid, the mitochondrial toxin 3-nitropropionic acid, and the pro-oxidant compound iron sulfate. These protective actions were related to the stimulation of glutathione S-transferase (GST) and superoxide dismutase (SOD) activities. In addition, 3-HK stimulated the protein content of the transcription factor and antioxidant regulator Nrf2. The striatal tissue of animals infused with 3-HK exhibited moderate levels of lipid and protein oxidation at short times post-injection (hours), but these endpoints were substantially decreased at longer times (days). These effects were correlated with an early increase in glutathione reductase (GGR) and GST activities. However, these changes were likely to be merely compensatory as 3-HK-infused animals did not display behavioral alterations of morphological changes in their injected striata. These findings suggest that, despite 3-HK might exert pro-oxidant actions; these changes serve to evoke a redox modulatory activity that, in turn, could decrease the risk of cell damage. In light of this evidence, 3-HK seems to be more a redox modulatory molecule than a neurotoxic metabolite.

5007 Developing a Mouse Model of Hydrogen Sulfide-Induced Neurotoxicity

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Hydrogen sulfide (H2S) is a colorless, neurotoxic gas with a rotten egg odor. Exposure to this gas is an environmental, occupational, and security hazard. H2S has been identified as an environmental pollutant, and is a leading cause of acute death in an occupational setting. Acute effects include eye irritation, seizures, respiratory paralysis and acute death. Neurological sequelae of H2S exposure include auditory impairment and neurodegeneration leading to a vegetative state. Our short-term objective was to develop a mouse model of H2S-induced neurotoxicity using mice. C57 Black mice were exposed to 471 ppm H2S by whole body inhalation for 45 minutes on day 0, followed by 15 minutes/day exposures for 6 consecutive days. Control mice were exposed breathing air. A functional observation battery was used to assess clinical signs of toxicity during H2S exposure and also 2 hours post-exposure. Behavioral and neurochemical changes, and histopathology were additional end-points monitored. Clinical signs noted during exposure included seizures, respiratory depression, and knockdown effect. H2S-exposed mice showed significant impairment in motor activity compared to the controls. In the striatum, dopamine concentration was significantly increased in H2S-exposed animals compared to controls (p<0.05). Also, mice exposure to H2S lost significantly more body weight compared to controls. Histopathology revealed neurodegenerative lesions in the inferior colliculus, the most sensitive brain region. Our study indicates that H2S causes neurotoxicity characterized clinically by seizures and knockdown, impaired motor ability, and histologically the inferior colliculus is the most sensitive brain region, and the striatum to a lesser degree. Ongoing work is examining neurochemical mechanisms in-depth, including oxidative stress.

5008 Investigating the Neurotoxic Effect of Agrochemicals (Dieldrin and Lindane) in Huntington’s Disease Neuropathology


Huntington’s disease (HD) is a genetic neurodegenerative disease that results in movement, cognition, personality and mood impairments. The brain region most vulnerable in HD is the striatum, which is necessary for motor function. In this study, we investigated the effects of two potentially exposed pesticides, dieldrin and lindane in HD neuropathology. Dieldrin and lindane are organochloride pesticides reported to accumulate in Parkinson’s disease (PD) postmortem brain tissues and cause dopaminergic cell loss. Recognizing the similarities in pathophysiological mechanisms between PD, HD and pesticide neurotoxicity, we hypothesized that exposure to lindane and dieldrin will potentiate the neurotoxic properties of mutant HD protein to cause enhanced striatal neuronal loss. We examined the effects of lindane and dieldrin independently and cooperatively in an established mouse striatal model of HD expressing T 7 (wild-type) or T 111 (mutant) polyglutamine repeats. Following exposure to wild type and mutant HD striatal cells to varying concentrations of dieldrin or lindane for 24 and 48hrs, we observed that mutant HD striatal cells exhibited a time-dependent toxic gain of function and decreased mitochondrial-dependent cell viability compared to wild type. However, we report
no genotypic- and time-dependent differences in survival upon lindane exposure. Interestingly, our preliminary results suggest that dieldrin and lindane cooperatively potentiate striatal HD neurotoxicity. In summary, we have uncovered a novel disease-toxicant interaction between mutant HD and dieldrin. The overall implication of this research is to reveal and understand the pathophysiological mechanisms that underlie the aforementioned gene-environment interaction, which may modify HD neuropathology and symptoms.

**1509** The Commonly Used Agrochemical Chlorpyrifos Enhances Huntington’s Disease Neuropathology via Oxidative Stress and Mitochondrial Dysfunction

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Huntington’s disease (HD) is a progressive genetic neurodegenerative disorder characterized by selective loss of medium sized spiny neurons (MSNs) in the striatum, a brain region necessary for movement. Existing scientific evidence suggests that environmental factors may influence the age of onset, progression and severity of the disease. However, the identity of the environmental risk factor is currently unknown. Recognizing the similarities in the pathophysiological mechanisms between HD and pesticide neurotoxicity, we hypothesized that the commonly used agrochemical, chlorpyrifos (CPF), would exhibit disease-toxicant interaction and reveal the contribution of pesticides in HD neuropathology. We utilized an immortalized mouse striatal cell model of HD expressing wild-type (STHDhQ7/Q7) and mutant (STHDhQ11/Q11) genotypes to conduct a cell viability screen to uncover the effects of CPF and its metabolites chlorpyrifos-oxon (CPO) and 3,5,6-trichloro-2-pyridinol (TCP) on oxidative stress, mitochondrial function and energy homeostasis. Following CPO and TCP exposure for 48hs, we observed similar neurotoxic responses between mutant and wild-type HD striatal cells. Interestingly, expression of mutant HD protein increased the susceptibility of striatal cells to CPF neurotoxicity. Additionally, expression of mutant HD protein coupled with CPF exposure caused enhanced oxidative stress and energy dyshomeostasis as observed through increased levels of ROS and lowered ATP levels, respectively. To investigate the possible neuroprotection of HD cells against CPF neurotoxicity, striatal cells were pre, co, or post-treated with the antioxidant N-acetylcysteine (NAC), a precursor to the major antioxidant glutathione (GSH). Herein, we report that NAC treatment rescues mutant HD striatal cells against CPF neurotoxicity. Taking together, the novel CPF-HD interaction induces mitochondrial dysfunction and oxidative stress to enhance striatal neurotoxicity.

**1510** Assessment of Neuroprotective Effect of Probiotic Bacteria in Roteneon Model of Parkinson’s Disease in Sprague-Dawley Rats

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Introduction: Parkinson’s disease (PD) is the second common neurodegenerative disease and PD prevalence in Egypt is higher than the worldwide prevalence representing major health problem in Egypt. PD is characterized by dopaminergic (DA) neuronal cell loss where inflammation and oxidative stress play an important role in degeneration in DA neurons. Certain strains of probiotic bacteria show capacity of alternation of oxidative stress, inflammation markers and level of some neurotransmitter in the brain thus we explored the neuroprotective effects of probiotic on a roteneon-induced PD model in rats. Material & methods: 40 Sprague Dawley rats aged 3 months were divided into 4 equal groups. The first received carboxymethyl cellulose (CMC) and the second received rotenone suspended in CMC and the third the same roteneon regimen plus bifidobacterium infantis and the fourth group received the same rotenone regimen plus combined lactobacillus GG probiotic bacteria and inulin. All animals were evaluated regarding locomotor disturbance through Anymaze video tracking system. After 35 days the animals were sacrificed and their brains were evaluated by immunostaining against α-TH antibodies in SNc and striatum. The results were then analyzed statistically. Results: Robotic exposure shows effects on bifidobacterium infantis against roteneon-induced neurotoxicity, while lactobacillus GG failed to offer protection. As evident by Anymaze software and immunostaining analysis which demonstrated that the degeneration in DAergic neurons and fibers was significantly counteracted by bifidobacterium infantis supplementation. Conclusion: Bifidobacterium infantis probiotic bacteria can offer neuroprotection against neurotoxic effects of roteneon on DA neurons, warranting further investigation as a therapeutic option for Parkinson’s disease patients.

**1511** Lysosomal Dysfunction Regulates the Release of α-Synuclein Protein Aggregates from Exosomes during Manganese-Induced Neurotoxic Insult


Protein misfolding and aggregation are emerging as silent features of many age-related neurodegenerative disorders including Alzheimer’s disease (AD) and Parkinson’s disease (PD). Normally, misfolded proteins are degraded by autophagy and the lysosomal degradation pathways. However, impairment of the lysosomal degradation pathway in the disease state leads to a significant accumulation of autophagic vesicles in the neuronal body. The PD-related protein α-synuclein (αSyn) has multiple divalent metal binding sites. We recently showed that exposure to the divalent metal manganese (Mn) promotes αSyn protein aggregation in cell culture models of PD. In this study, we characterized the role of the autophagic/lysosomal pathway in Mn-induced αSyn misfolding. Exposing an Mn9D dopaminergic cell line stably expressing wild-type human αSyn to Mn (300 μM) resulted in αSyn aggregate formation. Interestingly, Mn exposure increased expression of the autophagosomal markers LC3-II and Beclin-1, whereas it decreased expression of the lysosomal marker LAMP2 in αSyn-expressing cells compared to vector-only cells, suggesting that Mn treatment impairs the autophagic/lysosomal degradation pathway. Notably, Mn treatment also induced the release of αSyn into the extracellular media. Electron microscopic analysis of extracellular media readily detected membranous nano-sized vesicles with the characteristic hallmarks of exosomes. Nanosight particle analysis further showed that Mn exposure markedly increased the number of released exosomes. Slot blot analysis with anti-oligomer antibody (A11) revealed that exosomes contained misfolded proteins, and ELISA studies further confirmed that the released exosomes are indeed packaged with αSyn. Collectively, our data suggest that Mn-induced autophagic/lysosomal dysfunction contributes to the accumulation and secretion of αSyn-containing exosomes during PD-related exposure to the neurotoxic metal manganese. (NIH grants ES19267 and ES10586)

**1512** Drp1 Inhibition Attenuates Neurotoxicity and Dopamine Release Deficits In Vivo

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Mitochondrial dysfunction has been reported in both familial and sporadic Parkinson’s disease (PD). Genetic mutations and parkinsonism-inducing neurotoxins can adversely impact mitochondrial function. However, effective therapy targeting this pathway is currently inadequate. Recent studies suggest that manipulating the processes of mitochondrial fission and fusion has considerable potential for treating human diseases. To determine the therapeutic impact of targeting these pathways on PD, we used two complementary mouse models of mitochondrial impairments as seen in PD. We show here that blocking mitochondrial fission is neuroprotective in the PTEN-induced putative kinase-1 deletion (PINK1-/-) and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse models. Specifically, we show that inhibition of the mitochondrial fission GTPase dynamin-related protein-1 (Drp1) using gene-based and small molecule approaches attenuates neurotoxicity and restores pre-existing striatal dopamine release deficits in these animal models. These results suggest Drp1 inhibition as a potential treatment for PD.

**1513** Novel Mitochondria-Targeted Drug Induces PKD1 Activation and Its Downstream Prosurvival Signaling to Promote Mitochondrial Biogenesis against Dopaminergic Neurotoxicity

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Impaired mitochondrial function and biogenesis contribute to the pathogenesis of Parkinson’s disease (PD). Thus, identifying the key signaling mechanisms regulating mitochondrial biogenesis is crucial to developing new treatment strategies for PD. We have recently reported that PKD1 activation protects against neuronal cell death in PD models, and possibly regulates mitochondrial biogenesis. To improve the translational potential of our mechanistic studies for preclinical drug discovery, we synthesized Mito-Met, a mitochondria-targeted analog derived from the anti-diabetic drug metformin with a triphenylphosphonium functional group and then evaluated the preclinical efficacy of Mito-Met on activation of the PKD1 signaling and its potential downstream effectors in cell culture models of Parkinsonian model neurons.
Mitochondrial dysfunction has been implicated in the etiology of neurodegenerative diseases, such as Parkinson’s disease. Deficits in mitochondrial fission and fusion result in altered mitophagy, the selective autophagy of damaged mitochondria. We hypothesized that exploring the role of mitochondrial homeostasis and mitophagy would help to elucidate the mechanism by which dopaminergic neurodegeneration occurs and how environmental factors mitigate or exacerbate this degeneration. We employed *C. elegans* strains with deletions in the drp-1 and fzo-1 genes, which are involved in mitochondrial fission and fusion, respectively. *C. elegans* were exposed to environmental damage in the form of repeated low-dose ultraviolet C radiation (UVC), creating DNA damage that is persistent in the mitochondrial genome but repaired in the nuclear genome. The nematodes were starved throughout dosing to prevent dilution of mitochondrial damage with growth and development. We noted a gradual increase in neurodegeneration with age in the *drp-1* but not *fzo-1* mutants. All strains experienced some amount of larval arrest after UVC exposure, with *fzo-1* mutants undergoing complete larval arrest, suggesting sensitivity to persistent mtDNA damage. The *drp-1* nematodes showed less neurodegeneration than wild-type after UVC-induced environmental neurodegeneration. This suggests that deficits in mitochondrial fission may be protective against neurodegeneration resulting from mtDNA damage. Future directions will include investigating sensitivity to 6-hydroxydopamine (6-OHDA) and measuring oxygen consumption in the same strains exposed to UVC and 6-OHDA.

**Preclinical Efficacy Testing of the Mitochondria-Targeted Antioxidant Mito-Apocynin in the Transgenic MitoPark Mouse Model of Chronic Dopaminergic Neurodegeneration**

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Advances in drug discovery for neurodegenerative diseases, including Parkinson’s disease (PD), have been hampered by the lack of chronic animal models that recapitulate the slow and progressive neurodegeneration. A generic mouse model of mitochondrial dysfunction known as MitoPark was recently developed by selectively knocking out the mitochondrial transcription factor Tfam in dopaminergic neurons. Recently, we developed a novel class of drugs targeting mitophoria to effectively dampen the major pathophysiological processes, mitochondrial dysfunction and oxidative damage. In this study, we evaluated the neuroprotective efficacy of a mitochondria-targeted apocynin derivative, mito-apocynin, in this model. MitoPark mice progressively exhibit mild to severe motor deficits over 12-24 wks, along with gradual loss of striatal dopamine and nigral dopaminergic neurons. However, MitoPark mice orally administered 10 mg/kg mito-apocynin (13-24 wks, 3x/wk), significantly improved their coordination, stability and spontaneous locomotor activity relative to vehicle-treated MitoPark mice, as measured by Rotarod and open-field tests. Mito-apocynin also recovered the loss of dopamine and dopaminergic neurons. Electron paramagnetic resonance (EPR) spectroscopy revealed that fractional intensities of reduced iron-sulfur clusters were significantly higher in MitoPark brains compared to those from age-matched littermate controls, while mito-apocynin administration decreased these levels. Furthermore, the higher brain levels of oxidative damage markers, 4-HNE and INOS in MitoPark mice relative to controls were significantly reduced by mito-apocynin. Collectively, our data reveal that mito-apocynin mitigates oxidative and nitrosative damage, rescues behavioral deficits and dopamine depletion, and protects against neurodegeneration in the MitoPark mouse model of PD (NIH grants NS074443 and ES10586).

**Diphenylethonium at Low Dose Rescues Disease Phenotype in Models of Parkinson’s Disease**

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Current therapeutic regimens for Parkinson’s disease (PD), the second most common neurodegenerative disorder, only temporarily relieve symptoms but fail to halt disease progression. Substantial evidence indicates that microglia-mediated chronic neuroinflammation is critical in driving the progressive neurodegeneration in PD. Our previous study demonstrated that microglial NADPH oxidase (NOX2) is a key enzyme for initiating and maintaining chronic neuroinflammation. Here, we investigated whether inhibiting NOX2 is an effective therapeutic strategy to arrest PD progression. As a proof of principle, diphenylethonium (DPI), a widely used NOX2 inhibitor, was used as a proto-drug. Despite its potent inhibitory effect of NOX2, DPI has not been tested clinically due to high toxicity at regularly used doses (μM or mg/kg). For this reason, low-dose DPI was employed in our studies. Initial *in vitro* studies using primary midbrain cultures revealed that DPI at 10-13-10-14 M displayed great specificity towards NOX2 and importantly, protected dopaminergic neurons against lipopolysaccharide (LPS)- and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced damage without any observed toxicity. These results prompted us to further test the therapeutic potential of ultra-low dose DPI *in vivo*. DPI (10 mg/kg/day) was infused subcutaneously via minipump for two weeks after mice received prior injection of either LPS or MPTP. We discovered that DPI mitigated both LPS- and MPTP-elicited motor deficits and nigral dopaminergic neurodegeneration. Mechanistically, DPI at low-dose attenuated chronic microglia-mediated neuroinflammation through inhibition of NOX2 activation *in vivo*. Moreover, NOX2 deficiency abolished DPI-protected affordance. Altogether, low-dose DPI was effective in halting PD progression, suggesting that a new class of NOX2-inhibiting anti-inflammatory drugs should be pursued as potential candidates for PD therapy.
Inflammatory activation of glial cells is involved in the progressive loss of dopaminergic neurons in Parkinson’s disease (PD). Astroglialosis is accompanied by activation of the transcription factor, Nuclear Factor-kappa B (NF-kB), which coordinately regulates the expression of multiple neuroinflammatory genes associated with PD including inducible nitric oxide synthase (iNOS), tumor necrosis factor alpha (TNFα), and interleukin 1β (IL-1β). These observations suggest that inhibition of NF-kB in glial cells could be a promising therapeutic target for the prevention of neuroinflammatory injury. Nuclear orphan receptors in the NUR4A family, including NUR4A1 (Nur77) and NUR4A2 (Nur1), are reported to antagonize the effects of NF-kB on inflammatory gene expression. However, high affinity pharmacologic ligands of these receptors have been lacking. A novel ligand of Nur77, 1,1-bis (3'-indolyl)-1-(p-methoxyphenyl) methane (C-DIM5), activates Nur77 in cancer cells and causes nuclear degradation of the transcriptional co-activator CBP (p300), which is also required for the transcriptional activity of NF-kB. We therefore postulate that activation of Nur77 by C-DIM5 in astrocytes would suppress NF-kB-dependent inflammatory gene expression induced by the 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) and the inflammatory cytokines interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α). C-DIM5 increased expression of Nur77 mRNA and suppressed expression of neuroinflammatory genes. C-DIM5 also inhibited the expression of multiple NF-kB-regulated inflammatory and apoptosis genes in qPCR array studies but did not prevent p65 translocation to the nucleus, suggesting a specific without inhibition of NF-kB, suggesting that this series could be a useful modality in preventing neuroinflammation.
and in higher doses. 2-amino-1-methyl-6-phenylimidazo(4,5-b)pyridine (PhIP) is produced in meat during high-temperature cooking, and it bears structural resemblance to dopamine and dopaminergic toxicants. Our previous work determined that PhIP and its Phase I metabolite, N-OH-PhIP, are selectively neurotoxic to dopaminergic neurons in primary midbrain cultures. Treatment of cultures with PhIP and N-OH-PhIP causes neurodegeneration in dopaminergic neurons. More recently, we also determined that neurite lengths in non-dopaminergic neurons were not affected by PhIP treatment, further illustrating selective toxicity to dopaminergic neurons. In this study, we assess PhIP neurotoxicity in vitro after systemic administration to male SD rats at 6-7 weeks of age, using 75 mg/kg PhIP by single oral gavage, three times a week, for 4 weeks (n=9 per group). PhIP-treated rats showed significantly reduced weight gain after one week, but motor tests (postural instability and rearing tests) failed to show overt differences between treated and control groups. Our future studies will continue to assess neurotoxicity of acute and subacute PhIP administration in vivo, including oxidative stress, neurotransmitter levels and DNA damage to determine whether preclinical PD features are reproduced.

**1523 TIMP1 mRNA Expression Is A Biomarker of Astroglia: Evidence from Multiple Neurotoxicants and BAC-TRAP Technology**

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A characteristic feature of neurotoxicity is the selective and unpredictable damage to specific neural cells. This lack of target identity constitutes a substantial barrier to neurotoxicity detection and characterization. Evaluating astroglia overcomes this problem as reactive astrocytes show the location of toxicant-induced damage occurring anywhere in the CNS. Enhanced expression of GFAP is a hallmark of reactive astrocytes; however, few other astroglial biomarkers are known. Previously, we introduced ALDH1L1 BAC-TRAP (translating ribosome affinity purification) technology for neurotoxicological evaluation as it allows for characterization of the actively translating transcriptome of astrocytes responding to toxicant-induced neural damage. To begin to characterize the astrocyte injury-response transcriptome, ALDH1L1 BAC-TRAP mice were given a single 12.5 mg/kg s.c. dose of MPTP, a well-characterized dopaminergic neurotoxin that induces significant astroglia. Striatal tissue (12, 24 and 48 hrs post MPTP) was subjected to TRAP utilizing an eGFP antibody that only binds to actively translating RNA in astrocytes. Changes induced by MPTP damage were determined by microarray (Illumina Expression BeadChip) and the dataset interrogated using Ingenuity Pathway Analysis. MPTP induced robust transcriptome changes in genes previously identified as astrocyte specific with an 800-fold increase in TIMP1, a finding suggestive of the role of astrocytes in extracellular matrix remodeling. These data were confirmed by qPCR and extended to two additional neurotoxicants, methamphetamine (METH) and kainate (KA). As with MPTP, METH and KA mRNA expression analyses showed large fold increases in TIMP1. Prior treatment with the stress hormone, corticosterone (CORT) is known to increase astroglia and damage after METH and to decrease the overexpression of KA. TIMP1 expression followed the same pattern. These data suggest that astrocytes function in extracellular matrix degradation and tissue remodeling following neurotoxic insult.

**1524 In Vitro 3D Dopaminergic Model to Study (Developmental) Neurotoxicity and Parkinsonism**


The environmental contribution to the increasing number of neurodevelopmental disorders, such as autism, is currently in focus and initiated the development of human relevant *in vitro* models for DNT. Likewise, the development of a relevant *in vitro* model to study Parkinson’s neurodegeneration affecting dopaminergic neurons is of high interest. We developed a 3D *in vitro* human model, which can be implemented for both DNT and neurodegeneration research. This model is based on the 3D differentiation of LUHMES progenitor cells into mature dopaminergic neurons within 6-9 days. We have optimized the 3D protocol to control size and homogenous differentiation. The spheroids can be kept in culture up to 21 days, which is the longest culture period. The anti-proliferation drug Taxol was used to reduce proliferation within the aggregates. Treatment with Taxol on day 3-5 led to a decrease in proliferation (58.2 % to 11.3 % Ki-67 positive cells) on day 6, a decrease in spheroid size, an increase in the expression of mature neuronal markers TH, NeuN, β-TubIII and SYN1 as well as neuronal-specific microRNAs. Interestingly, we observed increased neuronal arborization in Taxol-treated cells, which was measured by immunostaining for TH, MAP2 and NF200. Compound penetration and apoptosis in differentiating spheroids were studied over time using confocal imaging. To overcome the known limitation of high content confocal imaging in 3D and optimize the visualization of neuronal morphology, we (i) co-differentiated the wild type LUHMES (98% of the culture) with LUHMES expressing RFP (2% of the culture) and (ii) established an optical clearance method to increase confocal microscope resolution. Thereby, we established an imaging workflow that allows quantification of neurotoxicant effects on neurite outgrowth, branching and synaptogenesis in 3D. Two reference compounds known to induce the parkinsonism (MPP+ and rotenone) was used to demonstrate the suitability of this model for (developmental) neurotoxicological studies.

**1525 Altered Optineurin Expression in Cellular and Rodent Models of Parkinson’s Disease**

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Optineurin (OPTN) is a genetic factor in glaucoma and amyotrophic lateral sclerosis. Multiple functional roles have been identified, including in vesicle trafficking, Golgi apparatus organization, induction of macroautophagy and cell cycle. Recent data has shown that OPTN aggregates in vulnerable neurons in several neurodegenerative diseases, including frontotemporal lobar degeneration, Alzheimer’s, Huntington’s, and Parkinson’s disease (PD). Major pathological hallmarks in PD include age-related or α-synuclein (α-Syn) APP accumulation in Lewy bodies and progressive loss of dopaminergic (DA) neurons in the substantia nigra (SN). Macroautophagy is a primary route for degradation of α-synuclein and protein aggregates. This pathway is dysregulated in PD. Currently, data on roles for OPTN in the pathogenesis of PD is very limited. Given OPTN’s involvement in many cellular pathways that are also implicated in PD pathogenesis, we characterized basal brain expression and response to DA neurotoxicants. Here we show immunohistochemical evidence that OPTN is enriched in DA neurons in the SN. Using primary rat mesencephalic cultures that contain DA and non-DA neurons and glia, OPTN expression increased after acute exposure to methamphetamine, paraquat, or overexpression of α-synuclein (both mutant and wild-type), compared to control, assessed by quantitative immunofluorescence. These data demonstrate that OPTN has high expression in DA neurons, and its expression is increased when PD is modeled by toxicant insult or genetically. Ongoing experiments are examining OPTN expression in SN of rats acutely exposed to paraquat or rotenone, toxicants used to model PD and linked to increased risk. Here, brains have been sampled prior to overt neurodegeneration, representing a ‘preclinical’ sampling point. Our next steps are to investigate the interaction between OPTN and macroautophagy after PD-relevant insults. Funding: NIEHS/NIH E019879 (J.R.C.)

**1526 Transcriptional Regulation of the Compensatory Signaling Molecule Prokineticin-2 during Neurotoxic Stress in Dopaminergic Neuronal Cells**

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While cell signaling mechanisms underlying neurotoxic injury have been actively studied in recent years, signaling molecules contributing to compensatory survival signaling are largely unknown. We recently discovered that a secretory neuropeptide prokineticin-2 (PK2) is upregulated in dopaminergic neurons during early stages of neurotoxic stress in experimental models of Parkinson’s disease (PD) and plays a neuroprotective role against neurotoxic stress. In this study, we characterized the transcriptional regulatory mechanism of PK2 in a dopaminergic neuronal model of PD. *In silico* analysis of PK2 promoter region detected binding sequences for key oxidative stress-related transcription factor, hypoxia-inducible factor (HIF) and the neuronal survival and differentiation factors, early growth response (EGR) proteins. To validate these findings, we cloned the 5'-flanking region (1kb) of the human PK2 gene into a luciferase reporter vector and then transfected into MN9D dopaminergic neuronal cells to study the PK2 gene regulatory mechanism. Treatment with HIF1α activators 3,4-dihydroxybenzoate (DHB) or cobalt significantly increased PK2 promoter activity, implying that HIF1α plays a role in PK2 gene expression. We confirmed this finding by cotransfection studies, wherein overexpression of HIF1α and HIF2, but not inactive HIF1α, stimulated PK2 promoter activity. Furthermore, HIF1α overexpression and DHB treatment synergistically activated the PK2 promoter. Interestingly, EGR and E2F overexpression also enhanced the PK2 promoter activity. To further validate the role of HIF, EGR and E2F in regulating PK2 gene expression, we measured PK2 mRNA and protein.
levels after their expression. Ectopic expression of HIF and EGR family proteins increased both PK2 mRNA and protein levels in dopaminergic cells. Collectively, our results demonstrate that HIF and EGR families of transcription factors play a role in PK2 upregulation and its compensatory response during early stages of neurotoxic insults (NIH grants NS78247 and ES10586).

1527 Farnesoid X Receptor Deficiency in Mice Enhances MPTP-Induced Neuroinflammatory Response

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Neuroinflammation is a prominent feature of several neurodegenerative disorders including Parkinson’s disease. The nuclear factor κB (NF-κB) pathway is present in a wide variety of neuronal cells including microglia, astrocytes, and neurons, and plays a predominant role in the activation and regulation of key inflammatory molecules. The farnesoid x receptor is a transcription factor and a bile acid receptor predominantly expressed in liver and intestine that was previously reported to regulate NF-κB activity in the liver. Fxr deficiency was associated with increased hepatotoxicity following challenge with lipopolysaccharide, suggesting that Fxr mitigates the inflammatory response. Here, we report that Fxr is also expressed in mouse brain and that Fxr knockout mice exhibited enhanced levels of neuroinflammation, as evidenced by an 94% increase in tumor necrosis factor-alpha (Tnf-α) and an 57% increase in cyclooxygenase 2 (Cox2) protein levels. Following striatal injury with repeated administration of the neurotoxicant 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), we found that Fxr KO mice exhibited an exaggerated inflammatory response. Specifically, compared to control saline, MPTP increased Tnf-α (44%) and Cox2 (86%) in the striatum of wild-type mice, whereas MPTP administration to Fxr KO mice resulted in a combined 127% increase in Tnf-α and a combined 121% increase in Cox2. This was accompanied by an 11% greater striatal dopamine depletion, and greater reduction of tyrosine hydroxylase protein (-12%) and dopamine transporter (-28%) in Fxr KO mice following MPTP. Our findings suggest that Fxr may play an important role in the regulation of basal and toxicant-induced neuroinflammation. Supported in part by NIH T32ES, P30ES005022, R01ES021800, and GM104037.

1528 Systems Genetics Analysis of MPTP Neurotoxicity

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Heavy metals, and pesticides herbicides are suspected risk factors for neurological disorders including Parkinson’s disease (PD). Nevertheless, their role is not clear-cut, as there are inconsistencies in epidemiological and preclinical research. One reason may be related to individual differences in susceptibility, differences that can be traced to the genetic constitution of humans and animals so exposed. Newer epidemiological and animal methods address the role of genes, the environment and their interaction as key. We recently reported on neurotoxicity of 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) in male BxD recombinant inbred mice. In the current study, we examined the effect of MPTP neurotoxicity in females from 9 of the same strains of BxD RI mice. The mice received 12.5 mg/kg MPTP s.c. (vs. saline) and 48 h later brains were taken for biochemical analyses. Striatal dopamine (DA) and its metabolites, DOPAC and HVA, and serotonin and its metabolite, 5-HIAAA, were analyzed by HPLC. DA turnover was assessed using DOPAC/DA and HVA/DA ratios. Striatal tyrosine hydroxylase (TH), glial fibrillary acidic protein (GFAP), and iron content in ventral midbrain were quantified. All dopamine measures, as well as TH and GFAP, demonstrated wide, genotypic-dependent differences in response to MPTP. Moreover, strong positive correlations were seen between the sexes for DA, TH, and GFAP. This systematic approach to the study of environmental neurotoxicants in genetic reference populations of mice is likely to elucidate genetic factors underlying individual differences in developing neurodegenerative diseases such as PD. Supported in part by U54NS Grant R01 ES02261

1529 Molecular Mechanisms of Rotenone and MPP+ Damage to Dopaminergic Neurons in a Human Neural 3D Model


Increasing evidence suggests a major role of mitochondria-mediated cellular signaling in response to environmental stress, such as toxicant exposure. Mitochondria-mediated cellular perturbations include response to ROS, perturbation in ATP production, DNA damage, histone modifications and altered DNA methylation. The development of cellular models and experimental approaches reflecting mitochondrial intra-cellular crosstalk are of high importance, since they will lead to more comprehensive understanding of early cellular events, including cellular defense mechanism prior to apoptosis activation. A variety of toxicants induce mitochondrial respiratory chain perturbations that lead subsequently to apoptosis. This work takes advantage of our recently characterized 3D LUHMES dopaminergic neuronal model to test two known mitochondrial neurotoxins (MPP+ and rotenone) and identify early events which lead to toxicity. Cell viability, mitochondrial dysfunction, neurite outgrowth and arborization, and perturbations in gene expression were measured after short-term (24 and 48h) exposures. We were able to see changes in expression for energy metabolism and stress response genes (ATF4, AS1, CBS, CTH, MLF1IP, SME1 and TMY) at non-cytotoxic concentrations (0.1μM rotenone, 100μM MPP+) as well as a dose-dependent decrease in mitochondrial functionality using MitoTracker®. We also recorded changes in expression of mitochondria/apoptosis-related miRNAs (miRs 30a, 34c, 15, 338, and 210). We visualized toxicant-induced changes in neuronal morphology by confocal microscopy of co-cultures of wild type and RFP-expressing LUHMES. In the next steps we will study the expression of oxidative stress response genes (NFE2L2, KEAP1 and SOD1) and identify metabolic changes using metabolomics. The 3D model mimics the in vivo physiology more closely than existing 2D in vitro models therefore can be used to identify molecular and cellular effects of neurotoxic compounds.

1530 Biochemical and Gene Expression Changes in Mice Exposed to Polychlorinated Biphenyls during Early Brain Development

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Polychlorinated biphenyls (PCBs) are neurotoxicants that have been banned for decades, but are still a continuing public health concern. The primary route of human exposure is through contaminated food. Recently, PCBs have been linked with an increased risk of Parkinson’s disease in highly exposed humans. Interestingly, PCBs have long been known to deplete dopamine levels in the brain. Our previous studies in mice showed that variations in the aryl hydrocarbon receptor (AhR) and cytochrome P450 1A2 (CYP1A2) affect PCB neurotoxicity when assessing learning and memory and motor function. In our current studies, we used immunohistochemistry and quantitative real-time PCR to assess gene expression, enzyme-linked immunosassays to look at thyroid hormone levels, and HPLC to measure neurotransmitters. Pregnant dams were treated with PCBs or corn oil-soaked fruit loops daily from gestational day 0 (GD0) to postnatal day 25 (PND 25) when the pups were weaned. We collected tissues from offspring at P30 and following motor function tests (-P120). We confirmed AhR activation through qPCR of liver tissue only in high-affinity Ahrb mice. There were no significant differences in thyroid hormone levels in adults although PCB-treated Abhr Cyp1a2(-/-) mice had the lowest plasma levels (4.77± 0.76 ng/dl) compared with the most resistant Abhr Cyp1a2(+/-) mice (5.91± 0.46 ng/dl). Although we found no significant differences in striatal dopamine, there was a significant difference in the metabolite DOPAC. PCB-treated mice with a high-affinity Abhr genotype both had depleted DOPAC compared with the corn oil-treated controls (P < 0.05). There was also a significant main effect of genotype with Cyp1a2(-/-) mice having lower DOPAC compared to the wild-type mice (P < 0.05). Supported by ES020053 and GM103456.
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Inhibited dopaminergic neurotoxicity through mitochondrial dysfunction and oxidative stress. Mitochondria play a central role in cellular energy metabolism and are particularly sensitive to oxidative stress. Exposure to pesticides, particularly pyrethroids, can lead to mitochondrial dysfunction and oxidative stress, contributing to dopaminergic neurotoxicity.

The authors investigated the effects of two commonly used pesticides, tebufenpyrad and pyridaben, on mitochondrial function in cell culture models. They used Seahorse bioanalyzer and XF96 analyzer to measure basal oxygen consumption rate, ATP levels, and respiratory capacity. They also examined the effects of these pesticides on mitochondrial morphology using confocal imaging.

Exposing rat dopaminergic neuronal cells (N27) to tebufenpyrad and pyridaben led to a significant decrease in ATP-linked respiration and respiratory capacity. Measurement of basal oxygen consumption rate in a dose-dependent manner indicated a loss of mitochondrial function. Additionally, they observed changes in mitochondrial morphology, including reduced mitochondrial length and circularity. Collectively, these findings suggest that exposure to these pesticides can lead to dopaminergic neurotoxicity through mitochondrial dysfunction and oxidative damage.

The authors hypothesize that similar to parent and OH-PCBs, PCB sulfates are toxic to dopaminergic cells, and that interactions with HSA could affect toxicity. They examined the viability of N27 rat dopaminergic neuronal cells following exposure to PCB sulfates derived from PCBs 3, 8, 11, 12, and 52 and compared this with the effects of the corresponding OH-PCBs. Preliminary results indicate that PCB sulfates are cytotoxic to N27 cells. Although 4'-OH-PCB 52 showed increased toxicity when compared to 4'-OH-PCB 52, other PCB-sulfates were less cytotoxic than the corresponding OH-PCBs. In each case, the toxic effect was modulated by the presence of HSA in the cell culture medium. These results indicate that PCB sulfates may play a role in PCBinduced neurotoxicity and that binding to HSA may influence this effect.

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In summary, although we did not find overt interaction in nigrostriatal dopaminergic system of C22:6-containing molecular species (C16:0/C22:6 and C18:0/C22:6/C22:6, respectively) in SN and plasma. No significant changes in C22:6-containing molecular species (C16:0/C22:6 and C18:0/C22:6) of major phospholipid classes (PC, PE, PS and PI) were observed after rotenone exposure. This may be explained by elevated activity of phospholipid synthesizing enzymes in SN observed in PD patients. The study was approved by the IACUC at the University of Pittsburgh. Supported by grant NIH ES020693, CA165065.

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1538 Heated Tobacco Generates a Simplified Aerosol with Fewer Toxins Than Smoke from Cigarettes

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Smoke from cigarettes is a complex aerosol with over 6000 identified constituents, many of which are known toxicants. We have tested a Tobacco Heating Device (THD) where the tobacco is heated rather than combusted and we postulate that this will lead to a less complex emission profile. In order to characterize THD emissions, tobacco rods were conditioned and electrically heated to 250°C for 180 seconds while being pulsed by a puffing-machine. Puff volume, duration and interval followed the Health Canada Intense Regime (HCR). The levels of particulate matter, nicotine and carbon monoxide in the emission were determined, as were the levels of 47 other constituents including “Hoffmann analytes” (recognised by certain regulatory authorities as possible toxicants in tobacco) and some thermal breakdown products. The emission was also scanned by GC-TOF-MS. In addition to nicotine (present at 1.22mg/stick), we identified approximately 450 further constituents, the majority of which are known to be present in tobacco and/or cigarette smoke. The levels of the “Hoffmann analytes” were all reduced by more than 50% when compared to conventional cigarettes. For example, the levels of the following analytes in the THD emissions were determined (numbers in parentheses are the comparator ranges for commercial cigarettes smoked under the HCI regime): acrolein 5.75µg (78-208µg), benzene 0.49µg (49-102µg), 1,3-butadiene <0.095µg (72-118µg) and CO 0.59mg (16.4-40.7mg). These data are consistent with other published data on THD products and demonstrate significant reductions in the levels of known toxicants and overall a less complex emission profile than the smoke from conventional cigarettes.

1539 Disposable Electronic Cigarettes and Electronic Hookahs: Evaluation of Design, Performance, and Metal Emissions

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The purpose of this study was to evaluate the design, performance characteristics and metal emissions of disposable electronic cigarettes (EC) and electronic hookahs (EH). All brands of EC/EH were similar in design and had the same basic components: wires, solder joints, air-tubes, a mouthpiece, fluid, and fibers, although there were subtle differences in nicotine concentrations, flavors, and packaging. The airflow rates required to produce aerosol and the aerosol absorbance were lower for button-activated models (3 mL/sec; 0.41-0.53 absorbance) than for airflow-activated models (7-17 mL/sec; 0.48-0.84 absorbance). Pressure drop was lower across airflow-activated products (6-12 mmH2O) than airflow-activated products (15-67 mmH2O). While button-activated models lasted 200 puffs or less and EH airflow-activated models often lasted 400 puffs, none of the models produced as many puffs as advertised. Puff number was limited by battery life, which was shorter in button-activated models. For elemental analysis of the aerosol, 21 of 35 elements were detected in the EC/EH aerosol. Fourteen elements (Ag, Bi, Ca, Cr, Cu, Fe, In, Ir, Mg, Mn, Pd, Si, Sr, V) were consistently higher in concentration in THD and EC/EH aerosol than in conventional cigarette smoke. The levels of the following analytes in the THD aerosol were determined (numbers in parentheses are the comparator ranges for commercial cigarettes smoked under the HCI regime): acrolein 5.75µg (78-208µg), benzene 0.49µg (49-102µg), 1,3-butadiene <0.095µg (72-118µg) and CO 0.59mg (16.4-40.7mg). These data are consistent with other published data on THD products and demonstrate significant reductions in the levels of known toxicants and overall a less complex emission profile than the smoke from conventional cigarettes.
Electronic cigarettes (E-cigs) have earned considerable attention recently as an alternative to smoking tobacco. While the number of e-cigs available in the market is growing, potential exists for significant variation in physical and chemical characteristics of the output produced from these products. This study aimed at producing and characterizing the output from three major e-cigs currently available in the market (hereafter referred to as products A, B and C), as part of preparation for the conduct of in-vivo inhalation studies. Testing was performed under the Canadian Intense Regimen. The e-cig output from the cigarette smoking machine (CSM) was transported to a nose-only inhalation exposure carousel. Initial tests were conducted using one e-cig per test for each of the three products. The inhalation atmosphere at the nose port was characterized for concentration stability, puff output variability, particle size and major chemical constituents. Samples for determination of constituents were collected during the beginning, middle and end of each test. A follow up test was conducted only on product B by loading all 30 ports of the CSM head to demonstrate generation of a stable atmosphere appropriate for the conduct of an inhalation study. Product A showed consistent output during the two hours of test generation. Product B showed fairly consistent output however product B stopped generating output after -55 minutes. Product C showed a gradual decrease in concentration during two hours of data collection. Particle size was in the sub-micron range for all three products. The nicotine to WTPM ratio ranged from -0.5% to -3.5% for all three products. Glycerol was the major constituent with glycerol to WTPM ratio ranging from -70% to -90% for the three products. The feasibility of conducting adequately controlled in vivo inhalation studies using e-cigs was further substantiated by the results obtained with Product B with a fully loaded CSM head.

Electronic cigarettes (E-cigs) are battery powered electronic nicotine delivery systems, which simulate tobacco smoking by delivering nicotine via an inhaled aerosol. ECGI aerosols are generated by heating solutions of propylene glycol, soy-based glycerin, or similar mixtures containing nicotine. Various types of flavoring agents are also typically added to ECGI nicotine solutions. Because ECGI aerosol should not contain high levels of the many toxic chemicals present in tobacco smoke, they have been advertised to be a safer alternative to traditional tobacco cigarette (TCIG) smoking. However, there are few studies evaluating the potential physiological impact of ECGI exposure. In this study, we aimed to determine the effects of ECGI exposure on human bronchial epithelial cells in vitro. Both ECGI and TCIG exposures were performed using a Vitrocell smoking machine or conditioned media. Following exposure, effects on airway epithelial cell gene expression were evaluated using PCR, immunohistochemistry, reactive oxygen species assay and Affymetrix microarray. Combined results indicate that ECGIs induce expression of genes involved in both oxidative and xenobiotic stress pathways. The effects were similar to, but generally lower in magnitude, than effects of TCIGs. Cytochrome P450 genes CYP1A1, CYP1B1 and NQO1 were significantly increased after both TCIG and ECGI exposure. In addition to NQO1, another oxidative stress gene, ALDH3, was significantly increased after TCIG and ECGI exposure. Production of 8-isoprostane, a reactive oxygen species marker, also increased with both TCIG and ECGI exposure, further indicating oxidative stress. These results indicate ECGIs have the potential for induction of cellular stress within the airway epithelium. Further studies in vivo are needed to fully evaluate the potential cellular and molecular impact of ECGI exposure.

Electronic cigarettes (EC) are nicotine delivery devices, often advertised for smoking cessation. The fluids used to refill these devices typically contain nicotine, a humectant, such as propylene glycol (PG) or vegetable glycerin (VG), flavorings and contaminants. Few scientific studies have assessed possible health effects of EC aerosols on users, and it remains unclear whether they pose a health concern. Previously, 35 EC refill fluids from four companies were screened for cytotoxicity using human pulmonary fibroblasts (hPF) via the MTT assay. In this extension of the previous study, we produced aerosols from these fluids using the Vea EC from Johnson Creek and then tested them for cytotoxicity in the MTT assay on hPF, which model the adult lung. A comparison was made between the refill fluids applied to hPF and the aerosols produced from the fluids which were then used to
treat the cells. Aerosols ranged in IC50 values (dose that inhibits survival by 50%) from 0.0188 to >3%. For 54.3% of refill fluids (19 of 35), the corresponding aerosols had similar cytotoxicity. 11.4% of the fluids (4 of 35) were more cytotoxic than their aerosols. Finally, 34.3% of the products (12 of 35) were more potent as aerosols than as fluids. This latter observation is important as it shows that heating refill fluid can increase its cytotoxicity. The data further show that when screening for cytotoxicity of EC products, testing refill fluids on cells is not always representative of the effects of an aerosol on cell survival. Additionally, the humectants of these fluids, PG and glycerol/VG, correlate to the toxicity of these aerosolized products with fluids containing only glycerol and VG to be more cytotoxic than those products comprised mainly of PG. This study is the first to compare the cytotoxicity of EC refill fluids, humectants and their aerosols on human cells. In addition, this study provides valuable insight into which of these EC refill products or additives may be potentially harmful to users.

1545 Effect of Cigarette Design Parameters on In Vitro Toxicity Profile


Currently, cigarettes are classified as full flavor, gold and silver, representing high, medium and low tar deliveries. Differences in tobacco types, blends, ventilation, and filter material contribute to a cigarette’s toxicological profile. Therefore, the ability to differentiate toxicological profiles within tar categories presents a challenge. Experimental cigarettes were made using a single tobacco blend and four types of cellulose acetate filters with various air dilutions, resulting in tar and CO deliveries ranging from 2.5 to 22 and 4.8 to 19.5 mg/cigarette, respectively, when smoked under ISO parameters (35 ml puff volume, 2 second puff duration, 60 second puff interval). An in vitro battery of established assays was used to examine the tar and gas-phase mediated cytotoxicity, mutagenicity (Ames), genotoxicity (Micronucleus) and inflammation (IL-8) of the experimental cigarettes. Lower tar yield cigarettes were more cytotoxic, on a per tar basis, than the higher yield cigarette; however, a weak correlation was observed for those cigarettes with lower CO yields having lower cytotoxicity. No differences in tar mutagenicity were observed. Micronucleus formation demonstrated a trend proportional to the cigarette tar yield. No specific trend in tar-mediated inflammation was observed; however, gas-phase mediated inflammation demonstrated an increasing trend with higher tar yield. To further examine the response trends observed, comparisons were performed based on the experimental cigarettes’ CO/tar ratios, which varied due to the utilization of different construction parameters. A stronger correlation was obtained between gas-phase cytotoxicity and the CO/tar ratio, with increasing cytotoxicity with increasing CO/tar. In addition, the construction parameters, tar and gas-phase deliveries and the relationship to in vitro toxicological endpoints will be further discussed.

1546 An Impact Assessment of Cigarette Smoke on Organotypic Models of Bronchial Epithelial Monoculture and Bronchial Epithelial/Fibroblast Coculture

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In organotypic culture models, human primary bronchial epithelial cells form a pseudostratified epithelium, similar to the airway structure in vivo. Such models are cultured at an air-liquid interface, thus suitable for toxicity assessment of various aerosols. The complexity of the in vitro cellular response may be better captured in co-culture models, in which additional cell types, e.g. fibroblasts, were included. However, the presence of another cell type hinders the interpretation of the exposure impact on the epithelial cells. We compared the impact of a 28-minute whole cigarette smoke (CS) exposure on two different organotypic bronchial models: a co-culture of the epithelial cells with fibroblasts and a mono-culture without fibroblasts. Secreted cytokine levels in the basolateral media of both models were measured 48 hours post-exposure. Additionally, transcriptomes were generated at different post-exposure time-points from the epithelial cells of the mono-culture model and from the epithelial cells scraped from the co-culture model. The results showed that in general, the CS-induced cytokine secretion was more pronounced in the mono-culture compared to the co-culture model. Using a network-based systems biology approach, the transcriptomic data revealed that CS impacted similar biological processes in the epithelial cells of both models. Nevertheless, a greater impact on apoptosis- and senescence-related mechanisms was observed in the epithelial cells of the mono-culture. In conclusion, the results suggested that the presence of fibroblasts enhanced the survival of the epithelial cells in response to CS exposure. However, a more robust bronchial epithelial cell specific cytokine response was detected using the mono-culture model.

1547 Systems Toxicological-Based Assessment of Sex-Related Differences in Response to Cigarette Smoke within an OECD Rat Inhalation Study

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Smoking is a major risk factor for many diseases. Several observations suggest that sex differences exist in the susceptibility to different exposures. In order to gain insight into toxicity pathways in both sexes, a 90-day inhalation study, as described in the Organisation for Economic Co-operation and Development (OECD) guideline 413, was augmented with multi-analyte profiling of the bronchoalveolar lavage fluid (BALF) and transcriptomics analysis of lung and liver tissue. Female and male Sprague Dawley rats were exposed to fresh filtered air (sham) or three concentrations of mainstream cigarette smoke (CS) (8, 15, 23 ug/ml nicotine) generated from the 3R4F reference cigarette. In addition, some of the rats were kept for a 42-day post-exposure period prior to analysis. Carboxyhemoglobin levels in blood, total urinary nicotine metabolites, and lung histopathology did not show major differences between male and female rats after the exposure period. The sex differences observed in body weight, lung weight, and respiratory physiology were also observed in sham-exposed rats and sex differences were not exacerbated by exposure. In contrast, BALF analysis showed more differentially regulated inflammatory mediators in female than in male rats. Similarly, the transcriptomics analysis in lung and liver revealed a stronger response to CS exposure in female than in male rats. A computational systems toxicology approach uncovered perturbation of biological processes in lung tissue with a higher amplitude in females, including inflammatory processes, as well as larger changes in lipid metabolism-related pathways in liver of female rats compared with males. In conclusion, BALF and transcriptomic analyses showed a generally stronger exposure response in female compared with male rats.

1548 Electronic Cigarette- and Hookah-Induced Changes on Nasal Epithelial Cell Gene Expression Profiles

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Emerging alternative tobacco products, such as hookah and electronic cigarettes (e-cigs), have been gaining in popularity within the past decade. Although they are perceived as less harmful than traditional tobacco products, little is known regarding their toxicity and whether they cause similar or distinct health outcomes. Previous studies have shown that exposure to cigarette smoke results in permanent changes at the genomic level that induces the development of disease as well as suppresses host defense responses. However, there have been very few studies that identify exposure-related biomarkers associated with alternative tobacco products. The purpose of this study was to evaluate gene expression profiles of nasal epithelial cell mRNA from nonsmokers (n=14), cigarette smokers (n=13), e-cig users (n=8), and hookah smokers (n=9) using an immunology gene panel of 594 genes. Multivariable regression models were used to identify genes with significant change (p<0.05), and when compared to nonsmokers, e-cig users and hookah smokers displayed 198 and 267 differentially expressed genes, respectively. However, when e-cig users were compared to cigarette smokers there were only 14 distinct genes changed. These results suggest that e-cig users, the majority of who are ex-smokers, have patterns of expression most similar to cigarette smokers than nonsmokers, including the suppression of key responder genes such as IFR3, TRAFs, and IFN-α, indicating that even after using e-cigs for at least 6 months a nonsmoking phenotype is not restored. Overall, our findings provide a better understanding of the biological effects associated with alternative tobacco products as well as identify specific respiratory mucosal biomarkers.
Despite declines in use of cigarettes in the U.S., alternative tobacco products like hookah are gaining popularity. However, there is currently a lack of knowledge on the health impacts of both mainstream and secondhand hookah smoke. These pilot studies investigated the indoor air quality of 14 NYC hookah bars and their adverse health effects on hookah bar workers and patrons. Air quality within hookah bar establishments was evaluated for particle matter (PM2.5), black carbon (BC), carbon monoxide (CO), and nicotine during the work shift of 10 hookah bar employees and also during the bar visits of 13 pairs of study participants. Within each pair, one individual was assigned to actively smoke hookah while the other did not, enabling the assessment of both mainstream and secondhand hookah smoke health outcomes. Hookah workers were evaluated before and after their work shifts, while pairs of participants were tested immediately before and after bar visits. Physiological data collected of hookah workers and pairs of patrons included cardiopulmonary endpoints (blood pressure, heart rate, FVC, FEV1), markers of secondhand smoke exposure (exhaled CO (eCO) and salivary cotinine), inflammatory cytokines (IL-1β, IL-6, IL-8, INF-γ, TNF-α), oxidative stress (8-OHdG), gene expression, and epigenetic changes. PM2.5 and CO measurements in air samples were significantly elevated compared to ambient and background home exposures, with 3 bars at PM2.5 levels exceeding 1,000 μg/m³. In addition, inflammatory markers and eCO levels increased significantly post work shifts and after bar visits, with 2 workers and 1 patron presenting eCO levels greater than 90ppm. These studies suggest the air quality within NYC hookah bars may be hazardous to bar employees and patrons, warranting further investigation on a wider scale and also highlight the importance of increased policy regulation of hookah bars.
rush hour traffic in the morning and afternoon, while peak levels of ozone (O3), a photochemical reaction product, occur in the afternoon. Given the possibility that exposure to a single pollutant may sensitize an individual to the effects of a second pollutant, we hypothesized that pre-exposure to NO2 in the morning will exaggerate the cardiovascular effects of O3 exposure in the afternoon in rats. Rats were divided into the following groups that were each exposed for 3 hours in the morning (m) and 3 hours in the afternoon (a) on the same day: 1) m-Air/a-Air, 2) m-Air/a-O3 (0.3 ppm), 3) m-NO2 (0.5 ppm)/a-Air, and 4) m-NO2/a-O3. Rats were monitored for heart rate (HR), blood pressure, electrocardiogram (ECG), and heart rate variability (HRV), an indicator of autonomic tone. Sensitivity to arrhythmia was measured in a separate cohort as the threshold dose of acrolein required to elicit cardiac arrhythmia. Only m-NO2/a-O3 caused several ECG changes including decreased HR and increased PR, QRS and QTc intervals. In addition, only m-NO2/a-O3 exposure decreased systolic and diastolic blood pressures and core body temperature, and increased QTc relative to m-Air/a-Air. HRV and lung/systemic toxicity data will also be presented. These data indicate that priming effects from short term sequential exposure to air pollution components may underlie some adverse cardiovascular health outcomes. (This abstract does not reflect US EPA policy).

1554 Effect of Same-Day Sequential Exposure to Nitrogen Dioxide and Ozone on Cardiac and Ventilatory Function in Mice

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Although the mechanisms underlying air pollution-induced cardiopulmonary responses have been extensively studied, the impact one pollutant has on another is still unclear. Nitrogen dioxide (NO2) is released following combustion of hydrocarbon fuels peaking particularly in the morning of hot summer days. Ozone (O3) is produced by the reaction of ultraviolet rays from the sun on pollutants already present in the air and peaks in the afternoon. Therefore on any given summer day, individuals could be sequentially exposed to both gaseous air pollutants. We hypothesized that sequential exposure to NO2 followed by O3 on the same day would cause cardiac electrical dysfunction and ventilatory changes in mice. C57BL/6 mice surgically implanted with radiotelemeters were exposed to either m-Air/a-O3 or m-NO2/a-O3 and m-NO2/a-Air for 4 hours each day. These exposures caused greater sensitivity to acrolein-induced ventricular premature beats and tachycardia relative to m-Air/a-Air. HRV and lung/systemic toxicity data will also be presented. These data indicate that priming effects from short term sequential exposure to air pollution components may underlie some adverse cardiovascular health outcomes. (This abstract does not reflect US EPA policy).

1555 Expression of Proinflammatory and Oxidative Stress Mediators Induced by Nitrogen Dioxide and Ozone in Primary Human Bronchial Epithelial Cells

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Air pollutants have been linked to insulin resistance (IR) and the rise of cardio-metabolic disease. Yet, pollutant effects on metabolism are poorly understood. We have recently shown in rodents that acute O3 exposure alters systemic metabolic homeostasis likely through a stress response pathway. The goal of this study was to examine if humans exposed to O3 produce similar systemic metabolic alterations. Serum samples were obtained from a clinical study involving O3 exposure of healthy young-adults (18-40 years age, n=24) with or without a history of drug usage, smoking, or respiratory disease. The crossover study included two clinical visits (each separated by more than two weeks) where each fasted volunteer was blindly exposed to either FA or O3 for 3 hours. Serum samples from each volunteer were divided into the following groups that were each exposed for 3 hours in the morning (m) and 3 hours in the afternoon (a) on the same day: 1) m-Air/a-Air, 2) m-Air/a-FACl) (0.5 ppm), 3) m-NO2/a-Air, and 4) m-NO2/a-O3. Sources for cytokines, oxidants, and metabolites were isolated from serum collected immediately after exposure. O3 exposure significantly increased serum lysolipids, monacoylglycerol, glycerol, and medium and long chain free fatty acids (FFA; C12-C20) likely from adipose lypoysis. O3 also elevated metabolites of sphingolipids involved in the transport of lipids and may consequently increase FFAs in the liver. Ketone body (3-hydroxybutyrate) and acylcarnitines associated with fatty acid β-oxidation increased significantly. O3-induced increases in dicarbonylic acids, azelaic and 2-hydroxylgluturinate might indicate a shift to fatty acid oxidation. Increases in n3 and n6 PUFAs may stimulate inflammatory processes in serum collected immediately after exposure. O3 exposure significantly increased serum oxidized low-density lipoprotein, monacoylglycerol, glycerol, and medium and long chain free fatty acids (FFA; C12-C20) likely from adipose lypoysis. O3 also elevated metabolites of sphingolipids involved in the transport of lipids and may consequently increase FFAs in the liver. Ketone body (3-hydroxybutyrate) and acylcarnitines associated with fatty acid β-oxidation increased significantly. O3-induced increases in dicarbonylic acids, azelaic and 2-hydroxylgluturinate might indicate a shift to fatty acid oxidation. Increases in n3 and n6 PUFAs may stimulate inflammatory processes. Serum samples from each volunteer were divided into the following groups that were each exposed for 3 hours in the morning (m) and 3 hours in the afternoon (a) on the same day: 1) m-Air/a-Air, 2) m-Air/a-FACl) (0.5 ppm), 3) m-NO2/a-Air, and 4) m-NO2/a-O3. Sources for cytokines, oxidants, and metabolites were isolated from serum collected immediately after exposure. O3 exposure significantly increased serum lysolipids, monacoylglycerol, glycerol, and medium and long chain free fatty acids (FFA; C12-C20) likely from adipose lypoysis. O3 also elevated metabolites of sphingolipids involved in the transport of lipids and may consequently increase FFAs in the liver. Ketone body (3-hydroxybutyrate) and acylcarnitines associated with fatty acid β-oxidation increased significantly. O3-induced increases in dicarbonylic acids, azelaic and 2-hydroxylgluturinate might indicate a shift to fatty acid oxidation. Increases in n3 and n6 PUFAs may stimulate inflammatory processes. Serum samples from each volunteer were divided into the following groups that were each exposed for 3 hours in the morning (m) and 3 hours in the afternoon (a) on the same day: 1) m-Air/a-Air, 2) m-Air/a-FACl) (0.5 ppm), 3) m-NO2/a-Air, and 4) m-NO2/a-O3. 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Sources for cytokines, oxidants, and metabolites were isolated from serum collected immediately after exposure. O3 exposure significantly increased serum oxidized low-density lipoprotein, monacoylglycerol, glycerol, and medium and long chain free fatty acids (FFA; C12-C20) likely from adipose lypoysis. O3 also elevated metabolites of sphingolipids involved in the transport of lipids and may consequently increase FFAs in the liver. Ketone body (3-hydroxybutyrate) and acylcarnitines associated with fatty acid β-oxidation increased significantly. O3-induced increases in dicarbonylic acids, azelaic and 2-hydroxylgluturinate might indicate a shift to fatty acid oxidation. Increases in n3 and n6 PUFAs may stimulate inflammatory processes.
1559 Susceptibility of Diabetic Rats to Pulmonary and Systemic Effects of Inhaled Photochemically Aged Atmosphere and Ozone (O3)


Introduction: Rodents with type 1 (T1) diabetes mellitus (DM) are considered a valuable model for understanding human insulin-dependent diabetes mellitus (IDDM). In this study, we investigated the effects of photochemically aged atmospheres (PAAs) on T1DM rats, compared to normal (NO) rats. PAAs were generated in a laboratory environment with a combination of ozone, nitrogen oxides, and secondary organic aerosols.

Methods: Male Sprague-Dawley rats were divided into two groups: T1DM and NO rats. Both groups were exposed to PAAs for 2 hours per day, 5 days per week, for 4 weeks. Blood glucose levels, insulin levels, and body weights were measured prior to and after the exposure period.

Results: The T1DM rats showed significantly higher blood glucose levels compared to the NO rats, indicating greater susceptibility to hyperglycemia. Insulin levels in the T1DM rats were lower than in the NO rats, indicating impaired insulin secretion. Body weights were also lower in the T1DM rats compared to the NO rats, suggesting greater susceptibility to weight loss.

Conclusion: The results of this study suggest that photochemically aged atmospheres can exacerbate the symptoms of T1DM in rats, indicating a potential link between air pollution and diabetes.

1562 Transient Receptor Potential Cation Channel A1 (TRPA1) Mediates Changes in Heart Rate Variability following a Single Exposure to Acrolein in Mice

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Human and animal studies show that short-term air pollution exposure modulates heart rate variability (HRV). HRV, a marker of autonomic influence on the heart, represents homeostatic control mechanisms which dynamically regulate cardio-
vascular function. Low HRV confers a poor prognosis in some clinical populations. Thus, HRV provides insight into cardiac regulation and may have clinical relevance. Our previous data showed that a single exposure to acrolein or ozone decreases cardiac mechanical function and increases dysrhythmia mediated by TRPA1. Sensory activation through TRPA1 by air pollutants triggers autonomic reflexes so we hypothesize that exposure to acrolein or ozone will cause autonomic imbalance as measured by changes in HRV. Conscious, unrestrained C57BL/6 (wt) and TRPA1 knockout (ko) mice were exposed to either 3 ppm acrolein, 0.3 ppm ozone or filtered air (FA) for 3 hours. Electrocardiography (ECG) was recorded continuously before, during, and after exposure. HRV, which included the standard deviation of the NN intervals (SDNN), the root-mean-square of the successive differences between adjacent NNs (RMSSD) and the low frequency/high frequency (LF/HF) ratio, was increased independent of HR in wt mice during acrolein exposure. Counter to our hypothesis, ozone did not cause any HRV changes in wt mice. There was no difference in the HRV of ko mice exposed to either acrolein or ozone when compared to FA. These data demonstrate that a single exposure to acrolein causes cardiac dysfunction through TRPA1 activation and autonomic imbalance, which may represent a shift towards parasympathetic influence due to sensory irritation. Furthermore, although ozone also causes cardiac effects through TRPA1, it appears to do so independently of HRV-related autonomic mechanisms. (This abstract does not reflect EPA policy)

1563 TRPV4 Inhibition Counteracts Edema and Inflammation and Improves Pulmonary Function and Oxygen Saturation in Chemically Induced Acute Lung Injury

S. Balakrishna2, W. Song1, S. Achanta1, B. Liu1, M. M. Kaelberer1, Y. Zhihong2, A. Sui2, M. Cheung4, E. Leishman5, H. S. Eidam4, G. Ye4, 2, 4, 5Cytokines, while improving tissue pathology. These effects were recapitulated in TRPV4-deficient mice. TRPV4 inhibitors had similar anti-inflammatory effects in inflammatory and occupational environments and in transportation accidents. Postexposure and to chlorine gas, a severe chemical threat with frequent exposures in domestic, industrial, and occupational settings. Here, we describe a single exposure of mice to chlorine gas, and the resulting effects on HRV and ventilation. HRV was measured by spectral analysis using the power spectrum. The results showed that HRV was decreased in response to chlorine gas exposure. This decrease was not due to changes in heart rate or respiratory frequency. These findings suggest that HRV may be a useful biomarker for assessing the severity of chlorine gas exposure.

Repeatability of whole-body inhalation exposure of F344 rats to ethylene (300-1000 ppm for 15 weeks: 10,000 ppm for 2 or 4 weeks) results in concentration- and time-dependent focal regions of nasal airway remodeling characterized by mucous cell hyperplasia/hyperplasia, eosinophilic inflammation, and the presence of ciliated cells. In the present study, the effects of repeated high-level exposures to ethylene on HRV were investigated in male C57Bl/6J mice exposed to either acrolein or ozone when compared to FA. These data demonstrate that a single exposure to acrolein causes cardiac dysfunction through TRPA1 activation and autonomic imbalance, which may represent a shift towards parasympathetic influence due to sensory irritation. Furthermore, although ozone also causes cardiac effects through TRPA1, it appears to do so independently of HRV-related autonomic mechanisms. (This abstract does not reflect EPA policy)

1566 In Vitro Dissolution of Libby Amphibole, Amosite Asbestos, and MMVF Using Acid and Synthetic Lung Fluid Media


Toxicity of inhaled fibers is dependent in part on biopersistence due to changes in size distribution after deposition and clearance in the respiratory tract. To model this in vivo behavior, respirable (PM10) Libby amphibole (LA) and amosite asbestos, and a reference material glass wool (man-made vitreous fiber; MMVF-11) were subjected to in vitro dissolution. Dissolution tests were conducted with strong acid (HF/HCl/citric acid) or synthetic lung fluid (SLF; EURIMA method, modified Gambles’ salt solution, pH 4.5). Samples were exposed to acid for 24 h or to SLF by continuous constant flow for 30 or 90 days at 37 °C. TEM analyses showed that up to 10 min acid exposure of LA fibers (representing a chronic exposure in the rat lung) caused no splitting or separation, and little change in size distribution. 30-60 min of LA acid exposure reduced numbers of the smallest paracrystalline particles (Ym1/2), indicating modest fiber dissolution with no apparent effects on fiber clearance and clearance mechanisms. These data suggest that LA fibers are more resistant to acid dissolution than MMVF fibers, which may explain their greater biopersistence in vivo.

1567 Strain Differences in Ethylene-Induced Nasal Lesions in Rats

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Repeated whole-body inhalation exposure of F344 rats to ethylene (300-1000 ppm for 15 weeks; 10,000 ppm for 2 or 4 weeks) results in concentration- and time-dependent focal regions of nasal airway remodeling characterized by mucous cell hyperplasia/hyperplasia, eosinophilic inflammation, and the presence of ciliated cells. In the present study, the effects of repeated high-level exposures to ethylene on HRV were investigated in male C57Bl/6J mice exposed to either acrolein or ozone when compared to FA. These data demonstrate that a single exposure to acrolein causes cardiac dysfunction through TRPA1 activation and autonomic imbalance, which may represent a shift towards parasympathetic influence due to sensory irritation. Furthermore, although ozone also causes cardiac effects through TRPA1, it appears to do so independently of HRV-related autonomic mechanisms. (This abstract does not reflect EPA policy)
Effect of Metal Composition and the Use of Adhesive and Anti-Spatter Chemicals on Lung Responses in Rats after Inhalation during Spot Welding


Spot welding (SW) is common in the automotive industry. Adhesives are used as sealers to the seams of metals that are joined. Anti-spatter compounds are sprayed onto metals to be welded to improve the weld surface finish. SW produces complex aerosols composed of metal and volatile compounds (VOCs) which have caused lung disease in workers. The goal was to evaluate the effect that different components of SW fumes may have on lung responses in an animal model. Sprague-Dawley rats were exposed by inhalation to 25 mg/m$^3$ of aerosol for 4 h/day x 8 d during SW of mild steel or galvanized Zn-coated steel in the presence or absence of an adhesive or anti-spatter spray. Controls were exposed to air. Particle size distribution and chemical composition of the generated aerosol were determined. Particle size distribution was tri-modal with a MMAD of 0.25 μm. The metal fraction of the mild steel fume was 99.9% Fe, whereas the Zn-coated steel was 90% Fe, 7% Zn, and 2% Mn. VOCs (e.g., siloxanes, benzene, toluene) were present when an adhesive was used. After exposure, bronchoalveolar lavage (BAL) was performed to assess lung toxicity. Lung resistance (R$_L$) was evaluated before and after challenge with inhaled methacholine (MCh). Lung toxicity and BAL neutrophils were significantly elevated compared to controls 1 d after exposure to the fumes from Zn-coated steel but not mild steel, indicating the development of acute lung inflammation. All markers of lung toxicity for the Zn-coated group returned to control values by 7 d. Immediately after exposure, baseline R$_L$ was significantly elevated in the group exposed when VOC levels were high. Basal R$_L$ returned to control level by 1 d. Reactivity to MCh was not affected at any time point after SW fume exposure. The use of an anti-spay compound had no effect on lung toxicity or function. Inhalation of SW fumes caused acute lung toxicity due to the presence of specific metals (e.g., Zn) as well as increase R$_L$ due to the use of adhesives.

Comparison of Inhaled Dose vs. Postexposure Time Period in Silica-Induced Pulmonary Toxicity in the Rats


Occupational exposure to respirable crystalline silica results in silicosis, cancer, autoimmune diseases, tuberculosis, and renal diseases. Currently, we have investigated and compared the effect of dose vs. post-exposure time period in the pulmonary toxicity induced by inhalation exposure of rats to crystalline silica. Rats were exposed by inhalation to respirable crystalline silica (Min-U-Sil 5 Silica, U.S. Silica, Berkeley Springs, WV) at a concentration of 15 mg/m$^3$, 6 hours per day, 5 days/week for 1 week or 12 consecutive weeks. The rats exposed to silica for 1 week were maintained under standard animal housing conditions for 44 weeks following termination of their exposure to silica and euthanized. The rats exposed to silica for 12 weeks were euthanized soon after termination of the silica exposure. The total amount of silica inhaled by the 12-week exposure group of rats was roughly 12-times more than that of the 44-week post-exposure group. Silica-induced pulmonary toxicity was determined in both groups of rats on the basis of bronchoalveolar lavage fluid (BALF) parameters of toxicity [lactate dehydrogenase (LDH) activity, albumin content, total number of alveolar macrophages (AMs) and polymorphonuclear leukocytes (PMNs), and inflammatory cytokine levels], lung histology, and global gene expression changes in the lungs. Induction of significant pulmonary toxicity was identified in both groups of rats based on the various pulmonary toxicity parameters analyzed. However, the magnitude of changes in the majority of the pulmonary toxicity parameters determined was significantly higher in the rats belonging to the 44-week post silica exposure time period group compared to the 12-week silica exposure group. These results collectively suggested that the post-silica exposure time period is more critical to silica-induced pulmonary toxicity than the inhaled dose of crystalline silica in the rats.

A Single Exposure to Photochemical Smog Causes Airway Irritation and Cardiac Dysrhythmia in Mice

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Smog, which is a complex mixture of particulate matter and gaseous irritants (ozone, sulfur dioxide, reactive aldehydes), as well as components which react with sunlight to form secondary pollutants, has recently been linked to increased risk of adverse cardiopulmonary responses. We hypothesized that a single exposure to photochemical smog would cause cardiac electrical and ventilatory changes in mice. Female C57BL/6 mice were surgically implanted with radiotelemetry for the measurement of heart rate (HR), electrocardiogram (ECG) and heart rate variability (HRV). Following recovery mice were exposed whole-body to either smog or filtered air (FA) for 4hrs. A photochemical reaction chamber was used to generate smog from a precursor mixture of hydrocarbons and nitric oxides which achieved concentrations of 337 μg/m$^3$ secondary organic aerosol, 0.072 ppm O3, and 0.131 ppm NO2. Ventilatory function was assessed before, and one and 24hrs after exposure in a plethysmograph while HR and ECG were measured continuously. Mice exposed to smog experienced a significant increase in HR during exposure when compared to FA; however, there were no differences or changes in ECG or HRV. Exposure to smog caused a significant increase in breathing frequency and decreased inspiratory time (i.e. rapid shallow breathing) and a significant decrease in HR 1hr post-exposure; these effects were gone 2hrs later. Mice exposed to smog also had cardiac dysrhythmia as well as non-conducted p-waves, which were not present in the FA group. The results of this study show that a single exposure to smog causes acute cardiac and ventilatory effects, which reverse over time. Although these responses likely do not represent serious or permanent underlying deficits, they clearly indicate the potential toxicity of complex multipollutant mixtures, particularly for those with cardiopulmonary disease. (This abstract does not reflect USEPA policy.)

The Role of Oxidized Low-Density Lipoprotein Receptors in Matrix Metalloproteinase Activity and Tight Junction Protein Expression in the Cerebral Microvasculature of Mice Exposed to Traffic-Generated Air Pollutants

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Epidemiologic studies report a positive correlation between environmental air pollution exposure and deleterious effects on the central nervous system, including neuroinflammation and onset/exacerbation of stroke. While the mechanisms involved have not been fully elucidated, one pathway may be through disruption of the blood brain barrier (BBB). We have reported that oxidized LDL (oxLDL) and its receptor, the lectin-like oxLDL receptor (LOX-1), are significantly elevated in the systemic vasculature of Apolipoprotein KO (ApoE-/-) mice exposed to mixed exhaust (ME). ME-exposure also increased BBB permeability, associated with matrix metalloproteinase (MMP)-9 activity and decreased tight junction (TJ) protein expression. To determine whether ME pollutants mediate disruption of the BBB through an oxLDL-LOX-1 pathway, 10 wk old male ApoE-/- mice on a high fat diet received either mouse IgG control or the neutralizing antibodies to LOX-1 (Lox-1 Ab). Mice were randomly assigned to inhalational exposure of either filtered-air (FA; n=12 LOX-1 Ab, n=12 IgG) or 30 μg PM/m$^3$ diesel exhaust + 70 μg PM/m$^3$ gasoline exhaust (ME; n=12 LOX-1 Ab, n=12 IgG) for 6 hr/d for 7 d. Treatment with the LOX-1 Ab led to a significant decrease of LOX-1 expression in the cerebral microvasculature. Exposure to ME resulted in a significant increase in MMP-9 and -2 expression and activity, which was attenuated by LOX-1 Ab treatment. Histological analysis showed that ME-exposure resulted in decreased expression of BBB TJ proteins occludin and claudin-5, which were normalized with LOX-1 Ab treatment. Such findings indicate that inhalation exposure to traffic-generated air pollutants results in BBB disruption associated with MMP activity and decreased TJ protein expression, which are mediated (at least in part) through the LOX-1 receptor. Funded by NIEHS R00ES016586 (AKL).
Development of an Inhalation Exposure System for Resistance Spot Welding Using an Anti-Spatter Spray


A common metal joining process is resistance spot welding (RSW). In RSW, two copper alloy electrodes squeeze pieces of sheet metal together and pass high levels of current through the metals to create a weld. The process generates a complex aerosol. The chemical properties of the aerosol are dependent on the metal profile of the welded sheet metal as well as the composition of anti-spatter agents used during the process. Anti-spatter treatment protects the electrodes and improves the welding surface finish. However, anti-spatter chemicals contain ingredients known to be harmful to health. Respiratory disease has been observed in RSW welders. The goal was to design a RSW inhalation exposure system that includes an anti-spatter spray system and determine if the anti-spatter agent contributes to lung responses associated with RSW fume. This system will be used for animal toxicology studies. The system is divided into different areas: (1) enclosed automated spot welder; (2) exposure chamber with aerosol characterization equipment; (3) sheet metal driving system; (4) computer control room and (5) anti-spatter spray unit. The anti-spatter agent was sprayed before welding on the surface of two strips of low carbon steel. A fume injector was used that is controlled by a data acquisition system. Generated RSW fume was delivered to the animal exposure chamber. A real-time aerosol monitor was used to measure and maintain a RSW particle mass concentration of 25 mg/m³. SEM/EDX revealed the RSW aerosols to be primarily composed of iron and arranged as chain-like agglomerates. Analysis of the size distribution indicated the MMAD of the generated particles was approximately 0.258 µm. Two distinct particle morphologies were observed; a reddish-brown metal particle (likely iron) observed in the nanometer size range and a yellowish particle in the larger micron/submicron range (likely from anti-spatter agent). The exposure system has been designed to assess the potential toxicity of anti-spatter spray used in RSW.

Acetaminophen Potentiates Acute Respiratory Responses to Oxidants and Environmental Tobacco Smoke

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Epidemiological evidence suggests that acetaminophen (N-acetyl-para-aminophenol, APAP) may play a role in the pathogenesis of asthma, likely through pro-oxidant mechanisms. However, direct data on the pro-oxidant effects of APAP in the airways is absent. To determine if APAP acts as a pro-oxidant in the airways, we administered APAP (100mg/kg, ip) to female C57Bl/6J mice and measured tissue non-protein sulphydryl (NPSH), and antioxidant response element (ARE)-dependent gene induction by qRT-PCR. APAP caused a 20% NPSH loss and significant ARE gene induction within 2hrs throughout the airways. An attenuated ARE response in Nfr2 null mice confirmed a role for the Nfr2 pathway in this response. We hypothesized that as a pro-oxidant, APAP may enhance the airway response to known pro-oxidant asthma causative factors such as environmental tobacco smoke (ETS). Therefore, we measured the effect of the combination of ETS and APAP pretreatment on airway NPSH, ARE gene expression, and sensory irritation. APAP and ETS caused greater NPSH loss (~40%) than either treatment alone, and potentiated the ARE gene response. The ETS induced irritation response was greatly enhanced by pretreatment with APAP. APAP enhanced the response to the pure-oxidant acrolein, but not to the non-oxidant cyclohexanone suggesting that this is not a generalized pro-oxidant phenomenon, but rather specific to oxidants. The increased acrolein response was blocked by giving a cytochrome p50 2E1 inhibitor, 5-phenyl-1-pentyne, indicating an APAP metabolite was responsible for the response. Taken together these data demonstrate that APAP acts at moderate doses, acts a pro-oxidant in the airways and enhances the airway response to ETS. Furthermore, our results support the novel concept that APAP may influence the pathogenesis of asthma by potentiating the effects of other oxidants such as ETS.

Preclinical Nebuliser Comparisons to Allow More Effective Decision-Making on Device Selection

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The generation output comparison of the 15 most common pneumatic or jet nebulisers was undertaken to assist in formulating a more informed decision on device selection and compound requirements when provided information on the disease target, test article and formulation properties, study design and dose levels. Each nebuliser was primed with 0.9% Phosphate buffered saline and the generation output (g/min) determined for a period of 10mins. The airflow through each device was then increased in 2L/min increments from 3 to 27L/min. The generation rate at 3L/min was <0.05g/min for the Wright and EVO nebulisers but was over 5 times (highest 9.25g/min for the Pari LC nebuliser). A linear increase from the Wright gave a linear increase in output from 3 to 7L/min. The Wright nebuliser gave an exponential increase in generation output over this range. At airflows between 7 and 12L/min, there were several devices that still gave a proportionate increase in output with increased airflow. These included the HEART, AeroMist, Pari LC D and Wright nebulisers. No increase in output was observed for the EVO nebuliser at airflow >9L/min. In summary, the mini-Heart gave the most consistent output over the airflow range evaluated. The Wright nebuliser gave the lowest output from those tested. The Pari LC plus gave the highest output over the manufacturers recommended working range for the device. In conclusion, this data has allowed improved prediction with greater accuracy of the formulation requirements and help make a more informed decision on device selection recommendations for a given study design. This is of particular importance when the active drug is very expensive and non-clinical programme time lines are critical.

A Controlled Human Exposure System for Di-(n)-Butyl Phthalate (DBP) in the Vapor Phase

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Phthalates are commonly used as plasticizers and are ubiquitous contaminants in indoor environments. Epidemiological studies have linked phthalates to development and exacerbation of asthma and allergy, but the contribution of the inhalation exposure route remains unclear. To perform a controlled human exposure study and assess the pulmonary and systemic effects due to phthalate inhalation, we have designed and built an exposure system to deliver a known concentration of DBP in vapor phase to the study participants through a mask. DBP was chosen as a model phthalate since some of the highest indoor air levels have been reported for this phthalate. The target DBP concentration, to be inhaled for 3 hours by study participants, is 4 times the maximal reported concentration within homes, i.e. 60 µg/m³. The DBP is contained in a flask, immersed in a temperature-controlled water bath where the temperature determines the DBP vapor concentration. A photo ionization detector (PID) monitors the level of total volatile organic compounds (TVOC) in the system. The major determinants for the measured TVOC levels are temperature, DBP volume and airflow through the flask. The desired TVOC level, calculated to correspond to 60 +/- 10 µg/m³, is reached within 90 minutes and kept stable for a 3 hour period, when applying the developed experimental procedure. However, since the PID provides a non-specific TVOC measurement, these levels are now calibrated by collecting air samples in Tenax tubes for measurement of the corresponding DBP concentration by thermal desorption GC-MS.

Precision-Cut Lung Slices As an Alternative Model for Repeated-Dose Inhalation Toxicity

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There is a growing need for appropriate alternatives for animal inhalation studies in order to test respiratory adverse effects of inhalable substances. These alternatives should comply with the three R principles. In this regard Precision-cut lung slices (PCLS) are a prevalent used ex vivo alternative reflecting the respiratory tract. However, most studies using PCLS have been conducted within a 72 h time window to investigate acute respiratory toxicity. In order to evaluate the feasibility of long-term PCLS cultivation to test e.g. toxicity of slowly metabolized substances, rat PCLS were cultivated for more than 14 days. Additionally, triple Triton X-100 treated rat PCLS were compared to double exposed PCLS in order to investigate their suitability for repetitive exposure studies. Markers for slice vitality.
were LDH and WST-1 assay. Moreover, physiological alterations were studied using methacholine-induced bronchoconstriction. Constant vitality and bronchoconstriction were observed over the 14 day period with slight decreases towards the end of cultivation. Repeated Triton X-100 exposure had no influence on the sensitivity of PCLS (AE50 [μg/mL] = 4 μM). Overall these results showed feasibility of long-term cultivation with a good preservation of vitality and physiology. Further it demonstrated that constant vitality remained despite repeated chemical exposure making PCLS a possible future model for slowly metabolized substances and repeated dose testing.

**1575 PCB-Mediated Estrogen Receptor-α-Dependent Histone Modifications: Possible Regulatory Link between PCB Exposure and Induction of Vascular Inflammation**

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Endothelial dysfunction is an early event in the pathology of atherosclerosis, which is an underlying cause in the majority of cardiovascular events. The early pathology of atherosclerosis includes endothelial cell dysfunction, including inflammation, and the pathology progresses throughout life. Exposure to persistent environmental pollutants, such as polychlorinated biphenyls (PCBs), may cause inflammation of the vascular endothelium leading to the development of atherosclerosis. We found that coplanar PCBs 77 and 126 induced nuclear factor-κB (NF-κB) target genes such as interleukin (IL)-6 and IL-1β, monocyte chemoattractant protein-1 (MCP-1), intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and C-reactive protein (CRP). Most interestingly, PCBs 77 and 126 also up-regulated the expression of Junmomi domain-containing protein 2B (JMD2B), a histone H3K9 trimethyl demethylase in an estrogen receptor (ER-α)-dependent manner. Thus, we propose that coplanar PCBs are atherogenic through pro-inflammatory factors, which are mediated by ER-α-dependent histone modifications. Further studies will focus on ER-α and JMD2B knockdown as well as chromatin immunoprecipitation (ChIP) to access ER-α and JMD2B binding and histone methylation markers on promoter regions of target genes. This work may have implications in understanding epigenetic regulation of PCB-induced vascular toxicity. Research reported was supported by the NIEHS/NIH grant P42ES007380. The content is solely the responsibility of the authors and does not necessarily represent the official views of NIH.

**1576 Real-Time Cell Analysis for Cytotoxicity Assessment of Coal Fly Ash for Air Quality Monitoring Applications**

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Environmental air pollution is assessed via two complementary approaches, physicochemical and bioassay-based analysis; the latter is most often determined using dye-based cytotoxicity assays. However, particulate matter (PM) interference with these assays is commonly reported. Here, we demonstrate the application of the novel impedance-based bioassay, real-time cell analysis (RTCA), for the cytotoxicity assessment of a PM, coal fly ash (CFA). CFA was sampled from a coal-burning power plant in China and size-fractionated into three fractions: PM2.5 (≤2.5 μm), PM10-2.5 (2.5 μm–<10 μm), and PM10 (≥10 μm). Fractions were sterilized via submersion in 70% ethanol and dried in a vacuum desiccator. A novel approach to reduce the effects of particle interference on the RTCA electrodes was developed by employing particulate well blanks (no cells present in wells) for background signal subtraction from measured impedance values of treatment wells (cells present). Two human lung carcinoma cell lines, A549 and SK-MES-1, were used in the RTCA system as the lungs are a primary target of PM pollutants. Cells were treated with CFA (1 μg/mL–20 μg/mL) and hourly impedance data was collected for 60 h post-exposure. Two-way ANOVA of 24 h IC50 values revealed the cytotoxic effects were both size- (PM2.5 > PM10-2.5 > PM10; p<0.05) and cell-dependent (SK-MES-1 > A549; p<0.05). Because PM2.5 is the most biologically significant PM fraction, PM2.5 CFA fractions from three additional power plants in China were also assessed in addition to the urban air dust standard reference material, SRM1642a, in A549 cells. One-way ANOVA of the 24 h IC50 values (106μgC–276±30 μg/mL) for the four PM2.5 samples indicated that cytotoxicity was location-dependent (p<0.05). In summary, RTCA is an impedance-based detection technique that overcomes particulate interference to provide an accurate cytotoxicity assessment and ranking of CFA PM, demonstrating the potential application of this technique in air quality monitoring schemes.

**1577 Detection of Airway Microbiota by Next-Generation Sequencing following Burn and Inhalation Injury**


Healthy airways contain defense mechanisms that keep them clear of colonizing bacteria; however, inhalation of smoke, a multi-pollutant mixture, bombards the respiratory tract with gases and particles that may facilitate bacterial colonization by impairing normal protective barriers. Airway bacterial colonization is associated with morbidity and mortality in burn victims with inhalation injury. Infection in these patients is diagnosed by culturing bacteria; however, this is limited by availability of species-specific tests and may give unreliable results. To overcome these limitations, we developed a method for characterizing the bacterial communities, or microbiota, of burn patient airways following inhalation injury. We identified bacterial families present in the airways of burn patients and correlated patterns of colonization with inhalation injury. Total DNA was isolated from bronchial washes obtained from 102 burn patients admitted to the North Carolina Jaycee Burn Center. Bacterial DNA was enriched by exclusion of CpG-methylated DNA prior to tagmentation with primers specific to the bacterial 16S rDNA locus. Tagged bacterial DNA was sequenced on the Illumina MiSeq and bacterial families were identified using the program MMTToolbox. Preliminary results from 11 patients identified 49.7% of the sequencing reads as the bacterial family Staphylococcaceae, 37.4% as Pasteurellaceae, and 4.9% as Streptococcaceae. The family Neisseriaceae was associated with inhalation injury. These results demonstrate that damage to the airways by inhaled smoke may be associated with colonization by certain bacteria. Characterization of airway microbiota after smoke inhalation may aid in risk assessment following particle and gas exposure. Further, it will serve as a starting point for mechanistic in vitro studies examining effects of particulate matter on susceptibility of airway epithelial cells to bacterial infection. This work does not reflect EPA policy.

**1578 Air Pollution (PM 2.5) in Fresno County Leading to Shorter Life Spans**


In 2010, the American Heart Association reported that long term exposure to PM2.5 caused higher cardiovascular disease (CVD)-related mortalities and shortened life spans by a few years or months compared to short term exposure. Fresno County (FC) is commonly known to have poor air quality. The objective of this project was to identify trends in CVD-related mortalities due to high levels of PM2.5 in children age <14, adults ages 45-64 and seniors 85+ living in FC compared to Monterey County (MC). MC typically has better air quality and is similar in size and population to FC. The California Air Resources Board iADAM website provided 24 hr average PM2.5 levels from an FC and MC sites every ten days. Individual data points for PM2.5 were collected and compared for FC and MC between Jan 1, 2009-Dec 31, 2011. Monthly CVD-related mortalities were obtained from the US Center for Disease Control Wonder database by specifying the years (2009-2011), age at death, and cause of death. A comparison of PM2.5 levels was made to CVD-related deaths in FC and MC from 2009-2011. A pattern in PM2.5 levels was observed for FC: high monthly season = Nov-Feb (9.5 μg/m3); low season = Mar-Oct (6.21 μg/m3). Averaged monthly PM2.5 levels for MC ranged from 0.10 μg/m3 for Nov-Jan and 3.7 μg/m3 for Feb-Oct. Highest PM2.5 levels for FC reached 60.6, 56.1, and 76.4 μg/m3 in each December of 2009, 2010, and 2011 with 3, 3, and 6 data readings above US EPA national standard (35 μg/m3). The CVD-related mortalities for FC were statistically significant greater than deaths for MC. No CVD-related deaths were reported in the <14 yo group for either county. CVD deaths in MC for the 85+ group reached 31 (Dec 2009), 36 (Sept-Aug 2010), and 42 (Sept 2011), while in age group 45-64, no enough mortalities occurred to be recorded. The highest mortalities for age 85+ in FC were 83 (Jan 2009), 87 (Jan 2010), and 95 (Dec 2011) while the 45-64 group reached 31 (Nov & Mar 2009), 37 (May 2010), and 25 (Mar & Nov 2011). Peak mortalities in FC tracked peak PM2.5 levels. The results suggest that the higher PM2.5 levels result in higher CVD mortalities in ages 45-64 and 85+.
Although air pollution is a complex mixture consisting of multiple gaseous and particulate components, current regulations are based on single pollutants and limited research is available on the health effects of realistic multipollutant atmospheres. To better assess the impact of air pollution mixtures on respiratory health, we investigated the effects of a smog mixture on allergic airway disease in mice. A photochemical reaction chamber was used to generate smog from a precursor mixture of hydrocarbons and NO which achieved concentrations of 337 μg/m3 secondary organic aerosol, 0.072 ppm O3, and 0.131 ppm NO2. Healthy and house dust mite (HDM)-sensitized (allergic) BALB/c mice were exposed 4 hr/day for 1 or 5 days to smog or clean air. Two days after HDM challenge, airway mechanics were tested in anesthetized ventilated mice. Airway resistance following methacholine aerosol challenge was significantly increased in HDM-allergic mice, but smog exposure did not further enhance the response. Bronchoalveolar lavage (BAL) macrophages, eosinophils, and cytokines (IL-4 and IL-5) were also significantly increased in HDM-allergic mice compared to non-allergic mice. Five days of smog exposure induced an increase in macrophages and eosinophils, and 1 day smog exposure induced an increase in BAL lactate dehydrogenase in the HDM-allergic mice, but these differences were not significant compared to air exposed HDM-allergic mice. Lung histopathology showed that 5 day smog exposure increased the incidence of intracytoplasmic particulate matter within alveolar macrophages, but did not alter allergic-induced inflammatory changes. No significant effects of allergic challenge or smog treatment were observed on nasal histopathology. Future studies will examine alternative mixtures of smog at different air quality levels to further explore which realistic atmospheres affect the severity of allergic lung disease. (This abstract does not represent U.S. EPA policy.)
Adverse cardiovascular effects after particulate inhalation exposures have been reported; however, the mechanisms involved are largely unknown. For mechanistic insight, we investigated global transcriptional alterations in the target organ (lung) as well as several extrapulmonary tissues (heart, aorta, whole blood cells) following inhalation (40 mg/m³ for 3 h/d for 5 d for a week on 10 d) to stainless steel welding fumes. Tissues were collected 4 h and 28 d post-exposure. RNA was isolated and microarray results were analyzed using Ingenuity Pathway Analysis for pertinent biological and molecular networks associated with effects. Utilizing the upstream regulator (i.e. transcription factors, cytokines, growth factors) analysis, the lung had 285, blood cells 30, aorta 39, and heart 32 significantly altered mediators 4 h post-exposure. There was a graded decline in the total mediators at 28 d with the lung decreasing by 3%, blood cells 37%, aorta 77% and heart 100%. When examining the connectivity of signaling at 4 h, 90% of the upstream regulators in the blood cells were reflective of the lung response, 90% in the aorta, and 44% in the heart. Specific mediators of interest in the aorta (Mt2, Sele, Hspa1b, Vcam1) predictive of adverse vascular effects were increased. Mitochondrial dysfunction was the top canonical pathway in the heart with the top three signaling networks centering on altered energy metabolism. PPARGC1A, a transcription factor regulating mitochondrial biogenesis and function and induced by oxidative stress, was increased and linked to many of the altered cardiac upstream regulators and genes associated with mitochondrial dysfunction. In conclusion, systemic signaling, suggestive of oxidative stress-mediated cardiovascular dysfunction, was strongly reflective of the ongoing pulmonary response although the altered signaling was not sustained compared to the lung.
Combustion derived particulate matter, including coal fly ash (CFA), has been shown to activate members of the transient receptor potential (TRP) family of ion channels. TRP melastatin-8 (TRPM8) is a cold-sensitive receptor that has been shown to respond to certain types of particulate matter (PM) via a mechanism that appears to be related to mechanical perturbation of the cell surface. The purpose of this study was to investigate the activation of TRPM8 by PM using site-directed mutagenesis, and treatment at various temperatures. N-terminally truncated mutants, the predicted TRPM8 glycosylation site (N934Q), a cysteine bridge (C929A and C940A), N-terminal (R247T), and the SNPs Y251C, S419N, and M462T were generated, transfected into HEK-293 cells, and functionality evaluated by comparing calcium flux relative to the wild-type channel. The effect of cooling on TRPM8 activation was also examined by administering soluble and insoluble agonists at different temperatures to cells maintained at 37°C. A drop in temperature due to ice-cold media addition did not activate TRPM8. However, responses to PM and the soluble agonists menthol and icilin were increased when applied as cold solutions. Mutations in the cysteine bridge region decreased responses to both soluble and PM agonists. Truncation of the N-terminus to M801 decreased activation by agonists and N-terminal R247T decreased response to PM only. The glycosylation site N934Q mutant increased responses to CFA, but decreased the response to icilin, and the SNP S419N showed increased activation by PM only. Essentially equivalent mRNA expression was shown for all mutants versus wild-type TRPM8, suggesting that the changes in activity were functionally based. These findings contribute to our general understanding of components of TRPM8 that are necessary for activation by mechanical stimuli that could result in the production of inflammatory cytokines in lung cells. Thus, these studies provide new insights into mechanisms by which PM may cause lung irritation and toxicity. Support: ES017346.

1589 Particle Size Distributions of ENDS Aerosol at Various Voltages Utilizing 90 Degree White Light Spectroscopy
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As the use of ENDS (Electronic Nicotine Delivery Systems) continues to rise, research regarding their electrical properties and associated aerosol characteristics remains limited. ENDS deliver an aerosol to the user by evaporating a Propylene Glycol and Glycerin solution containing various proportions of nicotine and flavoring compounds. The electrical properties associated with ENDS, including the current and associated voltage used for aerosol generation have been shown to have some of the most significant effects on aerosol production, particularly in regard to particle size and count. Moreover, various voltages can be generated in ENDS, such as by the steady voltage drop related to battery loss over time and by versions with voltage variation features that give users the ability to choose their preferred voltage. A novel ENDS aerosol generator was developed to help study the physicochemical properties associated with ENDS aerosols at various voltages. Two voltages were chosen for propagation, one higher voltage of 5.2 V, simulating a freshly charged battery, and one lower voltage of 2.5 V, simulating significant battery loss. Ten, 4 s puffs of a 55 mL puff volume were generated every 30 s using the developed generator and NJOY tank system with a 15 mg/ml nicotine content, “Classic Tobacco” PG/VG solution. The mouthpiece of the ENDS was connected to the inlet of a 90 degree, white-light scattering spectrometer (Pals GmbH) operating continuously at 5 L/min. Clean, dry, particle free dilution air was provided to minimize coincidence errors associated with particle measurement. Larger particle sizes were generated at the higher of the two voltages, indicating a 16.7% difference in particle size between the two. The ENDS aerosol generated at 5.2 V had a optical diameter of 0.28 um (0.06), as compared to an optical diameter of 0.23 um (0.008) measured at 2.5 V. More research is needed to determine if the difference in particle size is due to particle agglomeration or by thermal degradation of temperature dependent constituents formed at the higher voltages.

1590 Early Kidney Damage Induced by Continuous Exposure to Concentrated PM2.5

Cardiorespiratory diseases are associated with particle matter (PM) exposure. Inflammation and oxidative stress are involved in PM toxicity and with cardiorespiratory effects. We recently demonstrated that PM up regulates angiotensin (RAAS) and bradykinin (KKK) system components, in lung and heart, these endocrine systems are also regulated by the kidney. We hypothesized that continuous PM2.5 exposure could contribute to early kidney damage. Sprague-Dawley male rats were exposed for 8 w, 4 d/w, 5 h/d, to concentrated PM2.5 and filtered air (FA) using a particle concentrator. We collected 12-hour urine, measured water consumption and blood pressure, and performed general urine exam and blood chemistry. We analyzed urine early kidney damage markers (albumin, AGP, β2M, Cis-C, EGF and NGAL) from week 1, 2, 4, 6 and 8, and cytokines (IL-1β, IL-6, TNF-α, IL-4, IL-10, INF-Y, IL-17a, MIP2 and RANTES) in total kidney cortex protein by LUMINETX. We evaluated markers of RAAS by qPCR, AT1 and AT2, from KKS: B1r, B2r and Klk1, the antioxidant enzyme Hmxox-1, and SOD2 and γGCsc proteins by Western blot, finally, we performed kidney histology. We observed that PM2.5 exposure increased blood pressure; water intake; urinary flow rate; serum creatinine and parameters related with nitrogen excretion and ion-balance, and decreased glomerular filtration. We observed an augment of AGP, EGF and NGAL at week 2, and Cis-C and β2M augment in all weeks, except in week-4, in urine from the PM2.5 group. We also observed a depletion of the immune response in the kidney. Also, we observed down-regulation of At1r and Klk1; however B1r was up regulated. Antioxidant response showed that Hmxox-1 and SOD2 were down regulated; however γGCsc was up regulated. Kidney histology showed tubular damage. We here demonstrate that PM2.5 exposure induced early kidney damage perhaps associated with blood pressure increase, the activation of endocrine systems with imbalance of immune response and antioxidants that could feedback lung and heart damage. (Funding: CONacyt 167778).

1592 Vanadium Inhalation Effect on Lung Mast Cells in a Murine Model

Inhalation of particulate matter (PM) as a consequence of air pollution has become a health threatening reality. Some PM sources are industry and petroleum-derivates combustion products, which carried metals on its surface. One of these metals is vanadium that has been associated with symptoms similar to asthma and mast cells (MC) are involved in this event. Mast cells active degranulation liberates histamine by IgE, while reactive oxygen species (ROS) and peroxidation of MC membrane causes a passive degranulation. This event has been studied in nervous system ischemia-reperfusion events. Carnosine has been identified as an effective antioxidant normally found in nervous system reducing MC degranulation and histamine re-
leak in cases of ischemia-reperfusion. Our objective was to demonstrate the effect of carnosine in the lung after vanadum inhalation. Twenty male-C57 mouse 35±5g were distributed in 4 groups: Inhalation of saline, inhalation of V5O2 (1h/ twice a week), Inhalation of saline and carnosine 1mg/kg/day orally, and inhalation of V5O2 and oral carnosine. Mice were sacrificed at week four. Lungs were fix by intracardiac perfusion and processed for histologic evaluation, stained with toluidine blue looking for peribronchial metachromatic mast cells. Five fields at 40X from each mouse were counted. Our results indicated an increase of MC in Vanadium exposed mouse and a decrease when carnosine was administrated. We conclude that carnosine decreases the presence of peribronchial MC possibly by reducing ROS generated by vanadium exposure.

1593 Characterization and Acute Toxicity of Airborne Particles in an Electronic Waste Recycling Facility
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Improper disposal of electronic wastes (e-waste) can lead to the release of toxic chemicals into the environment and increase health risks. Improved recycling processes are evolving to dispose e-waste and recover valuable materials. While these e-waste recycling operations represent vast improvement, little is known about environmental releases, exposures, and potential health impacts at these facilities. In this study, a particulate matter (PM) sampling was conducted at a modern U.S.-based e-waste recycling facility that employs mechanical processing operations. Size-fractionated PM samples were physicochemically analyzed and then given by oropharyngeal aspiration to mice or cultured with lung slices for lung toxicity tests. Chemical analysis showed that the fine and ultrafine PM had higher levels of copper, lead, and zinc (up to ~81 times) than ambient PM collected in the same manner in the recycling facility, with the coarse PM having even greater levels (on an equivalent mass basis) of the metals (up to ~600 times) than the ambient PM. The lung toxicity test results showed that the coarse PM significantly elicited pro-inflammatory responses in the mouse lung at 24 h post-exposure compared to fine and ultrafine PM, and similar toxicity outcomes were observed in the lung slice model. We conclude that exposure to the coarse PM caused substantial inflammation in the mouse lung and the toxicity appeared to be associated with higher concentrations of heavy metals produced by the e-waste recycling operation. Although the exposure levels to total PM as well as specific metal components were well within current Occupational Safety & Health Administration (OSHA) guidelines, the enrichment of these metals compared to levels normally in ambient PM could be of potential health concern. (This abstract does not represent U.S. EPA policy).

1594 PM2.5 Exposure Results in Endothelial Damage and Altered Immune Cell Populations
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Exposure to fine airborne particulate matter (PM2.5) is associated with cardiovascular morbidity and mortality, but the basis for this is not clear. In addition, there are few indices of incipient cardiovascular disease arising from acute or chronic exposures. To identify early biomarkers of exposure we characterize blood cells levels and circulating microparticles in both humans and mice by flow cytometry. We also measured plasma cytokines in samples obtained from human subjects on high and low PM2.5 days. Human blood samples were obtained from a cohort of young, healthy subjects living in the Wasatch (Utah) Valley, during times of low, intermediate, and high PM2.5 levels from January to March of 2013 and 2014. Murine blood samples were obtained from mice exposed (6h/d x 5d) to HEPA-filtered air or concentrated ambient particles (CAPS) generated by a versatile aerosol concentration enrichment system from downtown Louisville, KY air. In human blood samples, we observed a statistically significant, positive association between PM2.5 levels and CD4+ T cells, CD8+ T cells, CD14+ monocytes, and CD16+ neutrophils. Conversely, we observed an inverse association with CD19+ B cells. Like the human samples, we observed a positive association between CAPS levels and circulating CD4+ and CD8+ T cells in mice. Microparticle analysis of human plasma samples revealed increases in endothelial (Annexin V+, CD31+, CD41+) - derived microparticles, while the mouse samples demonstrated a positive association with activated endothelial microparticles (Annexin V+, CD62E+). Multiple cytokines demonstrated a 1.5 fold or greater increase in human plasma on high PM2.5 days. These data suggest that acute exposure to PM2.5 results in endothelial damage and changes in blood cell populations and cytokines. These factors may contribute to the cardiovascular pathology resulting from exposure.

1595 Sepiapterin Supplementation Fails to Ameliorate Diesel Exhaust Particle Exposure-Related Erectile and Coronary Artery Dysfunction in Young Lewis Rats
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The health hazards linked to diesel exhaust particles (DEPs) are of interest because of their nano-scale size, increased respiratory tract deposition, and their complex physio-chemical properties. The exposure to DEP is known to cause a myriad of vasculopathies through increased inflammation and oxidative stress including oxidation of the nitric oxide synthase (NOS) cofactor, tetrahydrobiopterin (BH4) and uncoupling of the NOS complex. The effect of combustion particle exposure on erectile response is under-investigated. We hypothesized that: 1) Instillation of DEPs would induce erectile and coronary vascular dysfunction. 2) The erectile dysfunction would manifest prior to coronary vascular dysfunction in a model of repeated exposure. 3) Increasing bioavailability of BH4 would ameliorate DEP-induced dysfunction. Erectile function in young vehicle control (14 weeks old; n=12) and groups of 1x, 2x, & 3x 125 µg DEP-instilled over 14 days (14 weeks old; n=3) male Lewis rats was assessed in situ by measuring the maximum intracaverno- ral pressure (ICP) and mean arterial pressure (MAP) in response to electrical field stimulation of the cavernosal nerve. DEP exposed groups (1x and 3x) displayed depressed ICP levels at all voltages including a rightward shift in the EV50. Erectile responses after intracavernosal injection of 10 µM sepiapterin, a BH4 precursor were unaffected. In vitro coronary artery responses were revealed impaired serotonin-dependent vasconstriction and endothelium-dependent relaxation in all DEP exposed groups that was not relieved by sepiapterin treatment. Based on these data, IT instillation of DEP is associated with both coronary artery and erectile dysfunction and this dysfunction is unrelieved by supplementation with sepiapterin to increase BH4 bioavailability. Supported in part by a Sexual Medicine Society of North America fellowship to D.P.B., East Carolina University and NIH U19 ES 019525.

1596 Mitochondrial microRNA Dysregulation Contributes to Acute Cardiac Dysfunction following Pulmonary Mountaintop Mining Particulate Matter Exposure
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Heart disease is the leading cause of mortality worldwide and is exacerbated in areas surrounding active mountaintop mining operations. Mountaintop mining generates particulate aerosols and thus creates unique air pollution. mitochondrial dysfunction has been identified following exposure, yet the causative mechanisms are unidentified. MicroRNAs contribute to homeostatic and adaptive mechanisms and dysregulation contributes to the progression of heart disease. The goal of this study was to determine the effect of mountaintop mining particulate matter (PMTIM) on cardiac function and microRNA dysregulation. Adult male Sprague-Dawley rats and FVB mice were exposed to PMTIM collected from areas surrounding active mountaintop mining operations using intratracheal instillation and pharyngeal aspiration, respectively. Twenty-four hours post-exposure, cardiac functional measurements and mitochondrial isolated were performed. Cardiac dysfunction was indicated in both species compared to their sham control by decreased ejection fraction and fractional shortening. Following mitochondrial isolation, RNA was isolated and RT-qPCR was used to assess microRNA levels within the mitochondrion in both species, miR-378 was increased within the mitochondrion following PMTIM exposure. Finally, we identified ATP synthase F0 subunit 6 (ATP6) as a potential target of miR-378 regulation. Immunoblotting identified a decrease in ATP6 protein content in the mitochondria of both species. In conclusion, this study provides evidence that PMTIM exposure increases mitochondrial microRNA content that contributes to mitochondrial dysfunction leading to cardiac dysfunction. AHA 13PRE16850066; NIH R01ES015022; NSF DGE1144676; AHA 14PRE19890020; NIOSH NTRC; NIH R01ES015022.
Nerve agents have been employed by Iraq and Syria and were released by terrorists in Japan on 11 occasions in 1994–1995. These releases indicate that countries must be prepared to treat civilian as well as military casualties. This requires an understanding of the mechanisms of toxicity of these agents, the factors that influence their clinical impact and knowledge of potential treatments. Much research is underway to improve the current treatment regimens, which include an anticholinergic drug (e.g., atropine) to antagonize the effects of excess acetylcholine (ACh) at muscarinic effector sites, the use of an oxime to reactivate nerve agent-inhibited acetylcholinesterase (AChE), and an anticonvulsant benzodiazepine to prevent or stop nerve agent-induced seizures. A series of novel phenoxylpyridinium oximes that show efficacy in the brain have been tested and found to reduce brain AChE inhibition and attenuate seizures. The in-service (military) medical countermeasure provision is based on carbamate pretreatment; such an approach is not possible in the case of a civilian population who are also not likely to be wearing personal protective equipment (PPE). The concept of employing physostigmine, hyoscine, and HI-6 in a single autoinjector in the absence of any form of pretreatment may reduce incapacitation significantly. In addition, the potential of human recombinant butyrylcholinesterase alone, and in combination with standard therapy, as a postexposure treatment, and the use of antinicotinic drugs to reduce the effects of accumulated ACh, could offer additional benefits. Finally, a beta-cyclodextrin with an attached oxime function may offer an alternative approach by enhancing detoxification of nerve agents.

While pyridinium oximes, such as pralidoxime (2-PAM) or HI-6, are effective reactivators of organophosphate (OP)-inhibited acetylcholinesterase (AChE), their quaternary ammonium groups prevent appreciable, if any, entry into the brain, and therefore they cannot independently prevent AChE inhibition in the brain and the consequent seizures and brain damage that result from prolonged high level AChE inhibition. Our laboratories have invented a series of novel phenoxylpyridinium oximes that show efficacy in the brain for reactivating AChE inhibited by two nerve agent-relevant OP structures. These oximes have been tested with surrogates for sarin (nitrophenyl isopropyl methylphosphonate) and for VX (nitrophenyl ethyl methylphosphonate), both of which leave AChE phosphorylated by the same chemical moiety as their respective nerve agents and therefore are highly relevant for reactivation studies. Exposures to these surrogates, when administered at a high sub-lethal dosage to result in 80% brain AChE inhibition, result in seizures and brain damage as indicated by glial fibrillary acidic protein (GFAP) accumulation in a laboratory rat model. The novel oximes, when administered at the time of peak AChE inhibition following surrogate administration (1 hr), reduce the brain AChE inhibition by up to 35%, attenuate the surrogate-induced seizures and retard the accumulation of GFAP with both surrogates. 2-PAM cannot prevent these adverse effects on brain AChE, seizures or GFAP accumulation. These novel oximes have future potential as therapeutics that can reactivate AChE both peripherally and centrally and that can prevent or attenuate central adverse effects. Therefore these novel oximes could assist with prevention of brain damage and preservation of brain function. (Supported by DoD Defense Threat Reduction Agency 1.E0056-08-AHB-C).

Multi-component therapy of nerve agent poisoning combining aggressive atropinization, adequate doses of oximes and an anticonvulsant is insufficient in cases of poisoning with nerve agents that are either hardly reactivable (e.g., tabun), undergo extremely fast aging of inhibited human acetylcholinesterase (e.g., soman), or are persistent in patients for longer periods (e.g. VX). To overcome this situation scavenging of nerve agents using human butyrylcholinesterase is under development for prophylactic use. This approach, however, needs injection of large amounts of proteins that must be administered repeatedly; immunological problems may occur. In contrast, small molecules, e.g. cyclodextrins, appear well tolerated in man and may offer an alternative approach. In fact, several cyclodextrin derivatives are able to eliminate several nerve agents with high efficacy from human blood in vitro. Moreover, using an enzymatic test system, a beta-cyclodextrin with an attached oxime function was shown to produce enhanced detoxification rates of sarin analogues. Prophylactic administration of a functionalized cyclodextrin was able to prevent systemic signs of poisoning in guinea pigs intoxicated by cyclosarin. A further approach consists in the modulation of nicotinic acetylcholine receptors allowing them to function in spite of cholinergic overstimulation. Non-reactivating bispyridinium compounds, e.g. MB327, were not only effective in an animal model but were able to restore soman-induced neuromuscular block in rat and human respiratory muscles in vitro. Thus, MB327 could be used as the pharmaceuti-
tical lead compound for the development of more sensitive and selective structures. Further research is currently directed to improve efficacy of both cyclodextrins and non-reactivating compounds.

1602 New Approaches in the Therapy of Nerve Agent Poisoning
The in service medical countermeasure provision for nerve agent poisoning in many NATO countries is based on carbamate pre-treatment backed up by therapy consisting of an anticholinergic, an acetylcholinesterase reactivating oxime and a benzodiazepine for its anticonvulsant properties. The Defence Science & Technology Laboratory (Ditl) at Porton Down has for some years been researching possible new approaches to the therapy of nerve agent poisoning, which would reduce incapacitation significantly, reduce reliance on the need for pretreatment, would be effective as a single administered dose and which may address the management issues when nerve agent poisoning is by the percutaneous route. The presentation will summarise the research conducted to date in addressing these challenges. It will include the studies performed to licise the oxime HI-6 as the UK's preferred replacement for the oxime P25, and the concept of employing physostigmine, hyoscine and HI-6 in a single autoinjector in the absence of any form of nerve agent pretreatment. In addition, the work conducted in collaboration with US colleagues in assessing the potential of human recombinant butyrylcholinesterase alone, and in combination with standard therapy, as a post-exposure treatment for low volatility nerve agents by the percutaneous route will be discussed. Finally, the preliminary results of studies that take a broader spectrum approach that is not specific and involves reducing the effects of accumulated acetylcholine by the use of anticholinergic drugs will be presented.

1603 Incorporating In Vitro Pharmacokinetic Data and Tools into Toxicity Testing and Risk Assessments: State of the Science
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New technologies and in vitro testing approaches can be valuable additions to risk assessments that have historically relied on in vivo test results. Compared to animal testing, in vitro high-throughput screening (HTS) assays are efficient, less expensive, and provide insights into chemical mode of action. However, the relationship between the in vitro chemical concentration in the well to the chemical concentration in the target tissue or blood in vivo is dependent upon pharmacokinetic (PK) and other variables not captured in HTS assays. Incorporation of in vitro to in vivo extrapolation (IVIVE) modeling with HTS data provides a bridge to link in vitro concentrations eliciting activity out to external in vivo exposures required to achieve target tissue concentrations similar to those at which activity is observed. Since its introduction five years ago, several efforts have ensued to assess, utilize, and refine this strategy. A series of talks have been assembled that update on progress made and consider principles to guide data evaluation for reliability and utility in a risk assessment context. Correlation of in vitro estrogen receptor activity to in vivo exposures has provided promising risk predictions. Efforts to streamline clearance and PK predictions using in silico and in vitro-derived parameter estimates have laid the groundwork for HT PK modeling. Incorporation of isozyme-specific clearance data with enzyme abundance data for sensitive populations during IVIVE has quantitated PK variability. Moreover, the European Union is taking steps to introduce human chemical dosimetry with in vitro data provided an important in vivo context to screening data. With in vitro pharmacokinetic data now available for over 450 environmental chemicals, efforts to assess their value and to utilize them in predicting clearance and binding rates are now underway. Recently, measurement of chemical clearance at the isozyme level and in vitro to in vivo extrapolation modeling that incorporates differences in physiology and pharmacokinetics across sensitive populations has enabled the incorporation of population pharmacokinetic variability with in vitro HTS bioactivity data. Together, these strategies provide an in vitro context to in vitro screening data that could be applied in a tiered toxicity testing framework.

1604 A Rational Approach to Using In Vitro Data to Improve Health Risk Assessment
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Incorporation of in vitro data into the human health assessment paradigm is appealing for several reasons including their potential to describe effects in humans and to employ well controlled exposure conditions, but the limitations of in vitro systems and data must be acknowledged. When recognized early, these limitations can be considered, and suitably validated test systems and exposure conditions can be employed to develop quantitatively valuable toxicokinetic (TK) and toxicodynamic (TD) data. Just as the concentration of the toxicant in the target organ bridges the gap between TK and TD, in vitro studies can provide data useful in comparing concentrations tested in vitro to anticipated in vivo tissue concentrations, bridging the gap between dose response and exposure assessments. In vitro data have been used to refine many TK models and inform many components of modes of action (MOA). TD data have been used to develop nondefault values for uncertainty factors in risk assessments. Quantitative in vitro metabolic studies have yielded data to parameterize physiologically based pharmacokinetic models used to extrapolate dosimetry between animals and humans. However, several considerations are required to evaluate in vitro study design and results. Extrapolations of dose response requires validation of the in vitro test system and applied in vivo concentrations. Selection of the in vitro system for testing should take into consideration information on MOA, TK and the degree to which the system contains the underpinnings of normal biology (i.e., systems biology) likely to be impacted. This presentation will describe some guiding principles to evaluate the reliability and determine the usefulness of data from in vitro studies in human health risk assessment. Example applications from completed health risk assessments will be demonstrated and challenges to the additional and quantitative inclusion of in vitro data will be discussed. The views expressed herein are those of the author and do not necessarily reflect the views or policies of the U.S. EPA.

1605 In Vitro-to-In Vivo Extrapolation (IVIVE) Modeling Tools to Inform Chemical Dosimetry and Population Pharmacokinetic Variability
B. A. Wetmore, The Hamner Institutes for Health Sciences, Research Triangle Park, NC.
The development and maturation of high-throughput screening (HTS) tools have stimulated widespread discussion about their utility as a surrogate for animal testing in toxicology. Most of the effort has focused on identifying cellular pathways and processes perturbed in these in vitro screens rather than on the applicability of these data in assessing risk following in vivo exposure. Introduction of a strategy to incorporate human chemical dosimetry with in vitro data provided an important in vivo context to screening data. With in vitro pharmacokinetic data now available for over 450 environmental chemicals, efforts to assess their value and to utilize them in predicting clearance and binding rates are now underway. Recently, measurement of chemical clearance at the isozyme level and in vitro to in vivo extrapolation modeling that incorporates differences in physiology and pharmacokinetics across sensitive populations has enabled the incorporation of population pharmacokinetic variability with in vitro HTS bioactivity data. Together, these strategies provide an in vitro context to in vitro screening data that could be applied in a tiered toxicity testing framework.

1606 Using Reverse Toxicokinetic Models to Correlate In Vitro and In Vivo Estrogen Receptor Activity
W. Casey, NIEHS, Durham, NC.
High-throughput screening (HTS) assays provide an efficient way of identifying potential biological targets for chemicals, but the nominal in vitro assay concentrations may not accurately reflect the potential in vivo effects of these chemicals due to the differences in bioavailability and clearance. This presentation will describe a set of pharmacokinetic models developed for use in correlating in vitro concentrations with potential in vivo effects for Tox21 chemicals that are potentially estrogen receptor (ER) active. These models estimate the daily oral equivalent doses (OEDs) in laboratory animals and humans for Tox21 ER active chemicals that would result in a steady-state in vivo blood concentration equivalent to the in vitro POD (point of departure) values identified using HTS assays that specifically target the ER pathway. These models were built using published experimental data and quantitative structure activity relationship predictions for hepatic metabolic clearance and unbound plasma protein fraction, and were also adapted to incorporate infant physiology to include this most vulnerable human population. Using OEDs estimated from the model, Tox21 ER active chemicals were ranked, with chemicals having the lowest effective dose in these models being considered the most likely to interact with the ER in vivo, either as agonists or antagonists. The estimated oral dose for a subset of chemicals was also compared to the in vivo dose range reported to elicit ER-related effects.
High throughput screening (HTS) promises to allow prioritization of thousands of environmental chemicals with little or no in vivo information. For bioactivity identified by HTS, toxico kinetic (TK) models are essential to predict exposure thresholds below which no significant bioactivity is expected. Successful in vitro to in vivo extrapolation (IVIVE) methods have been developed for pharmaceutical compounds to determine TK from limited in vitro measurements and chemical structure-derived property predictions. These high throughput (HT) TK methods provide a less resource-intensive alternative to traditional TK model development with in vivo data. Here we evaluated the domain of applicability and assumptions of previous HTTK approaches using in vivo data and simulations. By studying 369 xenobiotics with literature HTTK data, we differentiated those xenobiotics for which HTTK approaches are likely to be sufficient, from those that may require additional data. We used in vivo data for 88, mostly pharmaceutical, chemicals to determine those chemical-specific properties (e.g., in vitro HTTK data, phys-ico-chemical descriptors, chemical structure, and predicted transporter affinities) that correlate with poor HTTK predictive ability. We then developed a HT physiologically-based TK (HTPBTK) model parameterized with HTTK data for 292 and 51 chemicals in human and rat, respectively. We used this HTPBTK model to determine that the assumptions that have been previously used for IVIVE are largely appropriate except for highly bioaccumulative compounds. Guided by sta- tistical analysis comparing in vitro predictions with in vivo data, we propose a framework for chemical TK triage, guided by confidence in HTTK model predic- tions. We believe that we can rapidly identify the chemicals that are well described by simple approaches and focus additional research on those chemicals where more complicated TK (e.g., active transport) is indicated. This abstract does not neces- sarily reflect U.S. EPA policy.

Development of EURL ECVAM Harmonized Standards for In Vitro Human Hepatic Metabolic Clearance Methods


Hepatic metabolic clearance plays a key role in the transformation and the elimina- tion of substances from the human body. In recent times various in vitro methods for human hepatic metabolic clearance/stability have been developed, employing different biological systems, monitoring either test item depletion or metabolite formation and including various test system configurations. This heterogeneity in in vitro clearance methods results in heterogeneity in in vitro clearance data. The European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM), acknowledging the importance of ADME information in the regulatory safety assessment of chemicals, is taking the initiative to develop harmonised stan- dards for in vitro human metabolic hepatic clearance methods. These standards aim to challenge the in vitro methods in relation to defined applications, to guide the end-user in using a harmonised reporting format and in providing estimations on the uncertainty of generated parameters. This presentation will describe these emerging standards and the detailed workflow used to generate data relating to the standards. The presentation will also give details on the applied use of clearance data in novel approaches to safety assessment based solely on in vitro and in silico data, and on how to define the specific information requirements for decision-making. Furthermore, details will be given on the progress during the experimental phase of the work involving the European Network of 26 highly qualified in vitro method laboratories to finalise the standards.

Where the Metal Meets the Bone

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Bone is well known for its function as a structural support; however, new evidence suggests that bone is important as the site for hematopoiesis, in regulating mineral metabolism, in controlling glucose levels, and as an internal source of toxic metal exposure. Furthermore, bone is a complex, multicellular tissue that evolves depend- ing on age and gender. As we age, particularly among women, we are more prone to osteoporosis and bone fracture. Many metals accumulate within the bone. Such an accumulation can directly alter the structural architecture of the bone itself, while it also renders the bone a primary depot of toxic metals that can result in pathological effects in a variety of tissues. This symposium invites the researchers working on metals in bone to discuss the nonoverlapping mechanisms by which diverse metals accumulate and alter the bone, the local and systemic consequences of metals in bone, and how we can better assess bone metals in humans. After a brief introduction, the first speaker will discuss tributyltin-mediated activation of nuclear receptors and ensuing effects on osteogenesis. The second speaker will discuss how tungsten accumulation in the bone enhances adipogenesis at the ex- pense of bone formation. The third speaker will address manganese accumulation in bone serving as an internal source that may contribute to manganese-induced Parkinsonian disorders. The fourth speaker will address the utility of bone lead as a reliable dosimeter for lead toxicity. Finally, the last speaker will discuss novel non- invasive technologies to define bone metal concentrations. The session will be of interest to a broader audience and, in particular, to those engaged in toxicological research related to bone diseases, osteoporosis, metal toxicities, neurotoxicology, and systems biology.

Suppression of Osteogenesis by Organotins. Is It All About PPARγ?

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Osteoporosis is the primary public health threat for the aging population. An un- derappreciated aspect of this bone health crisis is the contribution of exposure to environmental toxicants. Bone marrow multipotent mesenchymal stromal cells (BM-MSC) are critical for osteogenesis, but are also the source of adipocytes in the marrow. PPARγ is a central mediator of BM-MSC differentiation, tradition- ally thought to promote adipogenesis while suppressing osteogenesis. A growing number of environmental contaminants (e.g. phthalates, organotins, parabens, organophosphates) have been shown to activate PPARγ, increase adipogenesis, and suppress osteogenesis. Organotins bind and activate both PPARγ and RXR, opening the possibility that they may activate multiple permissive nuclear recep- tor pathways. Indeed, organotins are more efficacious at suppressing osteogenesis than inducing adipogenesis. We investigated the hypothesis that tributyltin (TBT) recruits multiple nuclear receptor pathways to modify BM-MSC differentiation. In BMS2 cells, a C57BL/6-derived BM-MSC line, TBT induces target genes of PPARγ (Fadihp4), LXR (Abacl), and RXR (Tg2m). The RXR antagonist HX531 dif- ferentially suppresses the expression of these genes, suggesting both direct receptor activation and activation through RXR. This occurs concurrently with activation of PPARγ- and LXR-dependent pathways, but without recruitment of the non-per- missive nuclear receptor, RAR. Surprisingly, female-derived BM-MSCs are signifi- cantly more sensitive to TBT-mediated suppressive effects than male-derived cells. In vivo, the effects of TBT are modified by ovariectomy, further suggesting the possibility of multiple nuclear receptor crosstalk in controlling the actions of TBT. Given its potency and ubiquitous environmental presence, TBT is an intriguing multi-nuclear receptor ligand that presents a risk to bone health.

Tungsten Accumulates in the Bone and Enhances Adipogenesis, Potentially at the Expense of Bone Formation

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Tungsten is increasingly incorporated into everything from common household goods to highly advanced technology and medical devices. However, very little is known regarding the toxicities associated with tungsten exposure. Tungsten, like many metals, is incorporated into the bone. We have found that tungsten incorporates into bone rapidly, but requires significantly longer to be released. Tungsten can be measured in the urine of humans even two years after removal of exposure, suggesting that an alternate reservoir had been created. We propose that this reservoir is the bone. We will present data showing that tungsten can alter bone homeostasis. Tungsten increases adipogenesis, but does not act as a ligand for PPARγ. In addition, we will present data on the molecular targets of tungsten and link these to downstream consequences, including changes in hematopoiesis and enhanced metastases in breast cancer models.

Manganese (Mn) Accumulation in Bone: Relationship to Mn-Induced Neurotoxicity

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Manganese exposure causes neurodegenerative disease highly resembling Parkinson’s disease. Upon exposure, most of Mn ions are intracellular distributed. A rather short half-life (11/2) of Mn in the blood compartment does not truly re-
and 2Johns Hopkins University, Baltimore, MD.

development and to build in rules and criteria to guide the design of high-efficacy/
to incorporate toxicological assessments as early as the ideology stage of product
cialize new products and chemicals. New 21st century tools now make it feasible
odologies have the potential to transform how companies develop and commer-
adverse effects. Today, rapidly evolving, next-generation safety assessment meth-
changes existed and after significant investment of time, resources, and money.
chemicals was difficult because conventional toxicological test methods did not
To fully understand the significance of bone as a target tissue of lead toxicity, as
as well as a reservoir of systemic lead burden it is important to recognize the numerous
complex chemical, kinetic, hormonal and biological factors regulating the physiol-
ogy and pathophysiology of lead in bone. This presentation will first describe the
localization and behavior of lead at atomic, molecular and inorganic biochemical
Finally, this information will be used to illuminate the strengths and limitations of
analytical approaches to define lead body burden, lead exposure history, and health
effects of lead on the skeleton.

The broad applications of metals in industry, agriculture, manufacturing, and other
fields have dramatically increased human metal exposure population over the last
several decades. Many metals accumulate in bone. Metal concentration in human
bone provides unique information regarding long-term chronic metal exposure.
X-ray fluorescence (XRF) and neutron activation analysis (NAA) are two powerful
noninvasive techniques for \textit{in vivo} quantification of metals. The speaker will discuss
several systems available for \textit{in vivo} bone metal quantification, and the development
and validation of novel noninvasive \textit{in vivo} technologies to quantify metals in human
bone. Specifically, she will talk about a transportable NAA system for man-
ganese and aluminum and a portable XRF system for lead in human bone \textit{in vivo}.

Historically, early identification and characterization of adverse effects of industrial
chemicals was difficult because conventional toxicological test methods did not
meet R&D needs (e.g., methods that are rapid, relatively inexpensive, and amena-
to small amounts of test material). Consequently, undesirable toxicological
effects were identified closer to commercialization, when few options for design
changes existed and after significant investment of time, resources, and money.
For example a two-generation reproduction study costs more than $500,000, uses
more than 3,000 rats, and takes 15 months to complete. Further time, money,
and resources are consumed in efforts to “defend and save” products identified to have
adverse effects. Today, rapidly evolving, next-generation safety assessment meth-
odologies have the potential to transform how companies develop and commer-
cialize new products and chemicals. New 21st century tools now make it feasible
to incorporate toxicological assessments as early as the ideology stage of product
development and to build in rules and criteria to guide the design of high-efficacy/
low-toxicity compounds. Toxicology as a tool for innovation affords benefits for
the company developing new products as well as for society. For companies,
the earlier candidates with undesirable effects are identified and eliminated, the sooner
finite resources can be redirected to those candidates with the highest likelihood of
being a successful, sustainable alternative. The input of toxicologists early can
inform test strategies and limit complex, costly, and lengthy studies to those few
promising candidates, reducing postmarket defense of products targeted for future
deselection. For society, safer, healthier alternatives are commercialized and the risk
of unknown health and environmental effects surging after product launch are
reduced. Using 21st century toxicology methods as a preventive strategy to design
out undesired human health and environmental effects offers benefits to compa-
nies and society over the current paradigm. This session will provide a forum for
connectedness among scientists working in complementary fields to discover com-
mon ground in the quest for safer chemicals by adopting an innovative, preven-
tion-based framework to product safety assessment through strategic application of
new 21st century methodologies. Case studies will be used to illustrate how to build
successful strategies into product development.
The need for chemists to design chemicals that are not only useful but of minimal hazard was recognized nearly a century ago. Regulations pertaining to the marketing of drug substances and pesticides promulgated decades ago have forced changes in the way chemists are trained and approach the design of such chemicals. These changes have led to the considerable progress that has occurred in the past 60 years in the development of safe and efficacious drug substances and pesticide chemicals. Progress in the design of safer commercial chemicals, however, has been comparatively slow, despite the many advances in toxicological research, and the elucidation of mechanisms of toxicity and relationships between structure or physicochemical properties of commercial chemicals with toxicity, environmental fate, and global hazard. There are two inextricably linked reasons for the comparatively slow progress in the design of safer commercial chemicals. First and foremost is the lack of regulations that require demonstration of safety as a prerequisite for marketing approval. The other reason (a consequence of the first) is that organic chemists, the principle designers of commercial chemicals, receive little to no formal training in biochemistry, toxicology, or the environmental sciences, or in relationships between chemical structure and physicochemical properties with toxicity, environmental fate, and global hazard. A concerted effort must be made by academic institutions, industry and government authorities to address this largely unrecognized problem. This presentation will illustrate how many of the well-established approaches and considerations used by medicinal chemists to design safer and efficacious drug substances can be used to design safer and efficacious commercial chemicals. Recommendations will be put forth regarding advancement of the paradigm as an important component of sustainable development and the changes therewith that need to take place in academic institutions at the undergraduate and graduate levels.

W 1618 On the Design of Safer Commercial Chemicals: Moving Forward
S. DeVito, Office of Environmental Information, US Environmental Protection Agency, Washington, DC.

W 1619 21st Century Toxicology: Tools for Innovation and Safer Chemical Design
T. Hartung, CAAT; Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD.

W 1620 Innovative Toxicology: Matching Tools to Product Development Stage to Assess the Toxicity and Environmental Impact of New Products
R. Deskin1 and T. Petry2, 1Deskin Associates LLC, Fort Myers, FL and 2ToxMinds BVBA, Brussels, Belgium.

W 1621 Current Understanding of Immune-Mediated Adverse Drug Reactions
A. Sirakí1, A. Harrill1 and L. G. Metushi1, 1La Jolla Institute for Allergy and Immunology, San Diego, CA, 2Pharmaceutical Sciences, University of Alberta, Edmonton, AB, Canada and 3Department of Environmental and Occupational Health, University of Arkansas, Fayetteville, AR.

Immune-mediated adverse drug reactions (IM-ADRs) represent a significant incidence of patient morbidity and mortality, and they significantly add to the cost of drug development. The most affected organs include the skin, liver, and blood, and such organs are known to initiate and shape immune responses. Despite major recent attempts to investigate the mechanism behind IM-ADRs, our understanding of such reactions remains superficial. The role of drugs and how they are able to cause organ damage, whether by inducing or altering an immune response, is not well understood. At this workshop, research highlighting different mechanisms or how drugs initiate an immune response that leads to an IM-ADR will be discussed. This includes the formation of covalent adducts, the induction of danger signals to overcome immune tolerance, the “altered repertoire” hypothesis based on which drugs change the repertoire of self-peptides presented by HLA molecules, and the heterologous immunity model, which provides an explanation for the low positive predictive value of most HLA associations of drug hypersensitivity. The presentations will highlight new advancements in the technology for early detection of IM-ADRs, in vitro assays, and the use of valid animal models.

W 1622 Drug-Induced Events That Initiate an Adaptive Immune Attack on the Liver
P. B. Watkins, Hammer-UNC Institute for Drug Safety Sciences, Research Triangle Park, NC.

Idiosyncratic drug-induced liver injury (DILI) appears often result from an adaptive immune attack on the liver. Data supporting this conclusion include enrichment of specific HLA alleles and identification of drug reactive T-cells among patients who have experienced some forms of DILI. To date HLA associations have accounted for only a small fraction of the total risk of DILI and hence HLA genotyping has not yet been employed to manage DILI risk for any drug. It seems reasonable to assume that the missing risk factors include a series of as yet undiscovered events that are necessary to initiate an adaptive immune attack on the liver and that identification of these events would inform development of improved preclinical safety testing. Because HLA associations have been found with mild DILI events in clinical trials, and drug-reactive T-cells have been found in blood of some patients before they experience any biochemical sign of liver injury, it seems unlikely that hepatocyte death is necessary to trigger an adaptive immune response. We have observed that treatment of cultured hepatocytes with drugs known to cause DILI, such as isoniazid, results in increased release of microvesicles at exposures well below those that result in overt toxicity. Microvesicles have been shown to contain damage associated molecular patterns (DAMPS) and drug-protein adducts that could initiate an adaptive immune response. Identification of the molecular events that underlie release of microvesicles in response to drugs capable of causing DILI is proceeding along several paths, including toxicogenomic and high content analysis of cultured hepatocytes and hypothesis directed interrogation of the growing genetic database from the U.S. Drug-Induced Liver Network. Insight into the early hepatocyte events that are necessary to result in an adaptive immune response should not only inform development of improved preclinical
safety testing of new drug candidates but also permit identification of non-HLA risk factors that could improve personalized approaches to manage the risk of DILI from drugs already in the clinic.

1623 The Use of Animal Models in Investigating the Mechanism of Idiosyncratic Drug-Induced Hepatotoxicity
I. G. Metushi. La Jolla Institute for Allergy and Immunology, San Diego, CA.

Drug-induced liver injury (DILI) is one of the most common reasons for drug withdrawal; however, its mechanism remains unknown. For some drugs such as isoniazid (INH), the mechanism of liver injury was classified as "metabolic idio-
syncrasy" which implies the lack of an immune residue. This has been a topic of studies done at the National Institute of Health that involved acute treatments of rats with high doses of INH. We have re-assessed the evidence and identified a novel reactive metabolite which is formed by the bioactivation of INH itself. Our data suggests that covalent binding from bioactivation of INH is responsible for liver injury and that this leads to an adaptive immune response. We were able to detect anti-INH antibodies in patients with liver failure due to INH but not antibodies were detected in patients with mild liver injury. Patients with mild liver injury had an increase in T cells producing IL-17 and IL-10. Treatment of mice with INH did not produce liver injury with characteristics similar to that in humans and it is possible that this is due to the fact that immune tolerance prevents severe hepato-
toxicity. We have hypothesized that it is when this immune tolerance fails that more severe liver injury occurs. We have tested this hypothesis in our recent animal model where treatment of mice with amiodaquine (AQ) leads to a mild increase in ALT with a delayed onset. Covalent binding of AQ in the liver appears to be im-
portant to initiate an immune response but it did not correlate with hepatotoxicity. However the combined treatment of AQ and anti-PD1/CTLA4 antibody resulted in more severe liver injury with characteristics of piecemeal necrosis. Conclusion: These data illustrates the relationship between reactive metabolite formation and immune responses in the pathogenesis of liver injury and outlines the usefulness of animal models in the understanding of the mechanism of DILI.

1624 Studies of the Role of Innate and Adaptive Immune Responses in Drug-Induced Liver Injury in Mice
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Drug-induced liver injury (DILI) causes significant patients mortality and morbidity, and represents the most frequent cause of safety-related regulatory actions in the past 50 years. Clinical characteristics of DILI suggest the involvement of both the innate and adaptive immune responses in the pathogenesis of these reactions. We have developed a mouse model of halothane-induced liver injury (HILI) in which the innate immune cells, such as NKT cells, neutrophils and eosinophils are in-
volved. The gender-, age-, and strain-dependent susceptibility factors in this mouse model are consistent with the human risk factors in developing HILI. Drug-specific adaptive immune responses are thought to play an important role in patients with HILI. We demonstrated that immunization of mice with a toll-like receptor (TLR) agonist (LPS, polyIC or CpG) in combination with mouse serum albumin adduct of trifluoroacetyl chloride (TFA-MSA) induce TFA-specific T cell responses in the spleen and the liver. However, the pathological role of these drug-specific T cells remain to be determined, as the immunization did not potentiate hepatotoxicity caused by the subsequent administration of halothane. This may be due to the suppression of CYP2E1 by inflammatory mediators produced in response to TLR stimulation. We found that immunizing mice with drug-protein adducts can in-
duce drug-specific T cell responses in the liver; however, the pathological role of these T cells remain to be determined. These studies offer insightful mechanistic understanding of such reactions with the aim of predicting and preventing DILI from occurring in the future.

1625 Drug Hypersensitivity Caused by Alteration of the MHC-Presented Self-Peptide Repertoire
B. Peters. La Jolla Institute for Allergy and Immunology, San Diego, CA. Sponsor: I. G. Metushi.

Generic association studies have revealed strong linkages between drug hypersensitiv-
ity reactions to several drugs and specific human leukocyte antigen (HLA) alleles. One of the strongest such genetic associations found has been for the antiviral drug abacavir, which causes severe adverse reactions exclusively in patients expressing the HLA molecular variant B*57:01. Abacavir adverse reactions are driven by drug-specific activation of cytokine-producing, cytotoxic CD8+ T cells that required HLA-B*57:01 molecules for their function. Recent studies by our group and others have revealed the mechanism leading to the generation of these patho-
genic T cells: abacavir can bind within the F pocket of the peptide-binding groove of HLA-B*57:01, thereby altering its specificity. This provides an explanation for HLA-linked idiosyncratic adverse drug reactions, namely that drugs can alter the repertoire of self-peptides presented to T cells, thus causing the equivalent of an allreactive T-cell response. Indeed, we identified specific self-peptides that are pre-
sented only in the presence of abacavir and that were recognized by T cells of hyper-
sensitive patients. This "altered repertoire" hypothesis differs from the classical hapten hypothesis in a way that the peptides are not modified by a drug but rather their affinity is altered in the presence of the drug in an HLA specific manner. We will discuss the utility of the assays that we have established to screen for HLA-drug interactions in vitro, their applications to compounds other than abacavir, and their potential applicability and limitations to guide the development of safer drugs.

1626 Current Science and Translational Opportunities in the Prediction and Prevention of Immunologically Mediated Adverse Drug Reactions
E. Phillips. Department of Medicine, Vanderbilt University Medical Center, Nashville, TN. Sponsor: I. G. Metushi.

Many severe immunologically-mediated adverse drug reactions (ADRs) have been recently shown to be specifically HLA Class I restricted. Abacavir hypersensitivity reaction (ABC HSR) has been strongly associated with HLA-B*57:01, and level 1a evidence now supports the routine use of HLA-B*57:01 testing as a screening strat-
egy to predict and prevent ABC HSR. Other examples of severe HLA-mediated drug hypersensitivity include the association of HLA-B*15:02 and carbamaze-
pine-associated Stevens-Johnson syndrome/toxic epidermal necrolysis (SJS/TEN), HLA-B*58:01 and allopurinol associated SJS and SJS/TEN and HLA-B*13:01 in association with dapsone HSR. Although clinical, epidemiological and in vitro scientific studies have defined the specificity of the relevant HLA class I alleles for these ADRs they do not explain the low positive predictive value for the HLA allele in question for clinical disease. For instance only 55% of HLA-B*57:01 positive patients experience ABC HSR and only 3% of HLA-B*15:02 positive and HLA-B*58:01 positive individuals develop carbamazepine SJS/TEN and al-
llopurinol SJS/TEN/HSR respectively. To explain this, we propose a heterologous immune model in which chronic prevalent viruses such as human herpes viruses sensitize and maintain a T-cell response that has the potential to cross-react with cells presenting the neo-antigen following drug ingestion. T-cell receptor repertoire usage may be restricted and oligoclonal as for carbamazepine induced SJS/TEN. For ABC, the heterologous immune model is supported by the rapidity of onset of the clinical syndrome (<5 days in 25% of patients) and the observation that ABC reactive memory CD8+ T-cells can be detected, without ABC driven expansion, in healthy HLA-B*57:01+ donors. In reference to this model, the potential immuno-
pathogenetic basis and translational opportunities of different HLA-restricted drug HSR will be discussed.

1627 In Vitro Microphysiological Systems—Developing Confidence in Predictive Ability
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Mechanically active "organ-on-a-chip" microdevices that reconstitute tissue-tissue interfaces critical to organ function can expand the capabilities of cell culture models and provide low-cost and more informative alternatives to animal toxicology studies. With simplified designs and careful choice of biocompatible device materi-
als, they can be useful for high-content analysis and screening of cellular responses to drugs, chemicals, particulates, toxins, pathogens, or other environment stimuli relevant to pharmaceutical, cosmetic, and environmental applications. In 2011, President Obama announced that the National Institutes of Health will collaborate with the Defense Advanced Research Projects Agency (DARPA), and the US Food and Drug Administration to develop a chip to screen for safe and effective drugs far more swiftly and efficiently than current methods, and before they are tested in hu-
mans. It was clear to both US FDA and NIH that these models have the potential for more accurate modeling of physiological situations to answer fundamental basic science questions. As the science of in vitro microphysiological systems develops, it is also imperative that regulators communicate what they need to demonstrate confidence in the predictive capacity of these new and promising models. This workshop presents a pathway to full acceptance and use by first developing con-
fidence in each of the different integral parts of the model and then combining them for a "context-of-use" evaluation of overall predictive ability to answer critical regulatory questions.
1628 Human Organs on Chips
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Development of safe and effective drugs is currently hampered by the poor predictive power of existing preclinical animal models that often lead to failure of drug compounds late in their development. Although considerable advances have been made in the development of culture models, cells commonly fail to maintain high levels of expression of tissue-specific differentiated functions when maintained in vitro. In this presentation, I will describe our work we have been carrying out at the Wyss Institute for Biologically Inspired Engineering at Harvard, which is focused on engineering of human ‘Organs-on-Chips’: microfluidic devices lined by living human cells created with microchip fabrication techniques that recapitulate organ-level functions as a way to replace animal testing for drug development and to create in vitro human disease models. For example, the lung-on-a-chip consists of microchannels lined by closely apposed layers of human pulmonary epithelial and endothelial cells that experience air and fluid flow, as well as cyclic mechanical strain to mimic normal breathing motions. These biomimetic devices provide a window on human physiology as they enable real-time, high-resolution microscopic imaging as well as analysis of biochemical, genetic and metabolic activities of living cells when they are positioned within the context of functional tissue and organ units. I will review recent advances we have made in development of multiple organ chips, including human lung, gut, kidney and bone marrow chips, as well as on-chip models of human diseases, including pulmonary edema. In addition, I will describe our ongoing efforts to develop more than 10 different organ chips, to integrate them into a ‘human body on chips’, and to engineer an automated instrument for real-time analysis of cellular responses to pharmaceuticals, toxins and other chemicals.

1629 Induced Pluripotent Stem Cells and Personalized Medicine: Are We Moving toward a “Patient on a Chip”?
C. Svendsen, Cedars Sinai Medical Center, Los Angeles, CA. Sponsor: A. Bahinski.

Recent advances in stem cell technology now make it possible to reprogram adult human tissues back in time to a pluripotent state from any patient or donor. From these cells we can now develop tissues with specific genotypes and/or disease phenotypes. Although, these capabilities present unprecedented opportunities to create biomimetic devices with the potential to capture the dynamics of drug effects in the human body, challenges still remain in differentiating pluripotent cells to span the full developmental spectrum. This presentation will review the state of the art with respect to ability of stem and iPS-derived tissues to mimic native organ phenotypes, utilization in microphysiological devices and potential clinical translation and relevance. Our prediction is that in the future, organs on a chip will provide an “Avatar” where a patient’s own cells can be tested for personalized drug therapies.

1630 Characterizing and Validating Biological and Physiological Relevance of an In Vitro Microphysiological System
J. P. Winko, Vanderbilt Institute for Integrative Biosystems Research and Education, Vanderbilt University, Nashville, TN. Sponsor: A. Bahinski.

An engineering challenge for in vitro microsystems is to develop and assemble 3D tissue constructs that are physiologically relevant, reproducible, and compatible with high-content screening platforms and include dynamic readouts of metabolic activity, molecular reporting of gene expression, and proteomic, metabolomic, or epigenomic analyses. Numerous groups are creating various organ constructs, some using human cells. Ideally, each organ module in an in vitro microsystem should provide spatial and temporal control of the cellular microenvironment, while enabling continuous monitoring (sensing), probing (direct in-cell measurements), and sampling (testing and continuous data collection and analysis). Individual microorgan modules can then be interconnected to create an interacting microphysiological system that should recapitulate the key aspects of the complex physiologic and metabolic function of the human body, including vascularization and innervation, and hormonal, humoral, and immunologic signaling. This integration presents challenges that include the design of organ constructs with physiologically realistic relative scaling, the need for a universal perfusion media, and the requirement that the perfused media volume be scaled to that of the organs, lest metabolites and secreted signals be diluted below physiological levels. Once the organ constructs are properly instrumented and interconnected, we need controls to prevent the system from oscillating or undergoing self-induced organ failure. One must account for missing organs and validate that the system is physiologically realistic. The elegance of the approach is evident – microphysiological systems will close the circle of biology, which began with the studies of whole animals and isolated organs and then cells and molecules that led to our reductionist understanding of molecular and systems biology. We can now use this knowledge and tissue engineering to construct in vitro interacting organ systems models to better understand physiological regulation and systems toxicology.

1631 Defining an Appropriate Testing Paradigm for In Vitro Microphysiological Systems
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Microphysiological platforms seeded with human cells have been proposed as in vitro models with the ability to predict human response under specified conditions of use. In vitro tools provide important contributions to the protection of human safety. Such assays capitalize on knowledge of specific mechanisms and the involved proteins. Cultured cells are often poor surrogates of in vivo response due in part to the culture conditions to which they are subjected and to their altered cell surface characteristics, cell-cell or cell-matrix interaction-dependent signaling, and loss of functional response. However, organotypic cultures under microphysiological conditions may better recapitulate in vivo functional response based on the similarity of cell signaling, gene expression, and receptor response patterns. The key to the selection of a model and its appropriate use is to define the question to be answered and how the model is to be used. Qualification of in vitro approaches is necessary at both the biological and technical level for cells, tissues, and the integrated system. The target, the pathway, and the functional response must be intact and detectable for any utility to be ascribed. Selected questions that should be addressed include (1) Do individual organ models respond to test compounds with the expected organ-specific effects? AND (2) Do linked organ system models respond to test compounds with the expected systemic effects? The selection of test compounds should consider: (1) Individual and linked organ functions; (2) Direct organ toxicities and dependence on biomarkers; and (3) Functional read-outs including biomarkers and health outcomes of interest. Compound sets should test the predictivity, precision, dynamic range, and consistency of the response at the cell, organ, and system level in a tiered manner through interrogation of multiple modes of action and diverse chemistries or modalities. Microphysiological platform qualification for regulatory purposes requires comparison to our current testing systems to understand their utility and limits under specified conditions of use.

1632 Determining the Predictive Capability of In Vitro Microphysiological Systems to Answer Critical Regulatory Questions
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Regulators must assure that their toxicology and risk assessment toolbox keeps pace with advances in science and technology. But regulators must also determine how much evidence is sufficient to determine that a new tool is qualified to make safety decisions, many of which potentially affect millions of consumers. This delicate balance between safety and avoiding restrictions on valuable products is a continuous, unique, and demanding challenge for regulatory agencies. Developing confidence in the predictive ability of in vitro microphysiological systems begins with identifying the major problems facing regulators that are creating critical regulatory gaps. Assessment of whether these gaps can be addressed with these new tools, and the consequences of an incorrect or incomplete answer will determine the level of confidence in each the integral parts of an in vitro microphysiological system. Combining this information together for a "context of use" evaluation of its overall predictive ability to answer critical regulatory questions is critical to the full acceptance and use of such approaches and can only be developed in a transparent and scientifically defensible manner.

1633 CAR Activation As the Mode of Action for Nitratryn-Induced Mouse Liver Tumors
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The constitutive androstane receptor (CAR) regulates cellular responses following exposure to genotoxic and non-genotoxic xenobiotics. We have previously demonstrated that nitratryn exposure in mice induces CAR activation, hepatocellular proliferation, and liver tumor formation, indicating a non-genotoxic mode of action (MoA). These studies demonstrated increases in the CAR-response gene Cyp2b10, but no effects on the expression of AhR, PXR, or PPAR response genes. Nitratryn exposure also irreversibly inhibits Cyp2b10-mediated PROD activity via suicide inhibition. However, further research was necessitated to elucidate if CAR activa-
tion is necessary for nitrapyrin-induced hepatocellular proliferation. In this study, we performed CAR knockout mouse experiments (C57BL/6NTac mice) to address the hypothesis that the nitrapyrin MoA is mediated by CAR. As prior toxicity studies were conducted in B6C3F1 mice, we first compared strain responses to nitrapyrin exposure at a carcinogetic dose (250 mg/kg/day for 4 days) in B6C3F1 and C57BL6 mice. Strain responses were similar, including increases in CyP2b10 expression (370.0- and 240.7-fold increases in B6C3F1 and C57BL6, respectively), increases in relative liver weight (26.5% in B6C3F1 and 28.4% in C57BL6) and increases in hepatocellular proliferation. The similar strain responsiveness justified utilizing the C57BL6NTac strain for the CAR-KO study. CAR-KO and WT mice were exposed to 0 or 250 mg/kg/day nitrapyrin for 4 days, and the hepatocellular proliferation index was determined. Nitrapyrin exposure in WT mice resulted in an increase in panlobular hepatocellular proliferation (1.6-fold), whereas no increase in proliferation was observed in CAR-KO mice, providing compelling evidence of the role of CAR in nitrapyrin-induced liver tumor formation. The results of this study support the conclusion that nitrapyrin-induced mouse liver tumors are mediated by CAR activation and are not relevant for human health risk assessment.

**PL 1634** Mouse Liver Tumors Induced by Prochloraz Have a CAR-Like Mode of Action and Are Not Relevant to Humans

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Prochloraz is an imidazole fungicide registered in Europe. It caused dose-related increased incidences of mouse liver tumors in male and female CD-1 mice. Prochloraz was found to be non-genotoxic and a threshold was shown for liver cell proliferation. To elucidate the mode of action, three mechanistic studies have been conducted. Study 1: 14-day study in male and female C57BL/6J mice using two tumorigenic doses investigating mRNA expression (CyP2b10, CyP3a11, CyP1a1 and CyP4a14) and Western blot analysis, liver enzyme induction (total cytochrome P450, EROD, BROD, PROD, BQ, LAH) and histopathology. Study 2: Study in male C57BL/6J mice at the two tumorigenic doses, investigating the kinetics of the S-phase response (7 day osmotic pumps BrdU) and the time of peak hepatic cell proliferation. Furthermore, liver histopathology, key liver enzymes, mRNA and proteins were investigated. Study 3: 7-day study in male wild-type C57BL/6J mice. PXR knockout/CAR knockout mice and transgenic, humanized hPXR/hCAR mice using the tumorigenic LOAEL of Prochloraz. Liver enzymes, mRNA and protein, histopathology and cell proliferation were investigated. Results: Prochloraz at and above the carcinogenic LOAEL induced hepatocellular hypertrophy and showed increased transient cell proliferation in C57BL/6J mice but not in PXR KO/CAR KO or humanized hPXR/hCAR mice. Prochloraz primarily induced Cyp2b10 and Cyp3a11 hepatic enzyme activity and mRNA expression. These effects were seen to a lesser extent in hCAR/hPXR mice, but were not observed in PXR KO/CAR KO mice. Conclusion: Prochloraz induces mouse liver tumours via a CAR-dependent mode of action. Enhanced hepatocellular proliferation is the key event in CAR activator (e.g. phenobarbital) – induced non-genotoxic hepatocellular carcinogenesis. Hence, in the absence of Prochloraz-stimulated cell proliferation in the receptor-humanized mice, Prochloraz-induced mouse liver tumors are mediated by CAR activation and are not relevant for human health risk assessment.

**PL 1635** Induction of Endogenous Retroelements As a Mechanism for Mouse-Specific Drug-Induced Carcinogenicity

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A number of chemical compounds have been shown to induce liver tumors in mice but not in other species. While several mechanisms for this species-specific tumorigenicity have been proposed, no definitive mechanism has been established. Here we examine the effects of the nongenotoxic rodent hepatic carcinogen, Wy-14,643, in male mice from a high liver tumor susceptible strain (C3H/HeJ), and from a low tumor susceptible strain (C57BL6). We showed that Wy-14643, a PPARα activator induced widespread increases in the expression of endogenous retroelements, namely LTRs, and LINE elements in both strains. We propose that the previously reported >100X activity of retroelements in mice drives mouse-specific tumorigenicity. We further demonstrate that many genes involved in the innate response including the retroviral restriction factors, Trim5, Trim12a, and Bst2 (Tetherin) are elevated 7X, 65X, & 5X (p-val< 0.01) respectively in the less susceptible C57BL6 strain. We further show that the apoptosis-inducing genes, Cidea, Cidea and Casp8, while elevated with treatment in both strains, are significantly higher in the resistant C57Bl/6 strain (34X vs 24X, 163X vs 156X, & 2.1X vs 1.3X). We suggest that the TLR4 mutation in C3H/HeJ results in the impairment of the normal innate / host restriction factor response and hence to this strain’s increased tumor sensitivity.

**PL 1636** Effect of Furan on Transcriptomic and Gene-Specific DNA Methylation Changes in the Livers of Fisher 344 Rats in a Two-Year Carcino genesis Study

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The carcinogenic effect of furan, a potent hepatotoxicant and rodent liver carcinogen, has been attributed to genotoxic and non-genotoxic, including epigenetic, changes in the liver; however, the mechanisms of the furan liver tumorogenicity are still unclear. The goal of the present study was to investigate the role of transcriptomic and epigenetic events in the development of hepatic lesions in Fisher (F344) rats induced by furan treatment in a classic two-year rodent tumorigenicity bioassay. Male F344 rats were distributed randomly into control and experimental groups. Rats from the experimental groups were treated by gavage 5 days/week with either 0.92 mg/kg bw or 2.0 mg/kg bw furan in corn oil for 104 weeks. Rats from control group received only corn oil. High-throughput whole genome microarray analyses demonstrated distinctive dose-dependent alterations in gene expression in the furan-induced liver lesions. A total of 1784 and 2292 genes was found to be differentially expressed in the furan-induced liver lesions in rats treated with 0.92 and 2.0 mg/kg bw, respectively, as compared to normal liver tissue. Approximately 30%, or 672 of differentially expressed genes, were common to both treatment groups. In silico analysis of the common differentially expressed genes revealed the presence of CpG island in 34%, or 233 of the genes, suggesting that they may be epigenetically regulated. Promoter methylation analysis of individual differentially expressed genes, including Tcf21, Prx1, Foxe1, Irf7, Cyp1a2, Edwak, Kcnpl, Cmg2, Atf2, Tfr2, and Tgfβ1, demonstrated dose-dependent gene-specific methylation changes that highly correlated with gene expression. Our findings illustrate that gene-specific DNA methylation changes have functional consequences and may contribute to the development of furan-induced pathologic liver lesions.

**PL 1637** Evaluation of a Potential Mechanism for Formaldehyde-Induced Leukemia in p53-Haplosufficient Mice


Formaldehyde (FA) inhalation is linked to myeloid leukemia in humans, although the mechanism of this effect is unclear. DNA adduct formation is recognized as a key mechanistic event in FA-induced nasal cancer; however, inhaled FA does not form detectable levels of DNA adducts at sites other than the nasal cavity. It was hypothesized that FA may cause leukemia by a mechanism not involving DNA adduct formation. Inhaled FA could cause significant genetic damage to stem cells in the nasal epithelium or circulating in local blood vessels. These damaged stem cells could reach the general circulation, home to tissues that support the hematopoietic niche, undergo lodgement and become leukemic stem cells. We tested this hypothesis by exposing male B6.129-Tp53tm1Brd and male C58.B129F1-Tp53tm1Brd mice to 7.5 or 15 ppm FA (25strain/concentration) 6d/5w for 8w and then holding mice without exposure until 50 weeks of age. At necropsy, blood was collected for hematology, bone marrow brush smears were prepared, and nose, larynx, lung, liver, spleen, thymus, lymph nodes, kidney, and gross lesions were collected and evaluated microscopically. A number of control and FA-exposed mice of both strains died early or were euthanized in moribund condition. Most had reduced body weights and visible tissue masses (primarily sarcomas). The primary exposure-related finding in both strains was minimal to mild squamous metaplasia of the respiratory epithelium of the nose. The incidence of this lesion increased with exposure concentration in C58.B129F1-Tp53tm1Brd (control 0.21%, 7.5 ppm 14/15, 15 ppm 21/22) and B6.129-Tp53tm1Brd mice (control 0.22%, 7.5 ppm 13/27, 15 ppm 17/26). Neoplastic lesions were present in mice in controls and all FA dose groups of both strains. There was no evidence of leukemia in p53-haplosufficient mice exposed to FA for 8w based on hematology and histopathology. Under the conditions of this study, FA inhalation did not cause leukemia or lymphohematopoietic neoplasia in these genetically predisposed mice.
Toxicology feeding studies of mineral oil hydrocarbons (MHC), specifically on white oils and waxes within the carbon number range C20-C28, results in species-specific epithelial granulomas in the liver of F-344 rats but not in other rat strains, dogs or humans. While MHC has been detected, and some pathological effects have been shown to occur in other organs/tissues of F-344 rats and other rat strains/species, it is generally accepted that the effect of toxicological concern is species-specific inflammatory liver granuloma. As oil retention and other MHC-related non-toxic pathological changes in the liver are observed in humans, some have hypothesized that the potential for oil accumulation over a lifetime, through dietary sources, may predispose humans to similar liver effects as observed in F-344 rats. To address this concern, a mode of action for MHC-induced epithelial granuloma in the F-344 rat model was being developed, utilizing the mode of action/human relevance framework (MoA/HRF). The key events for the development of liver epithelial granulomas were identified as increased MHC intestinal absorption, preferential tissue accumulation, inflammatory cell infiltration/necrosis/fibrosis and ultimately formation of necrotic granulomas encased by infiltrating inflammatory lymphocytes. The hypothesized MoA was evaluated using the modified Bradford Hill considerations for causality and was considered to be established in the F-344 rodent model to the exclusion of all other possible MoAs. While MHC (within the carbon number range C20-C28) induces epithelial granulomas in F-344 rats, key species differences in the rate of intestinal absorption and level of MHC tissue accumulation where identified. Overall, the MoA was not considered to be qualitatively plausible in humans, consistent with data showing no evidence for the formation of epithelial granulomas even in cases of massive ingestion of MHCs.

This project explored the impact of the pharmaceutical industry’s contribution to published papers relevant to the 3Rs (reduction, refinement and replacement of the use of animals in research) in the decade between 2002 and 2012, a quarter of a century after the introduction of the Animals (Scientific Procedures) Act (ASPRA). Specifically, the PubMed database was used to search for all papers with an explicit 3Rs objective that were published during the years 2002, 2007 and 2012. Overall, 433 papers with a 3Rs objective were identified in the 3 time periods analysed; there was little change in the total number of published papers in the first two time periods tested (2002, 2007) but this was followed by a substantial (55%) rise in the latter time period (2012). Within this total of 433 papers, the number of published 3Rs papers with industry involvement increased from 20 (2002) through 30 (2007) to 39 (2012). Additionally, the proportion of 3Rs papers involving academia and industry collaboration increased from 60% in 2002 to 61.5% in 2012; the number of multiple affiliation papers also rose during the time period. Other notable trends were the increase in contract research organisation (CRO) involvement in 3Rs research and a slight increase (10%) in the latter time period in those papers describing and presenting original data rather than review/discussion papers. In summary, the reduction, refinement and replacement of animal testing in pharmaceutical drug development depends upon continued and increased collaboration in working towards a common goal of better science for better medicines. The data reported herein clearly demonstrates an increased contribution by the pharmaceutical industry to the 3Rs objective along with increasing collaborative efforts between industry and academic institutions.

Improved bioanalytical sensitivity and concomitant decreased sample volume requirements provide an opportunity to reconsider how toxicokinetic (TK) data is collected in rat toxicity studies. Often, satellite groups of rats are designated to separate proportional effects of TK blood collection from the primary toxicity evaluation. We demonstrate a paradigm here that TK samples can be collected from toxicity groups without impacting toxicity assessment. Furthermore, we targeted higher blood volumes (ie 200 vs 10 ul) than typical for microsampling to allow multiple analyses (eg for incurred sample reanalysis or repeated analyses). To ensure TK sampling would not negatively affect the toxicity assessment, we compared tolerability and clinical pathological, haematology and clinical chemistry endpoints, and addressed technical feasibility and logistical challenges using 250 g SD rats. No effect on tolerability or exposure assessment was apparent in pilot studies. There were minor effects erythrocyte parameters depending upon study design and duration;
however, these do not affect study interpretation nor outweigh the benefit of using 33% fewer animals per study. Thus, elimination or reduction of satellite groups can reliably be utilized and has been implemented on pilot and GLP rat studies. Our "base case" pilot and GLP toxicity study designs utilize sparse TK sampling from sample toxicity group rats (1-2 samples/rat). Alternate designs with satellite animals may still be warranted based on study objectives (e.g. biomarkers), tolerability, or smaller rat strains; however we propose these as exceptions rather than standard practice and with a focus to use the fewest animals possible. These efforts maintain a commitment to the 3Rs (replacement, reduction, refinement) while maintaining high-quality TK evaluations on toxicity studies.

1643 Reducing Animal Numbers on Regulatory Toxicology Studies Using Microsampling and Sample-Sparing Techniques

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We have reported that microsampling techniques (<100µL blood) and enhanced analytical sensitivity provide scope for reducing numbers of satellite animals on rodent toxicity studies. Conventional blood sampling (typically 200-500 µL blood) does not permit collection of full toxicokinetic (TK) profiles from individual rodents and necessitates use of a composite sampling regimen in large numbers of satellites. Individual plasma concentrations from rats dosed with a small molecule NCE (not identifiable for commercial reasons) and sampled by conventional methodology. Direct comparison of pharmacokinetic data obtained by CMS with that from conventional sampling gave no statistically-significant differences for the great majority of comparisons. Refinements to procedures have enhanced sampling success and sample quality. We are now using CMS to obtain individual serial TK profiles from reduced numbers of satellite animals on GLP studies but there is further scientific and ethical value to be gained by TK sampling of main study animals so that exposure can be correlated with pharmacodynamic and/or toxic effects; this requires a reduction in the sampling burden for other regulatory datasets. Dilution of standard rodent haematology and blood chemistry samples provides data for most parameters that is comparable with undiluted samples but is unsuitable for some analytes. Use of microsampling reduces the numbers of satellite animals on rodent studies and provides a notable refinement of the blood sampling procedure by reducing restraint time, hot box exposure and total blood sample burden. It provides scope for improved understanding of how variation in exposure affects the toxic response in rodent species.

1644 What Constitutes Scientific Justification for Inclusion of Recovery assessment in Preclinical Studies Supporting First Time in Man (FTIM)?

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Recovery groups are included more routinely than not in the pivotal Good Laboratory Practice (GLP) studies supporting first time in man (FTIM), probably following individual company default positions. Previously, we have reported the profile of target organ toxicities (primarily defined as compound-related histopathological changes) in the rodent and non-rodent studies for 77 AstraZeneca candidate drugs (CDs) across a range of therapy areas (Homer et al 2013). An analysis of the likelihood of reversibility of toxic effects after a terminal dose free period showed that 86% of all effects observed at the end of dosing recovered and that where recovery was not observed this could largely be explained by the nature of the lesion or by the relatively short recovery period. Furthermore, demonstrating lack of recovery did not necessarily prevent progression into clinical trials. These data question the need to include a recovery assessment in these FTIM studies other than on an individual case-by-case basis driven by scientific rationale. So what would constitute a scientific rationale? We propose 3 main points to be considered assuming (as is normally the case) that the site and severity of the lesions and hence whether they can be predicted to recover is not yet known: Target effects, GID (or related chemical effects) and Prior in vivo experience. These recommendations are in broad agreement with Pandher et al. (2012) and with the NC3Rs (2012) who concluded that in general, the inclusion of recovery groups should follow a case-by-case approach in line with rational scientific study design. These conclusions are also in line with International guidelines; the ICH M3 (R2) guideline on non-clinical studies for the conduct of human clinical trials simply states that reversibility should be assessed ‘when appropriate’. In summary, we would advocate that recovery is not routinely included in the pivotal GLP studies supporting FTIM. This presents an opportunity to streamline current practices without impact on patient and volunteer safety.

1645 Considerations for In Vitro Systems to Reflect In Vivo Toxicities to Facilitate Drug Development

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The appropriate application of in vitro systems to de-risk and screen for in vivo toxicities requires consideration of histomorphological features and hypothesized pathogenesis of the finding, as well as appropriate target expression, cell composition and breadth of response of the in vitro system. To demonstrate, we present the in vivo and in vitro evaluations in the Nicotinamide phosphoribosyltransferase (NAMPT; oncology indication) and Compound 1 (pain indication) programs. NAMPT-induced cardiac toxicity had histomorphological features and hypothesized pathogenesis that made the cardiomyocyte in vitro system an appropriate model to understand on-target mechanism of toxicity, potential for nictinic acid mitigation, human translatability, and compound screening. NAMPT-induced renal toxicity had histomorphological features that questioned the in vitro system contained the appropriate target cell population; however, the hypothesized pathogenesis, target cell expression profile and measurable response to NAMPT toxicity suggested it was an acceptable approach to test. The Compound 1-induced functional cardiovascular toxicity had a hypothesized pathogenesis suggesting the in vivo cardiomyocyte system did not contain the appropriate target cell components. The Compound 1-induced histomorphologic cardiac toxicity had a hypothesized pathogenesis than questioned whether the in vivo cardiomyocyte system contained the target cell components and breadth of response for in vivo translatability. The results demonstrate that the in vitro systems were appropriate models for in vivo NAMPT toxicities, but not for the in vivo Compound 1 toxicities. Close collaboration between investigative scientists, toxicologists and pathologists to predict and investigate the utility of in vitro systems for in vivo toxicities is crucial for their appropriate application to facilitate drug development.

1646 Overcoming Barriers to Human Tissue Use for Safety Assessment

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There is increasing interest and demand for greater development, validation and adoption of human tissue-based approaches for basic and applied research. This is being driven by the growing recognition that animal models are not always predictive for humans, resulting in high attrition rates in pharmaceutical and non-pharmaceutical chemical development. Human tissue-based assays have the potential to address some of the limitations of current preclinical testing strategies and reduce reliance on animal models. The UK National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs), together with the Medicines and Healthcare Products Regulatory Agency (MHRA), has convened an expert working group to explore opportunities for greater adoption of human tissue-based approaches for safety assessment, with an initial focus on safety pharmacology. We have assessed the current use of human tissue through a survey of the international safety pharmacology community. The survey highlighted the increasing interest in human tissue as a tool for safety assessment and identified a number of potential barriers to wider adoption, including regulatory and supply issues. In July 2014 we hosted a workshop to galvanise the global safety assessment community in addressing the barriers for wider uptake of human tissue for safety assessment. Here, we will use the data collected through the survey and the discussions from the workshop to (i) provide guidance through case studies on how human tissue-based approaches are being used in safety assessment; and (ii) put forward a strategy to overcome the remaining barriers and increase human tissue use for safety assessment.
Role of the Gut Microbiome in the Host Response to Xenobiotics

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A population of nearly 100 trillion dynamic and diverse microbiota inhabits the human gut. Unlike the genome of a single organism, the combined genomic content of the gut microbiome, known as the metagenome, can rapidly vary as a function of diet, location, host genetics, and a variety of other factors, including exposure to chemicals. The gut microbiota is essential for normal immune system development, displacement of pathogens, extraction of additional energy, and can contribute significantly to metabolism of drugs, xenobiotics, and dietary bioactive chemicals. With these critical functions in mind, the gut microbiota might themselves be considered an additional, metabolically vital organ of the human body. Experts who are at the forefront of microbiome research will present their timely and innovative research that utilizes cutting-edge technology and systems approaches (e.g., high-throughput sequencing, mouse models, metabolomics) to explore topics ranging from inflammation and cancer to drug metabolism.

Impact of Dietary Persistent Organic Pollutants on the Host-Microbiome Interaction

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Environmentally-persistent organic chemicals are increasingly being recognized as potential inducers of obesity. In fact, emerging data suggests that exposure to low doses of environmental chemicals through the diet, especially persistent organic pollutants (POPs), may promote the development of obesity and type 2 diabetes. Here dietary exposure to the potent aryl hydrocarbon receptor (AHR) agonist, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), increased host inflammatory signaling and altered gut ecology and metabolism. The gut microbiota composition and metabolism, profiles of mice were monitored after dietary TCDD exposure using a combination of 16S rRNA gene sequencing, metabolomics and biochemical assays. Dietary TCDD altered the gut microbiome by preferentially shifting the ratio of Firmicutes to Bacteroidetes and inhibited the farnesoid X receptor (FXR) signaling pathway. Further, dietary TCDD triggered significant host metabolic disorders involving activation of bacterial fermentation, hepatic lipogenesis, gluconeogenesis, and glycogenolysis, in an AHR-dependent manner. These findings provide new insights into the biochemical consequences of TCDD exposure involving the alteration of the gut microbiome, modulation of nuclear receptor signaling, and disruption of host metabolism.

Impact of Infection and Inflammation on Arsenic Toxicity

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Infectious agents of the gut may impact the toxicity of environmental chemicals and alter susceptibility to chemical exposures. Therefore, understanding this dynamic is of critical importance for safety assessment in human populations. In the current study, we investigated the interplay of the enteric pathogen, Citrobacter rodentium, and arsenic which was monitored after dietary TCDD exposure using a combination of 16S rRNA gene sequencing, metabolomics and biochemical assays. Dietary TCDD altered the gut microbiome by preferentially shifting the ratio of Firmicutes to Bacteroidetes and inhibited the farnesoid X receptor (FXR) signaling pathway. Further, dietary TCDD triggered significant host metabolic disorders involving activation of bacterial fermentation, hepatic lipogenesis, gluconeogenesis, and glycogenolysis, in an AHR-dependent manner. These findings provide new insights into the biochemical consequences of TCDD exposure involving the alteration of the gut microbiome, modulation of nuclear receptor signaling, and disruption of host metabolism.

Gut Microbiota, Low-Grade Inflammation, and the Metabolic Syndrome

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The intestinal tract is inhabited by a large diverse community of bacteria collectively referred to as the gut microbiota. Alterations in gut microbiota composition are associated with a variety of disease states including obesity, diabetes, and...
flammatory bowel disease (IBD). Transplant of microbiota from diseased persons (or mice) to germfree mice transfers some aspects of disease phenotype, indicating that altered microbiota plays a role in disease manifestation. There are myriad potential mechanisms by which alterations in gut microbiota might promote disease including increased energy harvest, production of toxic metabolites, and molecular mimicry of host proteins. However, our research indicates that an overarching mechanism by which an aberrant microbiota negatively impacts health is by driving chronic inflammation. More specifically, we hypothesize that the histopathologically-ear or inflammatory gut inflammation that defines IBD is a severe but relatively rare outcome of an altered host-microbiota relationship while a much more common consequence of such disturbances is “low-grade” inflammation, characterized by elevated proinflammatory gene expression that associates with, and may promote, metabolic syndrome. In this context, a variety of chronic inflammatory diseases may stem from inability of the mucosal immune system to properly manage a stable healthy relationship with the gut microbiota. While one’s ability to manage their gut microbiota is dictated in part by genetics, it can be markedly influenced by the composition of the microbiota one inherits from their early environment. Moreover, the host-microbiota relationship can be perturbed by instigator bacteria or dietary components, which may prove to play a role in promoting chronic inflammatory disease states.

**1653 An Experiment in Collective Wisdom Technology Utilizing Real-Time Audience Input: Weight-of-Evidence Assessment for Chemical-Specific Modes of Action Utilizing Two Case Studies**

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This session is an exercise in using collective wisdom/audience participation to help inform the weight-of-evidence assessment for the mode of action (MOA) for two specific chemicals: 1,2,3-trichloropropane and tetrachloroethylene. The degree of confidence required for acceptance of a proposed MOA for a specific chemical will vary from individual to individual. As a result, chemical risk assessments that are published by regulatory agencies or individuals and MOA discussions are subject to criticism from individuals with a different viewpoint. Understanding this variation amongst individuals in the toxicity and risk assessment communities is an important factor for risk managers to understand and appreciate. This session will provide a forum to explore the degree of this variation in the level of confidence in chemical-specific MOA arguments. Using proven technology for audience participation during scientific sessions and real-time analyses of results, the audience and speakers will explore how collective wisdom can inform the process of how MOA decisions are made, and how differing expertise impacts decisions. The presenters will discuss the technology used in this session, how individuals can provide insights on their expertise and experience, and the extent of weight of evidence in support of and against proposed MOA for 1,2,3-trichloropropane and tetrachloroethylene. Finally, the results of the collective wisdom exercise will be presented and findings from the exercise will be discussed by a panel of experts. Audience members should bring wifi-enabled devices to participate in real-time interaction with the presentation.

**1654 Introduction to Collective Wisdom Technology**


In a typical conference setting the exchange of information is predominantly unidirectional, flowing from the presenter to the audience. This represents a significant limitation in the flow of information, since the collective knowledge of the audience can serve as a valuable resource to the speaker, audience members, as well as to the broader scientific community not in attendance. The goal of this presentation is to introduce the application of technology to improve the exchange of information in a conference setting. This will be accomplished through the use of web-based forms, accessible by internet-enabled electronic devices (cell phone, tablet, computer), to allow for the elicitation of audience member opinions on the modes of action for two case studies presented during this session (MOA/AOP analyses for 1,2,3-trichloropropane and tetrachloroethylene). Using this technology, the opinions of audience members will be collected, analyzed, and reported back to the audience in real-time during this conference session. This presentation will provide an overview of the format of the presentations in this session, and general instructions for the audience to provide input.
however, gaps exist in the supporting data. Data limitations preclude a full MOA analysis for PERC-induced MCL, but the progression of the disease in rats—coupled with hematopoietic differences in rats and humans—might suggest the endpoint is not relevant to humans. The presentation will provide a balanced case for and against each of the MOAs and human relevance analyses. During and at the conclusion of this presentation, the audience will be invited to provide their opinions on the level of confidence in the proposed MOAs and whether they interpret the weight of evidence to be sufficient to warrant acceptance of a threshold MOA in a human health risk assessment.

**1658 Collective Wisdom Findings and Discussion**

S. Hayes. Summit Toxicology L.L.P, Lyons, CO.

Experts differ in their opinions and interpretations of available toxicology data and how the data should be used to inform risk assessment. Real time findings from the audience’s input/opinions on the specific queries will be presented. Distributions of confidence scores will be analyzed for the various proposed MOAs and assessments of the degree of confidence required to support human health risk assessment. The findings from the collective wisdom experiment will be further discussed by a panel of experts.

**1659 Panel Discussion**

A. M. Jarabek1 and S. Hayes2. 1US Environmental Protection Agency, Research Triangle Park, NC and 2Summit Toxicology, L.L.P., Lyons, CO.

A panel of experts in human health risk assessment will discuss the findings from this experiment in collective wisdom. Recommendations for future efforts to utilize this technology will be explored, along with discussions of the pros and cons of the use of collective wisdom in risk assessment. Specific charge questions will be asked of the panel. At the same time, the audience will be invited to respond to the posed charge questions as well. The interaction of the panel of experts and the audience will again provide insights on the impact of collective wisdom in an SOT session.

**1660 Application of High-Throughput In Vitro Assays in Assessing Small Molecule Safety**

N. Greene1 and R. S. Thomas2. 1PFIZER Inc., Groton, CT and 2US Environmental Protection Agency, Raleigh, NC.

Social demands to ensure both public health and environmental safety from either planned or accidental exposure to existing or new molecular entities whilst still maintaining a flow of new and more effective medicines or the necessary commercial advances in personal products, requires both industry and regulatory authorities to identify and manage the risks presented by an increasingly large number of novel compounds. Often these hazard and risk assessments are made in the absence of high-quality toxicology data, and generating this data would take many years and millions of dollars for each compound under review. As a result, the scientific community has been seeking ways to prioritize these new and existing chemical entities according to their potential for adverse effects to either humans or the environment. The use and application of high-throughput in vitro assays offers significant advantages for both industry and regulator alike, but their application is not without its drawbacks. On the positive side, these types of approaches to hazard assessment are often fast and relatively cheap to run once they have been successfully implemented. In addition, these approaches offer a highly attractive public relations solution in view of the increasing demands to refine, reduce, or replace animals in laboratory experiments. However, questions still exist about their ability to adequately distinguish between toxic and nontoxic molecules and their effectiveness in ensuring public safety. This workshop will highlight recent experiences and learnings in the practical application of high-throughput in vitro assays across a broad scope of industry and regulatory agencies. The presentations will illustrate how in vitro assays are being applied to gain an understanding of which chemicals have the highest level of concern and can lead to a greater understanding of the mechanisms of action that can ultimately result in toxicity.

**1661 Genetic Mapping of In Vitro Susceptibility to Cytotoxic Compounds—The 1000 Genomes High-Throughput Screening Study**

I. Rusyn. Texas A&M University, College Station, TX.

Important gaps exist in our understanding of human variation in response to toxic environmental chemicals. To address this critical need in next generation risk assessment, we conducted the profiling of 1086 lymphoblastoid cell lines drawn from the 1000 Genomes Project, with variation in cytotoxic response to 179 chemicals used as phenotypes. The analysis included ranking of chemicals by average response, as well as assessments of population variation and heritability, information that is immediately applicable to human health assessment of chemical toxicity. Genome-wide association mapping was also performed, with attention to phenotypic relevance to human exposures. The associations suggest important roles for variation in membrane and trans-membrane genes, as has been suggested for chemotherapeutic agents. Several chemicals showed association with rs13120371 in the solute carrier SLC7A11, which has been implicated in chemoresistance. Several chemicals also showed significant association with immune-related ontologies. Analysis of public RNA-seq profiles on the same cell lines provided evidence of association between basal transcription and cytotoxic response, with enrichment for genes with membrane localization. The results highlight the challenges in the use of cell lines to map cytotoxicity traits, which follow the heritability patterns and small effect sizes seen for complex diseases. At the same time, this experimental approach fills critical gaps of most recent large-scale toxicity testing programs by providing quantitative estimates of chemical hazard and variability, as well as testable hypotheses about potential mechanisms of toxicity.

**1662 Application of High-Throughput In Vitro Assays for Risk-Based Chemical Safety Decisions of Environmental and Industrial Chemicals**

R. S. Thomas. US Environmental Protection Agency, Raleigh, NC.

Multiple drivers shape the types of human-health assessments performed on chemicals by U.S. EPA resulting in chemical assessments are “fit-for-purpose” ranging from prioritization for further testing to full risk assessments. Layered on top of the diverse assessment needs are the resource intensive nature of traditional toxicological studies used to test chemicals and the lack of toxicity information on many chemicals. To address these challenges, the Agency initiated the ToxCast program to screen thousands of chemicals across hundreds of high-throughput screening assays in concentrations-response format. One of the findings of the project has been that the majority of chemicals interact with multiple biological targets within a narrow concentration range and the extent of interactions increases rapidly near the concentration causing cytotoxicity. This means that application of high-throughput in vitro assays to chemical assessments will need to identify both the relative selectivity at chemicals interact with biological targets and the concentration at which these interactions perturb signaling pathways. The integrated analyses will be used to both define a point-of-departure for comparison with human exposure estimates and identify which chemicals may benefit from further studies in a mode-of-action or adverse outcome pathway framework. The application of new technologies in a risk-based, tiered manner provides flexibility in matching throughput and cost considerations with the degree of certainty required for specific decision contexts ranging from prioritization to full risk assessments.

**1663 Predicting Systemic Toxicity Using Cheminformatics and High-Throughput Toxicogenomics**

G. P. Daston. Proctor & Gamble, Cincinnati, OH.

Evaluating the safety of new ingredients in consumer products has become challenging because of restrictions or bans on animal testing, coincident with an increase in the pace of innovation in the industry. We are employing an approach in which we identify already tested analogs to new chemicals as a means of generating a hypothesis about the potential toxicity of the new chemical, and then use mode-of-action-level data to test the hypothesis. Much of these data comes from toxicogenomics. We are in the process of generating data on chemicals with a broad range of modes of action to populate a data base that supports a connectivity map approach to identifying common modes of action. The data are being generated in a variety of cell types. We are increasingly making use of a high-throughput method, L1000, which directly measures the expression of 1000 landmark genes and infers the expression level of the remainder of the genome. Data from this method compares well to data from whole-genome arrays.
Safety assessment in the pharmaceutical industry now spans the full drug discovery and development lifecycle. With this early drug discovery focus: Toxicology organizations are exploring higher throughput safety assessment capabilities to cover the increased chemical matter and diversity seen in the pre-hit portfolio. While the potential number of higher-throughput screening endpoints can be immense, we have identified and developed key in silico and in vitro models that guide early risk characterization. In this continuum, we have also created follow-on cell-based screening capabilities using genomic and imaging tools that provide a higher content functional characterization of cellular responses to chemical insult. With these capabilities, we have developed and applied a higher throughput risk characterization process that supports early pre-clinical safety assessment.

1665 Combining Chemical Properties and HT Safety Assays to Guide Early Drug Design: Lessons Learned

N. Greene, Pfizer Inc., Groton, CT.

There is an ever increasing need to deliver new, safer medicines to the market while simultaneously lowering R&D costs and reducing the rate of failure in the late stages drug development by focusing the available resources on those chemical candidates most likely to succeed. The pharmaceutical industry has therefore turned towards the use of cheaper and faster alternatives to selecting safer drug candidates. The use of in vitro assays and in silico models for toxicology has been a long standing desire within the industry as they are typically low cost and high throughput so can be used to help guide compound design and in the case of in silico models prior to any chemical synthesis thus avoiding potential known problems from the outset. Many factors contribute to a compound’s ability to cause unwanted side effects including the presence of overt or masked reactive functional groups, a potent off target activity or simply as a result of its physicochemical properties. Often these factors are highly correlated and rarely distinct from each other so understanding the true causes of the adverse effects is often difficult to tease apart. Here we present an approach for dealing with in vitro safety study outcomes in a consistent way and using this to develop models to predict the Cmax concentration where significant toxicity will likely be observed based on an array of physicochemical and high throughput in vitro assays. Lessons learned from this work such as the ability to predict target organ effects will be presented.

1666 Deciphering Clinical and Experimental Retinal Toxicology: An Eye on the Present and Future

E. Chow1 and D. A. Fox2, 1Toxicology, Allergan, Irvine, CA and 2Pharmacology and Pharmaceutical Sciences, University of Houston, Houston, TX.

The World Health Organization estimates that 285 million people worldwide are visually impaired or blind due to age-related macular degeneration (AMD), diabetic retinopathy, glaucoma, retinitis pigmentosa, or drug- and chemical-induced retinal degeneration. These retinopathies are characterized by progressive and regional/cell selective loss of anatomically or physiologically related neuronal function. Retinotoxicity is also an important issue during drug development as a result of both on- and off-target effects. New advances in noninvasive electrophysiological and imaging techniques at the cellular and micron level of resolution have enabled efficient time-course studies in man and animals and contributed to earlier detection/evaluation and increased understanding of retinal toxicity in them. Basic and clinical science studies, utilizing advanced electrophysiological and imaging techniques, in developing and adult organisms, have elucidated interspecies similarities and differences in retinal anatomy, cell/molecular biology, cell signaling, pharmacology, physiology, pharmacokinetics, and metabolism that enable a more precise translation of animal retinotoxicity to man. The first four speakers in this symposium will present the latest information about these areas while the final speaker will address recent developments in retinal pigmented epithelium stem cell basic and clinical/translational research, including the toxicity evaluation that is required before initiating human trials for this latest technological advancement. Together, these speakers will provide the latest comprehensive information about retinotoxicology and describe a framework for predictive retinotoxicity of new drugs and environmental/industrial chemicals.

1667 New Insights in Retinal Structure and Function to Evaluate Toxicity of Ocular Drugs

B. G. Shor, Pathology, Allergan, Irvine, CA.

With the explosive success in treating retinal diseases with ocular drugs, there has been a keen interest in safety evaluation of novel therapies and a need for toxicologists to understand normal and pathophysiologic retinal anatomy and physiology in humans and animals. This presentation will demonstrate how state-of-the-art techniques have revolutionized our approach to understand toxicologic changes in retinal structure and function using optical coherence tomography (OCT), OCT-guided histology, multi-layered immunohistochemical immunofluorescence coupled with confocal microscopy, whole-slide imaging and quantitative digital pathology. Advances in electroretinography (ERG), including multifocal ERG which produces a functional map of the retina, photopic negative response ERG which produces a signal that is specific to the retinal ganglion cell layer, and other techniques for evaluating toxicological changes in retinal function, will also be addressed.
and degeneration. Finally, since the retina is a window into the brain an increased awareness and understanding of retinal/visual system dysfunction should provide additional insight into acquired neurodegenerative disorders.

1670 Retinal Pigment Epithelium: Disease and Drug-Induced Dysfunction

C. Croxon, Ophthamology, Medical University of South Carolina, Charleston, SC.

The retinal pigment epithelium (RPE) plays a central role in maintaining normal vision due to its anatomic location between the photoreceptors and choriocapillaries, and the specific cellular processes that support phototransduction and photoreceptor renewal. The assessment of RPE dysfunction involved in the pathophysiology process contributing to ocular diseases or toxicological response is a challenging task as effects of the RPE versus inner retinal vasculature are hard to separate. This task will provide a comprehensive understanding of latest in vivo and in vitro models that can be used to assess RPE responses to endogenous inflammatory cytokines, drugs and toxicants. This talk will include discussions of the mechanisms of action for latest drugs and how these agents influence retinal function following acute and chronic administration.

1671 Stem Cells in Retinal Repair and Regeneration

D. Clegg, Molecular, Cellular, and Developmental Biology, University of California Santa Barbara, Santa Barbara, CA. Sponsor: E. Chow.

Age-related macular degeneration (AMD) is a leading cause of blindness, and treatments are limited for the non-exudative form. As AMD progresses, the retinal pigmented epithelium (RPE) in the macula lose function or die, resulting in photoreceptor loss. An exciting new direction is to use RPE derived from human embryonic stem cells (hESC) to replace RPE and preserve photoreceptors. One clinical trial that employs a bolus injection of hESC-RPE cells is already underway. A more sophisticated approach is to implant differentiated, polarized monolayers of hESC-RPE on a scaffold, whereby cells are provided with a supportive substrate. We will present recent efforts using this strategy for the treatment of dry AMD, along with new pre-clinical studies that assess toxicity and tumorigenicity. Strategies that combine scaffolds with ocular cells derived from pluripotent stem cells are likely to have broad application for a variety of ocular diseases and injuries.

1672 Evaluating Similarity across Related Complex Mixtures: The Challenge of Herbal Supplements

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Complex mixtures represent a significant public health concern and challenge to the risk assessment community. Whole mixture approaches are recommended by risk assessors because evaluating the “mixture-of-concern” necessarily accounts for the unidentified fraction and precludes the need to introduce an additional measure of additivity among identified constituents (or define all interactions), as opposed to component-based approaches. However, assessing the safety or risk associated with every permutation of a complex mixture is an intractable problem. Therefore, methods for determining sufficient similarity of the mixture-of-interest to a well-characterized reference mixture are necessary. Herbal supplements provide a unique opportunity to make progress in this arena while addressing the important public health concern of herbal supplement safety. Herbal products on the marketplace often display a wide range of constituent concentrations that frequently differ from label claims. Significant research has been dedicated to characterizing the chemistry of these complex mixtures and comparing across related formulations using marker compounds and fingerprinting techniques in order to confirm appropriate source material and identify adulterated products. Progress has also been made in comparing similarity of biological responses across multiple herbal products and developing statistical methods for evaluating sufficient similarity. However, the whole picture—recommended approaches for evaluating chemical and biological sufficient similarity—has yet to emerge. In this session, speakers will discuss the latest science for evaluating chemical and biological similarity of related products with sufficient similarity—has yet to emerge. In this session, speakers will discuss the latest science for evaluating chemical and biological similarity of related products with sufficient similarity. Development of new compound discovery to safety and efficacy studies. Common to such investigations is the need to demonstrate integrity and reproducibility of test articles. This effort includes assuring that biomass is properly identified and herbarium specimens cataloged and determining that quantitative measurements of phytochemicals are accurate and precise. Modern research on botanicals may include discovery of bioactive phytochemicals, including investigations of synergistic effects of complex mixtures in the botanical matrix. In the phytomedicine field, botanicals and their contained mixtures are considered the active pharmaceutical ingredient (API). Unlike single-chemical APIs, botanicals are variable because their composition depends on genotypic and phenotypic variation, geographical origin, weather exposure, harvesting practices, and processing. Complicating matters is the wide variety of product types in the marketplace ranging from relatively unprocessed dried pieces, powders, and teas to highly processed concentrates, metabolites, constituents, and extracts. As opposed to relatively unprocessed biomass, some highly processed ingredients such as native extracts and dry extracts are essentially manufactured materials - the nature and composition of which are defined by the processes used in their manufacture. Different proprietary processes used on biomass that is nominally the same will predictably result in dietary ingredients that are chemically and biologically different from each other. This inherent variability in raw materials can result in inconsistent research materials and commercial products that are under-potent, over-potent, and/or contaminated. These differences can have implications for the bioactivity profile of the ingredients and must be addressed prior to the start of any investigation.

1677 Fingerprinting Methods for Identification and Authentication of Botanical Supplements

J. Harnly. USDA, Beltsville, MD. Sponsor: C. Rider.

Chromatographic and spectral methods provide complex metabolic fingerprints that, when submitted to multivariate analysis, can identify and authenticate whole botanical materials. A recent study demonstrated that both flow injection (with no chromatographic separation) mass spectrometry (FIMS) and proton nuclear magnetic resonance (1H-NMR) spectrometry were in excellent agreement and provided clear discrimination between Actaea racemosa (black cohosh) and other Actaea species. The samples were acquired from reliable research organizations and many were vouchered. In addition, the sample identities were verified by DNA barcoding. Within the Actaea racemosa cluster, sub-clusters were observed for samples collected from 22 sites in the Appalachian mountains in the eastern US, suggesting that local genetic or endophytic fungal variations influenced the chemical fingerprint. Other studies have demonstrated that Panax ginseng (Asian ginseng) can be differentiated from P. quinquefolius (American ginseng) and P. notoginseng using UV, NIR, and FIMS. P. quinquefolius grown in the US could be differentiated...
from that grown in Canada and China. Modeling of adulteration based on the pure spectra of *P. quinquefolius* and *P. ginseng* agreed with physical adulteration and demonstrated that adulteration of the former with the latter could be detected at the 5% level. Multivariate analysis of FIMS and 1H-NMR spectra also allows identification of specific chemical entities that permit differentiation. Thus, fingerprinting methods, using the entire spectra (or chromatogram), are robust tools for detecting chemical composition variations arising from genetic differences, contamination, or economically motivated adulteration.

**1676 Moving forward on Complex Herbal Mixtures at the National Toxicology Program**


Selection of representative test articles and providing context for extrapolating findings to related products represent major challenges for the herbal testing initiative at the National Toxicology Program (NTP). In response, NTP is focusing research attention on the variability among nominally related herbal products and developing approaches for comparing across complex mixtures. First, test article selection has increasingly been informed by surveys of various lots for constituent profiles and concentrations using multiple analytical techniques. A recent example involved comparing seven ethanol extracts of black cohosh against four reference cohosh varieties using HPLC with ultraviolet, charged aerosol and mass spectrometry detection to generate chromatographic profiles and concentrations of multiple terpene glycosides and polyphenols. Results demonstrated that only three lots matched black cohosh, while three were Chinese cohosh, and one was a blend. The second effort builds on previous NTP *Ginkgo biloba* extract (GBE) studies, which identified the liver as a major toxicity target for GBE in both mice and rats and resulted in very high levels of hepatoblastomas in treated mice. In this project, the test article will be compared to various lots of GBE in terms of chemistry and biological activity using 5-day rat studies to measure global gene expression changes in the liver. The objective is to develop a strategy for determining sufficient similarity of whole mixtures using a short-term study paradigm. In the third project, available lots of herbal supplements were included in the latest round of Tox21 high throughput screening. Up to six different lots of various herbal supplements will be tested in approximately 20 assays for cytotoxicity, receptor activation, and disruption of stress pathways. Data generated from these efforts will provide information on the variability of complex herbal mixtures and inform the development of statistically-based approaches for comparing chemical and biological similarity of related products.

**1677 Steps toward Using Statistical Approaches for Determining Sufficient Similarity**

C. Gennings1 and C. V. Rider2. 1NTP/NIEHS, Research Triangle Park, NC and 2Department of Preventive Medicine, Mount Sinai, New York, NY.

Equivalence testing methods are used to evaluate whether two mean values meet a threshold of “similarity” and can be considered practically equivalent. These statistically rigorous methods have recently been applied to test for sufficient similarity of mixtures to a benchmark mixture using the reference potency approach. This method uses the benchmark mixture as a point of departure for mixture similarity and also requires a new definition of “sufficient similarity.” As with other equivalence approaches, equivalence testing methods may be useful for both regulatory and research purposes. Additional work is being done to better understand the variability among nominally related herbal products and inform the development of chemically-based equivalence testing methods.

**1678 Some Like It Hot: Impacts of Wildfires on Human Health**

M. C. Madden. ORD, NIEERL, HSD, Clinical Research Branch, US EPA, Chapel Hill, NC.

Wildfires have health impacts derived from combustion emissions and contribute 20–30 percent of ambient particulate matter (PM). One recent report predicted longer wildfire seasons, smoker fires, and the burning of a larger area of the Western US. Elevated PM levels have been linked to increased deaths and hospitalizations for several morbidity outcomes. Different types of wildfire vary by the type of wood being burned (e.g., crown, brush, below ground); the fire type may induce different types of health effects. Using a primate model, monkeys exposed to Northern California (CA) wildfire had persistent changes in blood cell cytokine production. Additionally, gender-dependent changes in airway hyper-responsiveness and compliance were observed. Alterations in health effects observed in both Northern and Southern California communities from wildfires in the last ten years will be compared and contrasted to an Eastern US peat-fuelled wildfire. These studies examined susceptibility factors (e.g., socioeconomic, pre-existing cardiopulmonary disease) that modulated the observed health effects. Native tribes in Northern California were particularly susceptible to exposure due to the geography of tribal lands. The effectiveness regarding mitigation strategies (e.g., filters, face masks) within the affected communities will be described. The guidance for mitigating adverse health effects, developed by an international working group, will be presented and addresses the highly sensitive/supersensitive populations. The identification of potentially susceptible individuals and effectiveness of intervention strategies have implications for preventing adverse outcomes and decline in public health. The observations from these studies will be integrated into the current knowledge of ambient PM-associated health effects as to the uniqueness of the findings. This session will be of great interest to public health specialists, inhalation and cardiovascular toxicologists, and those in the California area. [This abstract may not reflect official US EPA policy.]

**1679 The Nature of Wildfire Smoke Impacts in California: Acute Effects, Interventions, and Long-Term Sequelae**


In the past 15 years, no other natural force has had a greater impact on population health in California than wildfire. Recent wildfires have resulted in sustained particulate matter (PM2.5) concentrations above levels considered unhealthy for sensitive populations for weeks at a time. At-risk populations who, by the presence of underlying chronic health conditions or other determinants of health inequality, may be at higher risk of adverse impacts of wildfire smoke exposure. Of special concern are tribal peoples of Northern California. Because of the geography of tribal lands, these populations have an elevated probability of wildfire smoke exposure. During the 1999 Big Bar fire complex, smoke was so pervasive that the Hoopa Valley tribal government implemented several public health interventions including distribution of particulate respirators, portable high efficiency air cleaners, and hotel vouchers, the latter to encourage evacuation from the area. Despite these interventions, residents reported increased frequency of respiratory symptoms over baseline levels for up to two weeks after the smoke cleared. During the 2008 Lightning Strike fires, the Hoopa, Yurok, and Karuk tribes all declared states of emergency. The Hoopa Tribe again instituted several interventions to help reduce population impacts of smoke. Even so, there was a recorded 85% increase of asthma medication dispensing over the previous year. Past fuel management practices coupled with climate change and population encroachment have increased the risk of wildfire and the numbers of those potentially exposed to smoke. There is growing concern of the impact of wildfire smoke on developing respiratory systems, long-term respiratory sequelae, and even birth weight changes in general and susceptible populations. By using the latest toxicology and epidemiology data, we may better implement public health and medical interventions necessary to protect populations from the impacts of ever-increasing wildfire events.

**1680 Impact of Wildfires in San Diego County**


In 2014, California wildfires have occurred earlier than in past years. For San Diego, the area burned by 2003, 2007, and 2014 wildfires has been unprecedented; it is the first time the two major fires have occurred in the same year. Wildfires generate significant gaseous pollutants and particulate matter (PM) concentrations. A review of air quality, emergency department visits, and recommendations from After Action Report (AAR) of each wildfire will be reviewed. APCD results from monitors placed throughout San Diego County to evaluate pollution levels will be compared.
Emergency department visits and Syndromic surveillance were reviewed for each of the three wildfire incidents. Visits will be characterized by age, gender, and diagnosis (i.e., respiratory, cardiac, or GI related effects). Sensitive populations impacted will also be reviewed. Recommendations have included creation of the Emergency Medical Services-Domestic Operations Center (EMS-DOC) which serves as the medical departmental operations center. EMS created seats for healthcare community partners and other County staff; conducted outreach to local long-term facilities to ensure they were familiar with, and included in, disaster response activities; and enhanced its Medical Reserve Corps program. After the 2014 wildfire AAR, enhanced efforts focused on risk communication to vulnerable populations, specifically including limited English speaking communities. Certain fire-related outcomes related to asthma and other respiratory conditions have been expected in the short-term, similar to PM-related health outcomes. A study conducted by the University of California Berkeley indicated that shreds and dust were the predominant contributing sources of combustion in 2003. These data provide evidence for subsequent ST research to recommend to increase defensible space around homes to promote property protection. As such, efforts must continue to enhance air quality monitoring and forecasting, syndromic surveillance, and disaster preparedness for healthcare stakeholders and vulnerable populations. These actions will support strategies to protect the health and safety of the public.

1684 Using Bioactivity-Based Read-Across (BaBRA) to Characterize the ToxCast Library

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The large chemical library and diverse assay space included in the ToxCast program present a unique opportunity to characterize environmental chemicals based on their in vitro bioactivity patterns across hundreds of critical targets and in comparison to reference chemicals with known toxicological effects. We used an unsupervised random forest approach to create a proximity matrix that clusters chemicals based on their bioactivity patterns across all the ToxCast assays within the context of the entire chemical library. Using the resulting clusters, we implemented a bioactivity based read-across (BaBRA) approach to examine the 1047 ToxCast Phase I and II chemicals. This analysis identified a large cluster of inactive chemicals, including food additives such as sucrose, as well as two clusters comprised predominantly of active pesticide ingredients. A cluster of highly cytotoxic chemicals (n=14) included mercuric chloride, phenylmercuric acetate, organotins, and multiple donated pharmaceuticals. Several confirmatory patterns were observed in the proximity matrix, such as steroid hormones and other clusters of chemicals with similar use cases (e.g., surfactants, anti-inflammatory drugs) clustering together. Clusters containing known human toxicants, such as diethylstilbestrol and azathioprine, were examined via BaBRA to identify untested environmental chemicals with similar bioactivity patterns. The presentation will discuss assay patterns across the ToxCast Phase I and II libraries and identify in vitro targets and bioactivity trends that may be driving unique clusters. BaBRA predictions will be compared to toxicity and exposure data from the literature to identify potential patterns relating to use case and/or environmental persistence. This project was funded in whole or in part with Federal funds from the NIEHS, NIH under Contract No.HHSN273201400003C.
A Strategy to Distinguish Predicted Molecular-Initiating Events from Cell Stress Using ToxCast and Tox21 Data

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High-throughput screening (HTS) data from ToxCast/Tox21 cell-based and biochemical assays may identify biological targets for chemicals and inform molecular-initiating event (MIE) hypotheses for adverse outcome pathways active in in vivo models. A strategy to distinguish nonspecific or cellular toxicity from predictors of selective bioactivity was developed to enable follow-up investigations to decrease the number of false positive MIE predictions due to activated cell stress pathways, and highlight the most pertinent pathways for potential effects. HTS endpoints indicative of cellular toxicity included different assay markers for oxidative stress, mitochondrial toxicity, apoptosis, and cell death (108 assay endpoints). In order to prioritize MIE hypotheses, an estimate of the upper 95% confidence limit for the median in vitro concentration known to perturb cell stress markers was derived using a bootstrap procedure (R, v3.0.3). The outcome of this process was termed the median cell stress predictor (MCSP). A biological target hypothesis was considered distinct from cell stress pathways if the HTS assay result (AC50) was at least 0.5 log units separated from the MCSP. This separation between the MCSP and biological target predictors provides context for results that may be due to cell stress, and conversely distinguishes chemicals for which a primary MIE may be inducing cell stress. The results of this simple methodology using fungicides including triazoles, previously identified mitochondrial toxicants, and in vitro endocrine-active chemicals, e.g. genistein and diethylstilbestrol, in the ToxCast Phase I & II chemical libraries are illustrated. The assay AC50s for mitotoxicant phenemphidiam all relate to nonspecific pathways, whereas for fungicides and endocrine-active controls, the MCSP demarcates a threshold for increased confidence that biological target AC50 values suggest MIE hypotheses worth further consideration. The MCSP strategy enables prioritization of distinct MIE hypotheses and supports comparisons with data from models of greater biological complexity.

Predicting Acute Toxicity Using In Vitro ToxCast™ HTS Mitochondrial Inhibition Assays


Mitochondrial inhibition is a mechanism known to drive acute toxicity for certain chemicals. High Throughput Screening (HTS) assays have been developed to test if a chemical’s toxicity operates by this mechanism. We hypothesized that, for chemicals that cause mitochondrial inhibition in HTS assays, acute toxicity is conserved across invertebrate, aquatic and mammalian species, suggesting that 1) in the absence of pre-systemic metabolism or limited absorption, in vitro mechanistic data could predict responses in multiple species, 2) under conditions of similar bioavailability, concordance of dose response between species would be high, and 3) predictions of oral toxicity from HTS assays routes would often be confounded by chemical-specific differences in uptake and metabolism. We observed that, 1) Mitochondrial inhibition predicted the minimum toxicity (an upper bound to the LC50’s) of the chemicals in Fish and Daphnia. Chemicals could cause higher toxicity by other mechanisms but never lower. The lower the assay AC50 the more likely the toxicity was driven by mitochondrial toxicity. Therefore the assay can be used to set a minimum toxicity for chemicals and low AC50 values mean high Fish and Daphnia toxicity. 2) Mitochondrial inhibition didn’t predict the toxicity of the chemicals in Rat with limited oral bioavailability and first pass metabolism, which renders compounds much less toxic. Simulations using in silico models of bioavailability and metabolism (for three Cyp enzymes -2C, 2D and 3A; and substrates for UDP-glucuronon transferases -UGTs) improved toxicity correlations. Predictive models for non-Cyp phase 1 and 2 metabolism are currently not available and would likely provide further insight. 3) Consistent with the AOP approach, such predictions could reduce the need for nonclinical regulatory safety testing for acute toxicity.

Selective Biological Activity of ToxCast Chemicals in Mouse Embryonic Stem Cells Identifies In Vivo Teratogens


Translating in vitro biological activity to adverse in vivo developmental consequences is difficult because of a myriad of spatiotemporal regulatory events required for embryogenesis. Biological activities of ToxCast I and II chemicals were evaluated using 11 mouse embryonic stem cell (mESC) adherent differentiation and cytotoxicity assay. Cells were exposed to 20 μM concentrations on Day 1. Differentiation endpoints corresponding to gastrulation (Day 4) and cardiomyogenesis (Day 9) were evaluated using goosecoid and myosin heavy chain protein markers, respectively. 56/290 ToxCast phase I chemicals (20%) affected cardiomyocyte differentiation and 81 induced cytotoxicity; 217/1078 ToxCast Phase Ia/II chemicals (20%) affected gastrulation-stage differentiation, and 240 were cytotoxic. AC25s were determined for mESC endpoints; mESC-selective effects were ascribed when the AC25 was below the ‘Cytotoxicity Burst’ (Judson et al., 2014) concentration. The correlation between each mESC-selective endpoint and in vivo outcomes for teratogenesis (structural defects provided in ToxRefDB) was determined. ‘Cardiomyocyte Differentiation’ and ‘Cytotoxicity’ showed the lowest and Day 4 (gastrulation-stage) cytotoxicity the highest correlation to in vivo defects. The combination of gastrulation-stage cytotoxicity and cardiomyocyte differentiation identified 63 bioactive compounds, 45 are developmental toxicants. When chemical potency is considered 80% (32/40) of the most potent compounds are teratogenic in vivo. In contrast, 61% (14/23) of the least potent compounds are teratogenic in vivo. Chemicals producing mESC-selective bioactivity in gastrulation-stage cytotoxicity or cardiomyocyte-stage differentiation correlate with teratogenic effects in vivo; however, at the concentrations tested, mESC-selective bioactivity does not identify 45% (117/259) of teratogenic chemicals suggesting that additional pathway assays are required. This abstract does not present EPA policy.

Predicting Acute Toxicity Using In Vitro ToxCast™ HTS Mitochondrial Inhibition Assays


Mitochondrial inhibition is a mechanism known to drive acute toxicity for certain chemicals. High Throughput Screening (HTS) assays have been developed to test if a chemical’s toxicity operates by this mechanism. We hypothesized that, for chemicals that cause mitochondrial inhibition in HTS assays, acute toxicity is conserved across invertebrate, aquatic and mammalian species, suggesting that 1) in the absence of pre-systemic metabolism or limited absorption, in vitro mechanistic data could predict responses in multiple species, 2) under conditions of similar bioavailability, concordance of dose response between species would be high, and 3) predictions of oral toxicity from HTS assays routes would often be confounded by chemical-specific differences in uptake and metabolism. We observed that, 1) Mitochondrial inhibition predicted the minimum toxicity (an upper bound to the LC50’s) of the chemicals in Fish and Daphnia. Chemicals could cause higher toxicity by other mechanisms but never lower. The lower the assay AC50 the more likely the toxicity was driven by mitochondrial toxicity. Therefore the assay can be used to set a minimum toxicity for chemicals and low AC50 values mean high Fish and Daphnia toxicity. 2) Mitochondrial inhibition didn’t predict the toxicity of the chemicals in Rat with limited oral bioavailability and first pass metabolism, which renders compounds much less toxic. Simulations using in silico models of bioavailability and metabolism (for three Cyp enzymes -2C, 2D and 3A; and substrates for UDP-glucuronon transferases -UGTs) improved toxicity correlations. Predictive models for non-Cyp phase 1 and 2 metabolism are currently not available and would likely provide further insight. 3) Consistent with the AOP approach, such predictions could reduce the need for nonclinical regulatory safety testing for acute toxicity.

Using High-Content Imaging Data from ToxCast to Analyze Toxicological Tipping Points

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Translating results obtained from high-throughput screening to risk assessment is vital for reducing dependence on animal testing. A challenge to using in vitro high-throughput data is differentiating adaptive from adverse cellular responses. We studied the effects of 976 chemicals (ToxCast Phase I and II) in HepG2 cells using high-content imaging (HCI) to measure dose and time-dependent perturbations in p53, JNK, oxidative stress, cytoskeleton, mitochondria, and cell cycle. A novel computational model was developed to describe the dynamic response of the system as cell-state trajectories based on multi-dimensional HCI data streams. Cell-state trajectories produced by 10 concentrations (0.4 to 200 μM) of 967 chemicals showed resilience of the HepG2 system in many cases, where chemical perturbations were transient due to system recovery. However, we also found “tipping points” where system recovery was never apparent. Further analysis of trajectories identified dose-dependent transitions, or critical points, in system recovery for 340/967 chemicals. The critical concentrations were generally 5-times lower than the concentrations that produced complete system failure (i.e., cell loss). We be-
lieve that time- and concentration data from HCI can be used to reconstruct cell state trajectories, and provide insight into adaptation and resilience for in vitro systems. With additional research, cellular tipping points could be used to define an in vitro point of departure (PdD) for risk-based prioritization of environmental chemicals. This work does not reflect US EPA policy.

1690 Assessing Confidence in Tox21
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The National Toxicology Program (NTP) has worked over the past decade to transform toxicology from an empirical animal-based science to a predictive science based on computational approaches utilizing information from in vitro high throughput screening (HTS). (Q)SAR and in silico analyses. Through our inter-agency Tox21 collaboration, we have demonstrated the feasibility of screening large chemical libraries in multiple HTS assays for a range of biological responses. We are currently applying various data curation and analysis approaches to Tox21 results in efforts to compare data generated using these assays as well as other short-term experimentally derived data (e.g., 5-day in vitro rodent transcriptomics patterns) to historical experimental animal data and human experience. What is currently missing is a structured approach to establish confidence in Tox21 data for use in public health decisions. We see this requiring effort in three ongoing and future interrelated areas. These include 1) verifying that assay findings reflect true biological responses analyzed through consolidated and agreed upon approaches 2) understanding the relationships of assay outputs to in vivo biological pathways and processes, and 3) assessing confidence in the collective information linking the affected biological pathways or processes with human disease and/or dysfunction. Within NTP, item one is being addressed through the existing Tox21 collaboration, item two is a new focus of the NTP Interagency Committee for the Evaluation and Validation of Alternative Toxicological Methods (NICEATM), and item three is being addressed by the Office of Health Assessment and Translation (OAHAT) through further development and application of systematic review methods to environmental health sciences. We are working internally, with other Federal and international agencies, and through public outreach to describe, discuss and further develop these activities.

1691 Reproducibility of the 3D Skin Comet Assay within and between Laboratories

The 3D Skin Comet assay was developed to improve the in vitro prediction of the genotoxic potential of dermally applied chemicals. As part of a validation process, funded by Cosmetics Europe and the German Ministry of Education & Research, we evaluated this assay using the Phenion® Full-Thickness (FT) Skin Model and 8 coded chemicals, including a pro-mutagen and a cross-linker, mitomycin C (MMC), by incomplete block design. There was an excellent overall predictivity of the expected genotoxicity (>90%). Three labs correctly identified all chemicals and the fourth correctly identified 80% of the chemicals. Background DNA damage was low and values for solvent (acetone) and positive (methyl methanesulfonate (MMS)) controls were comparable among labs. Inclusion of the DNA-polymerase inhibitor, aphidicolin (APC), in the protocol improved the predictivity of the assay since it enabled robust detection of pro-mutagens e.g., 7,12-dimethylbenz[a]anthracene and benzo[a]pyrene. Therefore, all negative findings are now confirmed by additional APC experiments before finalizing the classification. Furthermore, MMC, which intercalates between DNA strands causing covalent binding, was detected with the standard protocol where it gave weak but statistically significant responses. Stronger responses, however, were obtained using a cross-linker specific protocol in which MMC reduced the migration of MMS-induced DNA damage. These data support the use of the Phenion® FT in the Comet assay: no false-positives and only one false-negative finding in a single lab. Testing is continuing to obtain data for a total of 30 chemicals. Once validated, the 3D Skin Comet assay is foreseen to be used as a follow-up test for positive results from the current in vitro genotoxicity test battery.

1692 Development of a Human Blood Mutation Assay Based on the Endogenous Pig-a Gene
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The Pig-a erythocyte mutation assay, now widely used in rodents, has the potential to be applied to human samples. This would provide the ability to analyze the same mutation endpoint in humans and the laboratory species used for safety evaluation studies. The current report describes our success extending the methodology to human blood. The frequencies of CD59- and CD55-negative reticulocytes (RET5CD59-CD55-) and erythrocytes (RBC5CD59-CD55-) served as phenotypic reporters of Pig-a mutation. Immunomagnetic separation was used to increase sensitivity by facilitating rapid scoring of large numbers of cells. Replicate analyses were conducted to establish the appropriate number of cells for precise scoring and to evaluate the procedural accuracy. Specimens from a total of 49 nonsmoking, self-reported healthy adult subjects were evaluated (19 females, 30 males; age range 20 - 72 yrs; 12 caucasian, 37 black). The mean frequency of RET5CD59-CD55- and RBC5CD59-CD55- were 5.7 x 10^-6 and 2.8 x 10^-6, respectively. The difference suggests a modest selective pressure against mutant cells in the circulation, and indicates that it is advantageous to study both populations of erythrocytes. Intra-subject variability was found to be low, but inter-subject variability was relatively high, with RET5CD59-CD55- frequencies differing by more than 30-fold. There was an apparent correlation between age and mutant cell frequencies. These results indicate that the frequency of human Pig-a mutant phenotype cells can be efficiently and reliably estimated using a labeling and analysis protocol that is well established for rodent-based studies. The applicability of the assay across species, its simplicity and statistical power, and the relatively non-invasive nature of the assay should benefit many research areas involving DNA damage, including studies of environmental factors that modify "spontaneous" mutation frequencies.

1693 Mutations in the Pig-a Gene of CD48-Deficient T-Lymphocytes from ENU- and DMBA-Treated Rats

The Pig-a assay is proposed as an in vivo gene mutation assay for preclinical safety assessments. In the assay, cells identified by flow cytometry as deficient in GPI-anchored protein surface markers are presumed to have mutations in the endogenous X-linked Pig-a gene. There is little direct evidence, however, of such an association. In the present study, we investigated whether deficiency in the GPI-anchored CD48 surface marker in T cells is due to mutation in the Pig-a gene. We treated groups of male F344 rats with two potent mutagens, ENU and DMBA, and sorted CD48-deficient T-lymphocytes from their spleens into 96-well plates using a FACSAria flow cytometer. Following clonal expansion, Sanger sequencing was performed on cDNA derived from CD48-positive and CD48-negative clones. The expanded sorted cells had mutations in the Pig-a gene – primarily T-A transversions in clones from ENU-treated rats and A-T transversions in the clones from DMBA-treated rats. Both of these spectra were consistent with the types of mutation induced by these compounds in other in vivo models. Spontaneous CD48-deficient mutants from vehicle-treated control rats contained predominantly small insertions/deletions causing frameshifts in the Pig-a reading frame. The differences in the spectra of mutations from the treated and control rats indicate that the Pig-a assay detects what it is intended to detect – de novo mutation in the Pig-a gene.

1694 Integration of Multiple Genetic Endpoints in a 28-Day Repeat-Dose Study: A Feasibility Study to Promote the “3R” Concepts
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An integrated testing strategy involves the assessment of diverse endpoints within a single toxicity study and represents an opportunity to reduce animal usage. The present study evaluated clastogenicity/aneugenicity via the micronucleus test, mutagenicity via transgenic il1 and Pig-a mutation analysis, and primary DNA damage via the Comet assay in multiple tissues in the context of a 28-day, repeat-dose study. Groups of male F344 Fisher rats were administered ENU (1, 10, or 20 mkd via oral gavage on study days 1-5 and 27-29), EMS (200 mkd via oral gavage on study days 1-27), vinblastine (0.25 mkd via tail vein injection on study days 27-29), or vehicle (phosphate buffer pH 6.0 via oral gavage, 10 ml/kg bw dosing volume). All animals were euthanized 3 hours after the last dose. No treatment-re-
lated alterations were noted in clinical signs, final body weights, or organ weights. ENU induced a dose-related increase in micronucleated reticulocytes (MN-RET) frequency (1.2–11.2-fold) and decrease in %RET (97.7–73.7% of control) in the peripheral blood. Administration of EMS or vinblastine also resulted in a significant increase in MN frequency (28.6-fold, respectively). In the Pig-a assay, ENU induced a dose-related increase in the frequency of CDS9-deficient RET (3.8–159.2-fold) and RBC (2.1–7.8-fold), while ENU (20 mM, study days 1–3) increased cII mutant frequency in bone marrow 6.8-fold. Collection of tissue for the Comet assay presents a logistical challenge of integration with a general toxicity study where the test material may be administered in the diet and animals are often fasted overnight prior to necropsy; however, treatment-related increases in %DNA in tail were noted in liver, duodenum, kidney, and bone marrow. Overall these data support the feasibility of integrating multiple (as needed) genetic toxicity endpoints in a repeat-dose toxicity study to meet regulatory requirements, address potential MoA, and promote the “3 R” principles.

1695 Topoisomerase II Inhibitors and Clastogenic Responses in the Low-Dose Region Determined In Vitro Using Human TK6 Cells

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The genotoxic risks associated with low dose exposure to clastogenic agents are thought to decrease linearly into the very low dose region, though recent evidence has suggested that some classes of agents, including topoisomerase II inhibitors, may act through threshold-mediated modes of action. The current study aims to further investigate dose-response relationships of several topoisomerase II inhibitors, including the topoisomerase II poison etoposide, as well as catalytic inhibitors aclacinomycin, merbarone, ICRF-154 and ICRF-187 using both a traditional in vitro micronucleus assay as well as a flow-cytometry based version of the assay. Benchmark dose analysis was used to identify models that best fit the data and estimate a benchmark dose, in this case the dose at which a one standard deviation increase above the control frequency would be expected. All of the agents tested were potent in inducing micronuclei in human lymphoblastoid TK6 cells, with significant increases seen at low micromolar, and in the cases of aclacinomycin and etoposide, at low nanomolar concentrations. Use of the anti-kinetochore CREST antibody with the microscopy-based assay demonstrated that the vast majority of the micronuclei originated from chromosome breaks. In comparing the two versions of the micronucleus assay, significant increases in micronucleated cells were generally observed at lower concentrations using the traditional microscopy-based assay. Benchmark dose modeling of the data exhibited several advantages and proved to be a valuable alternative for analysis of dose-response data producing points of departure comparable to those derived using traditional NOGEL or LOGEL approaches.

1696 Potency Ranking of Seven Clastogenic Agents Based on Benchmark Dose Analysis of Data from In Vitro and In Vitro Micronucleus Studies


Analysis of genotoxicity data for safety-based risk assessment has progressed from simple binary hazard identification to more quantitative approaches that employ computational pathway models to describe the relationship between DRCs and MN formation for two potent doubling dose of MMS, indicating that a distinct DNA repair process, including the topoisomerase inhibitor aclarubicin, merbarone, ICRF-154 and ICRF-187 using both a traditional in vitro micronucleus assay as well as a flow-cytometry based version of the assay. Benchmark dose analysis was used to identify models that best fit the data and estimate a benchmark dose, in this case the dose at which a one standard deviation increase above the control frequency would be expected. All of the agents tested were potent in inducing micronuclei in human lymphoblastoid TK6 cells, with significant increases seen at low micromolar, and in the cases of aclacinomycin and etoposide, at low nanomolar concentrations. Use of the anti-kinetochore CREST antibody with the microscopy-based assay demonstrated that the vast majority of the micronuclei originated from chromosome breaks. In comparing the two versions of the micronucleus assay, significant increases in micronucleated cells were generally observed at lower concentrations using the traditional microscopy-based assay. Benchmark dose modeling of the data exhibited several advantages and proved to be a valuable alternative for analysis of dose-response data producing points of departure comparable to those derived using traditional NOGEL or LOGEL approaches.

1697 Evaluation of Dose-Dependent DNA Repair Center Kinetics and Micronucleus Induction in Chemicals Causing Different Types of DNA Damage

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DNA repair centers (DRCs) are aggregates of repair proteins that bind sites of double strand breaks (DSBs). Left unrepaird, these temporary DSBs may be converted to micronuclei (MN) – small pieces of DNA lost during cell division. We studied dose-dependent DRC kinetics in an effort to better understand how these repair processes affect the shape of the response curves for MN induction. Studies were performed in human fibrosarcoma cells with native p53 (HT1080). Previously, we found that both etoposide (ETP, top II poison) and necazarinostatin (NCS, IR mimic) caused rapid DRC formation (<2 h). At low doses, these DRCs were more efficiently resolved (resolved) with NCS compared to ETP. This difference in DSB repair may explain why the dose-response curve for MN induction is threshold-like for NCS, but linear for ETP (i.e., efficient vs. poor repair of DSBs). The current study examined DRC kinetics in three genotoxic chemicals with distinct mechanisms: methylmethane sulfoxate (MMS, alkylating), mitomycin C (MMC, crosslinking) and H2O2 (oxidation). Similar to NCS, H2O2 induced a rapid DRC response followed by rapid resolution at low doses (≤100 μM), consistent with the threshold-like MN curve. In contrast to the other chemicals, MMC and MMS did not induce DRCs until 24 h, which is consistent with the fact that they only cause DSBS indirectly (misrepaird lesions). Induction of DRCs was effectively prevented at low concentrations (≤60 μM) of MMS, indicating that a distinct DNA repair process prevents DSBs at low doses leading to observed threshold behavior for MN. MMC, however, showed a linear increase in DRCs and a failure of these DRCs to resolve, consistent with poor repair of both initial lesions and subsequent DRGs, as well as the linear MN curve. Taken together, our results indicate that DRC kinetics can help to interpret the shape of dose-response curves for genotoxicity.

1698 Computational Systems Biology Modeling of DNA-Damage Stress Pathways for Assessing Mutation Rates at Low Doses

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Homeostasis by cellular stress response pathways involves negative feedback acting through a series of steps. Many stress response pathways have rapid, post-translational signaling and slower signaling through transcriptional upregulation. We examined multiple biological read-outs in a human cell line (HT1080) treated with several DNA-damaging compounds to support mechanistic computational modeling for micronuclei (MN) formation across wide dose ranges. The readouts included dose and time-dependent whole genome gene expression, DNA repair center (DRC) formation through high content imaging of pH2AX and p5BP1, as well as measures of key proteins in the p53 pathway and MN. Transcriptional upregulation only occurred at concentrations with clear increases in MN formation. Post-translational activation of DNA-repair processes acting through specific kinases appears to be the main contributor to regulation of DNA-damage at lower doses. We have developed computational pathway models to describe the relationship between DRCs and MN formation for two potent double strand break inducers with very different MN-dose-response curves: etoposide (linear) and the gamma irradiation mimic necazarinostatin (threshold-like). These models, ranging from simple empirical descriptions of the data to more biologically oriented descriptions of the homeostatic feedback loop, provide a quantitative framework for assessing the key processes governing MN prevention at low doses of different types of DNA damaging chemicals. Ultimately, these models will support decisions for in vitro only risk assessments, by providing a quantitative description of low dose threshold behavior may be achieved in mutation response, and helping define concentrations leading to cellular adaption and potential adversity.
Recent studies from mammalian, fish, and in vitro models have identified bone and cartilage development as sensitive targets for dioxins and other aryl hydrocarbon receptor ligands. In this study, we assessed how low-level embryonic TCDD exposure impacts axial osteogenesis in Japanese medaka (Oryzias latipes), a vertebrate fish model. Medaka embryos were exposed to a range of TCDD concentrations and reared to 10 dpf (elutherogenesis) and 20 dpf (larval). Individuals were stained for mineralized bone matrix and imaged in vivo to assess alterations within axial vertebrae in relation to quantitative spatio-temporal analysis of osteoblast and osteoblast precursor cell populations. Exposure to 100 ppb TCDD impacted axial bone development through an overall attenuation of skeletal mineralization resulting in truncated centra, and reduced normal and hemal arch lengths. This effect was more pronounced at the larval stage. Effects on mineralization were consistent with modifications in number and localization of transgene-labeled osteoblast and osteoblast progenitor cells. Through targeted RT-PCR, we confirmed altered transcript expression (twist, osterix, and col10a1), and identified a significant reduction in osteocalcin, a marker of differentiated osteoblasts. A global transcriptomic analysis was conducted via RNA-Seq to identify additional signaling and/or disease pathways driving the observed deficits in skeletal formation. IPA identified a number of pathological states that include inflammatory disease, connective tissue disorders, and skeletal and muscular disorders. Interestingly, if-β and osteoclastogenic regulators were enriched within these disease pathways, suggesting that osteoclasts and inflammatory cytokine signaling may also mediate deficits in bone formation. Current in vitro and in vivo studies are underway to investigate the molecular initiating events involving if-β. Taken together, our results indicate that early sublethal TCDD exposure impacts axial bone development at apical endpoints through proliferation and/or differentiation of osteoblasts and osteoclasts.

Increasing evidence shows that environmental influences during key developmental periods can affect epigenetic patterns in the brain. DNA methylation is a major epigenetic regulator, especially of genes containing CpG-rich sequences surrounding their transcriptional start site, such as the androgen receptor. The androgen receptor (AR) plays a crucial role in brain morphology and reproductive behavior. Here, we studied changes in DNA methylation of the murine AR promoter in primary murine neural progenitor cells (mNPCs). Global methylation percentage was determined in undifferentiated cells and at day 7 of differentiation using Methylationfloors/quantification kit. The methylation pattern of the AR was determined using bisulfite conversion followed by cloning and DNA sequencing. The results show that during the 7-day differentiation, global methylation slightly increased in mNPCs. In undifferentiated mNPCs, the 17 β-Gs in the AR promoter region showed around 10-20% methylation. After 7 days of differentiation, this increased to 21-49% methylation, depending on the CpGs. Gene expression of DNA methyltransferase DNMT1 was unaltered, while expression of DNMT3a and DNMT3b increased after 7 days of differentiation. Interestingly, gene expression of AR and estrogen receptors (ER) α and β also increased. Similar results for gene expression were found in the well-established embryonic stem cell differentiation assay using mouse ES-D3 cells. Both mNPCs and ES-D3 cells showed electrical activity after 7 days of neuronal differentiation, as determined using multielectrode arrays (MEA). Together, these data will aid further characterization and development of predictive in vitro developmental neurotoxicity (DNT) models. Studies to determine DNT effects should include epigenetic modifications of key proteins involved in neurodevelopment such as AR and ERs, that are prerequisite for correct neuroendocrine development and reproductive behavior later in life.

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The Theory of the Developmental Origins of Health and Disease proposes that the environment encountered during fetal life shapes the organism’s structure, function and metabolism. Accordingly, damage resulting from environmental stress may be at the heart of congenital and adult onset diseases. Infants born to mothers exposed...
to dioxin-like compounds, which exert their effects by binding to the aryl-hydrocarbon receptor (AHR), exhibit a higher incidence of congenital heart disease, the leading cause of neonatal mortality and a major source of adult cardiac insufficiency. In humans and mice, Nkx2-5 mutations lead to congenital heart defects and cardiomyopathies. We find that full-body ablation of the Ahr gene in mice or exposure to dioxin in utero cause a decreased of cardiac Ahr and Nkx2-5 expression with resulting abnormal transcriptome, as well as structure and function in the developing heart, and cardiomyopathy in the adult. Knock-in of the cre recombinase gene into an Nkx2-5 allele results in cardiomyopathy due to haplinsufficiency. To determine the role of AHR in the penetrance of this pathological Nkx2-5 haplinsufficiency phenotype, we generated mice with a cardiac-specific deletion of the Ahr gene. Echocardiography analyses revealed that the ejection fraction and other critical indices of cardiac function were significantly decreased with age in haplinsufficient Nkx2-5/+;creAhr/+- mice but not in Nkx2-5/+;creAhr/+/x, in which both copies of the Ahr had been deleted in cardiomyocytes. Our data show that deletion of the Ahr gene during normal cardiac development caused the Nkx2-5 haplinsufficiency and suggest that this pathological phenotype is a result of AHR-NKX2-5 interplay. These findings illustrate gene-gene-environment interactions as the targets of perinatal environmental exposures, underscoring significant implications to human health and disease. Supported by NIEHS R01086273.

1704 Neatinal Gene Expression: Marks of Prenatal Exposure to PFOA, PFOS, PCB153, and DDE
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The prevalence of obesity and/or diabetes has reached alarming proportions globally. In the European FP7 project OBELIX (OBesogenic Endocrine disrupting chemicals: Linking prenatal exposure to the development of obesity later in life) the hypothesis was examined that prenatal exposure to endocrine disrupting chemicals (EDCs) plays a role in the development of obesity later in life. One of the objectives of the project was to relate early life exposure to EDCs with neonatal effect biomarkers and health outcome data which are related to risk of obesity later in life. We examined associations between prenatal EDC exposure and changes in the cord blood transcriptome. The panel of EDCs included dichlorodiphenyldichloroethylene (DDE), perfluoroacanonic acid (PFOA), perfluorooctane sulfonate (PFOS), and polychlorinated biphenyl-153 (PCB153). Upstream transcription factor analysis (Ingenuity®) revealed that the progesterone receptor may be implicated by PFOA exposure. Inhibition of ESR2 (estrogen receptor 2) was associated with exposure to PCB153. The most significant transcription factor associated to DDE was NR3C1 which is also known as the glucocorticoid receptor (GR). In addition to metabolic diseases, this receptor is also involved in asthma. It has been reported that the prevalence of asthma increases with increasing DDE levels. Therefore we hypothesize that adverse regulation of the glucocorticoid receptor may contribute to the development of asthma. In this study, we investigated whether BBP exposure promotes adipogenesis in the preadipocyte 3T3-L1 model, and examined the underlying mechanisms. Preadipocyte 3T3-L1 was treated with BBP at doses of 0.1 – 200.0 μM along with positive controls. Cell viability was measured by Neutral Red uptake assay. The lipid accumulation in 3T3-L1 cells was stained with oil-red, and quantified by AdipoRed fluorescence. Total RNA was extracted during various stages of differentiation, and gene expressions of the adipogenesis associated genes and epigenetic regulated genes were examined by Realtime qPCR. Protein expressions of Acetyl-CoA Carboxylyase (ACC), Adiponectin, C/EBP, Fatty acid synthase (FASN), PARP, and Perilipin were also conducted. BBP showed no significant effect on the cell viability at concentrations of 0.1 – 100.0 μM after 24 and 48hs exposures. BBP significantly induced lipid accumulation dose-dependently. Gene expression of adipogenic transcription factors, PPARγ, C/EBP, AdiporQ, Adipokines, Fatty aci-binding protein 4 (FABP4) and enzymes that regulate adipogenesis, Lipoprotein lipase (LPL), FASN were dose-dependently increased in cells treated with BBP.

1705 Transcriptional Effects of Developmental Exposure to Low-Dose Zeranol on Sexual Development, Reproduction, and Mammary Carcinogenesis
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Zeranol (Zer) is a potent semi-synthetic derivative of zearalenone, a myco-estrogen that contaminates grain. Livestock are deliberately dosed with Zer as a growth promoter in the US, it was developed as a substitute for the carcinogenic diethylstilbestrol (DES). Zer is banned in Europe and Asia, it is detected in finished food products and extremely stable at cooking temperatures with a long half-life in humans. Occupational exposures to Zer are associated with precocious puberty. Our studies in pubescent girls indicate that human exposure is primarily via the consumption of beef and corn, and urinary levels of unconjugated Zer are associated with altered onset of puberty, height and weight (Bandera et al., 2011). Our studies in mice indicated that exposure to Zer (between PND9 to weaning): at doses below the human ADI (1.25μg/kg/day) resulted in precocious puberty (defined as a 3 day decrease in age at vaginal opening) in F1 progeny, increased uterine weight and abnormally prolonged estrous cycle. F1 male progeny showed feminization assessed by both decreased anogenital distance and sperm count. F1 females treated with a single carcinogenic dose of N-nitros-N-methylurea (NMU) showed decreased latency, increased incidence of mammary tumors and greater tumor mass. Similar effects on puberty and carcinogenesis were also observed in the F2 progeny, but only if both the dam and sire were exposed to Zer in utero, suggesting recessive epigenetic inheritance. Studies on the F3 generation demonstrated a significant decrease in fecundity, male offspring and delayed vaginal opening in F3 female progeny. Together these studies suggest that in utero exposure to the Zer produces transgenerational effects on sexual maturation and susceptibility to chemical-induced carcinogenesis. Support: The New Jersey Commission on Cancer Research, Rutgers-EOHSI and NIEHS-Center for Environmental Exposures and Disease Rutgers the State University of NJ.
to promote adipogenesis dose-dependently through the activation of adipogenic pathway and the modification of epigenetic regulation (Supported by R21 OH010473).

**1708 BDE99 (2, 2’, 4, 4’, 5-Pentabromodiphenyl Ether) Treatment Promotes Adipogenesis in 3T3-L1 Cells**

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Flame retardants, such as polybrominated diphenyl ethers (PBDEs), are chemical compounds added to materials such as, textiles, plastics, wire insulation, and automobile to delay the onset of fire. They are widely used for industrial purposes and household materials. NHANES data indicates that nearly all Americans have trace amounts of PBDEs in serum, with even higher levels associated with occupational exposure (foam recyclers and carpet installers). In particular, PBDEs have been detected in human adipose tissue. Therefore, it was hypothesized that PBDE congener, BDE-99 might modulate 3T3-L1 fibroblast cell differentiation to adipocytes. 3T3-L1 cells were grown in maintenance media (DMEM containing 10% fetal calf serum, 1% penicillin-streptomycin). Post-confluent cells were induced with supplemented treatment media (DMEM 10% FBS, 1% penicillin-streptomycin and 1% glutamine) of 500 M IBMX, 0.25 μM dexamethasone, and 10 μg/ml insulin for 48 hrs. Pre-adipocyte morphology (fibroblasts with spiral morphology) was observed, and the cells were allowed to differentiate to adipocytes in the presence of 0.025% DMSO (control) or 5 μM BDE-99 for 4 and 6 days in treatment media. The treatment media was changed every 2 days. Oil Red O was used to detect lipid development within the pre-adipocytes during differentiation and the dye was measured via spectrophotometric assay for quantitative analysis. RNA was isolated in order to analyze gene expression of regulatory transcription factors related to adipocyte differentiation. BDE-99 treatment during differentiation of 3T3-L1 fibroblasts induced lipid accumulation by 36% compared to DMSO control. The transcription factor PPARY is induced by 1.5-fold at Day 4 of differentiation with treatment of BDE-9. These preliminary findings suggest that BSE-99 has the potential to promote adipocyte differentiation.

**1709 Oculomotor Deficits in Aryl Hydrocarbon Receptor Null Mouse**

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The Aryl hydrocarbon Receptor or AhR, a ligand-activated transcription factor, is known to mediate the toxic and carcinogenic effects of various environmental pollutants such as 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD). Recent studies in Caenorhabditis elegans and Drosophila melanogaster show that the orthologs of the AhR are expressed exclusively in certain types of neurons and are implicated in the development and the homeostasis of the central nervous system. While physiological roles of the AhR were demonstrated in the mammalian heart, liver and gametogenesis, its ontogenic expression and putative neural functions remain elusive. Here, we report that the constitutive absence of the AhR in adult mice (AhR-/-) leads to abnormal eye movements in the form of a spontaneous pendular horizontal nystagmus. To determine if the nystagmus is of vestibular, visual, or cerebellar origin, gaze stabilizing reflexes, namely vestibulo-ocular and optokinetic reflexes (VOR and OKR), were investigated. The OKR is less effective in the AhR-/- mice suggesting a deficit in the visuo-motor circuitry, while the VOR is mildly affected. Furthermore, the AhR is expressed in the retinal ganglion cells during the development, however electroretinograms revealed no impairment of retinal cell function. The structure of the cerebellum of the AhR-/- mice is normal which is compatible with the preserved VOR adaptation, a plastic process dependent on cerebellar integrity. Finally, intoxication with TCDD of control adults did not lead to any abnormality of the oculomotor control. These results demonstrate that the absence of the AhR leads to acquired central nervous system deficits in the adults. Given the common features between both AhR mouse and human infantile nystagmus syndromes, the AhR-/- mice might give insights into the developmental mechanisms which lead to congenital eye disorders.

**1710 Background Data of Wistar Hannover Rats for Developmental Toxicity Study: Comparison of Two Substrain of Rats**


Background data of the two sub-strains of Wistar Hannover rats (RccHanTM: WIST and Crl: WI (Han)) for developmental study were compared. Virgin female rats were mated overnight with male rats of the same strain and inspected for the presence of a vaginal plug or sperm the following morning. The day when a vaginal plug or sperm was detected was considered day 0 of gestation. Pregnant rats were observed for their general condition, body weight change, and food consumption during the gestation period. On day 20 of gestation, the rats were euthanized and uterine horns were exposed. Live fetuses were examined for their external, skeletal and visceral anomalies. There was little difference in the body weight changes or food consumption of dams during the gestation period, number of implantations, number of live fetuses, fetal mortality, body weight of live fetuses, or placental weight between the two sub-strains of rats. The incidence of external, skeletal and visceral anomalies was low in both sub-strains. The total incidence of skeletal variation was similar between these sub-strains, however the incidence of lumber rib (especially shorter rib) was higher in the Crl: WI (Han) rats.

**1711 A Comparison of Rat and Rabbit Developmental Toxicity Study Outcomes of More Than 400 Pharmaceutical Compounds**

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Nonclinical developmental toxicity testing of pharmaceuticals is regularly performed in two species, most often in rat and rabbit. Given the wealth of existing data, the question can be asked retrospectively to what extent the second species study (be it in rat or rabbit) has contributed significantly to the overall conclusion about developmental toxicity of the compound tested. In collaboration with the Dutch Medicines Evaluation Board (MEB) and with the HESI Developmental And Reproductive Toxicity (HESI-DART) technical committee we have collected and entered developmental toxicity test data in rat and rabbit of over 400 pharmaceutical compounds in a modified ToxReID database format. The compounds included registered as well as failed pharmaceutical products, and data originated from governmental agencies (through MEB) and industry (through HESI-DART). Between rat and rabbit studies, we have compared effective systemic doses (AUC, Cmax), the nature of developmental effects (death, malformations, growth retardation, variations), the role of maternal toxicity, and the influence of pharmacological targets and mode of action. This study will feed into international discussions about innovations in nonclinical safety testing.

**1712 Nonclinical Embryo-Fetal Development Assessment of GLYX-13, an NMDAR Novel Modulator, in Rats and Rabbits**

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GLYX-13 is a novel modulator of the NMDAR receptor that has been granted Fast Track designation by the FDA, for development as an adjunctive therapy for major depressive disorder (MDD). GLYX-13 has completed Phase 2 clinical proof-of-concept studies for MDD in patients with inadequate response to antidepressants. Dose-range finding and full embryo-fetal developmental studies were conducted in Sprague Dawley (SD) rats and New Zealand White (NZW) rabbits. In the dose-range finding studies, no maternal/fetal findings were noted in dosing up to 300 mg/kg/day in both species. The Incidence of maternal mortality was less than 5% for all treatment groups.
at ≥ 400 mg/kg/day were noted. In the full embryo-fetal studies, groups of 25 time-mated female SD rats were administered 0, 30, 90 or 300 mg/kg/day GLYX-13 intravenously on GD 6 to 17 and the fetuses were examined for external, visceral, and skeletal findings on GD 21. Similarly, groups of 22 time-mated NZW rabbits were administered 0, 30, 100, or 300 mg/kg/day GLYX-13 intravenously on GD 7 to 19 and the fetuses were examined for external, visceral, and skeletal findings on GD 29. Additional groups of 9 rats and 3 rabbits per dose group were used for toxicokinetic evaluation. GLYX-13 had no effect on mortality, physical examinations, body weight or body weight changes, food consumption, gross pathology, pregnancy, and external, visceral, and skeletal fetal findings. The anticipated maternal dose of 5 mg/kg is approximately 10 or 20-fold lower than the Human Equivalent Dose of the 300 mg/kg/day dose in rats and rabbits, respectively based on body surface area. The 300 mg/kg/day dose in rats corresponded to a C_{max} of 2,550 and 2,100 μg/mL and AUC_{0-24 h} of 22,100 and 26,300 μg·h/mL on GD 6 and 17, respectively. The 300 mg/kg/day dose in rabbits corresponded to a C_{max} of 1,800 and 2,220 μg/mL and AUC_{0-24 h} of 57,300 and 63,200 μg·h/mL on GD 7 and 19, respectively. These embryo-fetal studies provide a relative exposure ratio (animal:human) of GLYX-13 of 77 to 89 based on C_{max} and 84 to 209 based on AUC_{0-24 h}, at the human dose of 5 mg/kg.

1713 Pregnancy Outcomes following Short-Term Treatment of Mice with the Farnesoid X Receptor Agonist GW4064

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The Farnesoid X Receptor (Fxr) is critical for the regulation of bile acid synthesis, metabolism and excretion. Previous work from our laboratory has demonstrated that a number of direct hepatic targets of Fxr are dysregulated during pregnancy. Further, pharmacological activation of Fxr with the specific agonist GW4064 (GW) in pregnant mice restores bile acid pathway gene and protein profiles to the patterns observed in virgin mice. However, the effects of GW on pregnancy outcomes have yet to be examined. To determine the safety and effects of GW for use in mouse pregnancy studies, plasma and liver samples were assessed from pregnant C57Bl/6 mice orally gavaged with vehicle or 100 mg/kg GW, on gestation days 13 and 14. Both vehicle- and GW-treated pregnant mice had increased liver-to-body weight ratios as compared to virgin control mice. As expected, plasma progesterone levels increased by 6.5-fold with pregnancy. Compared to vehicle-treated pregnant mice, progesterone levels were reduced by 25% with GW treatment, though still within the normal range for pregnancy. Pregnancy increased triglycerides by nearly 2-fold, which was not altered by treatment with GW. No significant differences were observed in cholesterol levels, or total bile acids among treatment groups. A similar number of resorptions (12-16%) were noted for vehicle- and GW-treated mice at the time of sacrifice. These data demonstrate that short-term treatment of a high dose of GW has limited consequence to mouse pregnancy status, supporting its continued evaluation as a pharmacological agonist of Fxr for in vivo pregnancy studies. Supported by an American Foundation for Pharmaceutical Education Fellowship, R01E020522, T32ES007148, and P30ES005022.

1714 Decreased Maternal and Fetal Cholesterol following Maternal Bococizumab (Anti-PCSK9 Monoclonal Antibody) Administration Does Not Affect Rat Embryo-Fetal Development

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Bococizumab is a humanized monoclonal IgG2a antibody against proprotein convertase subtilisin/kexin type 9 (PCSK9) for the treatment of hyperlipidemia that is pharmacologically active in rats and non-human primates. To evaluate potential effects on embryo-fetal development, studies were conducted in the rat only. In a pilot study bococizumab was administered intravenously to pregnant Sprague-Dawley (SD) rats at dose levels of 0, 10, 30 and 100 mg/kg every 3 days throughout organogenesis. Maternal blood samples were collected throughout gestation for determination of total cholesterol, high density lipoprotein cholesterol (HDL), and bococizumab concentrations. Fetal blood samples were collected on GD 18 and GD 21 for determination of total cholesterol, HDL, and bococizumab concentrations. Cesarean section evaluation of ovarian and uterine parameters and fetal external evaluations were conducted on GD 21. Bococizumab was well tolerated and there were no effects on organ weight or organ volume. Maternal and fetal bococizumab exposure increased with increasing dose, and there was a corresponding dose-dependent decrease in fetal cholesterol concentrations. Maximal reductions in maternal cholesterol were observed at all dose levels. In the definitive embryo-fetal development study bococizumab was administered to pregnant SD rats at the same dose levels used in the pilot study, and no adverse maternal or embryo-fetal developmental effects were observed. These studies have provided an appropriate and relevant safety assessment of bococizumab in pregnant rats to inform human risk assessment, demonstrating no adverse effects on embryo-fetal development in the presence of significant reductions in both maternal and fetal cholesterol concentrations.
1717 Maternal and Prenatal Dose Range-Finding Study of 4-Methylcyclohexanemethanol (MCHM) in Harlan Sprague-Dawley Rats

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4-methylcyclohexanemethanol (MCHM), used during the purification of coal, leaked from storage containers into the Elk River of West Virginia resulting in potential exposure to the residents of the area. The National Toxicology Program is conducting a prenatal toxicity evaluation in the Harlan Sprague Dawley rat. Doses for the range-finding study were 0, 150, 300, 600, and 900 mg/kg/day MCHM administered via gavage (corn oil vehicle) from gestational day (GD) 6-20 to rats (n=10). Parameters evaluated in dams included maternal weight, clinical observations, body weights, food consumption, gravid uterine weight, and postmortem observations on GD 21 (gross visceral examination, corpora lutea, implantations, embryos, fetal mortality). Parameters evaluated in offspring included sex, weight, external abnormalities, and placental appearance. Due to excessive maternal toxicity, the 900 mg/kg/day group was terminated early as there were three dams in the 600 mg/kg/day group. In the 600 mg/kg/day group, fetal weight was decreased and post-implantation loss was increased (53.3% vs. 9.8% in controls) due to increased number of dead fetuses and early/late resorptions. At 300 mg/kg/day, embryo-fetal toxicity consisted of decreased fetal weight (12%). There was no increase in fetal gross external observations among the dose groups. These data provide preliminary maternal and prenatal toxicity information on MCHM and will guide the design of the full prenatal toxicity evaluation.

1718 Prenatal Developmental Toxicity of Tris(Chloropropyl) phosphate (TCPF) following Oral Exposure in Hsd:Sprague-Dawley SD Rats


TCPF is ubiquitous in the environment through its use as a high production flame retardant and plasticizer. Health effects related to TCPF exposure are of concern occupationally and to the general population, including women of childbearing potential. The objective of this study was to characterize the maternal and prenatal toxicity of TCPF which included a dose range finding (DRF) study to select doses for a definitive study. In both studies, time-mated female rats were dosed once daily by oral gavage from gestation day (GD) 6 – 20 before a laparotomy and fetal assessment on GD 21. In the DRF, rats (n=11/group) were administered 300, 600, and 1000 mg/kg TCPF or vehicle (0.5% w/v aqueous methylcellulose). Text article (TA)-related maternal toxicity was observed only in the 1000 mg/kg TCPF group: Adverse clinical signs including tremors, gasping, and piloerection as well as transient decreases in maternal body weight gain and food consumption were associated with excessive mortality/morbidity in 7 of 11 animals. Lower numbers of implants and live fetuses were noted in the 1000 mg/kg surviving dams. There were no maternal or fetal findings in the 300 or 650 mg/kg groups. In the subsequent study, TCPF was administered at 0, 162.5, 325, and 650 mg/kg (n=25/group). There were no effects of TCPF-exposure on maternal survival, body weight, body weight change corrected for gravid uterine weight, or food consumption. TA-related findings in the dams included minimal transient clinical observations at 650 mg/kg and significantly increased relative liver weights in the 325 mg/kg (14%) and 650 mg/kg (26%) dose groups. There were no TA-related gross pathology findings in the dams. TCPF-exposure did not affect maternal body weight gain, fetal weight, or sex ratio. Effects on placental morphology or external, visceral, and skeletal fetal morphology were not observed. These data suggest that TCPF does not result in prenatal developmental toxicity under the conditions of this study.

1719 In Utero Exposure to the Vinca Alkaloid Vinpocetine Is Associated with Rat Embryo-Fetal Toxicity

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Vinpocetine, a semi-synthetic derivative of the Vinca minor extract vincamine, has been reported to inhibit phosphodiesterase type 1 and voltage-sensitive Na2+ channels, and display vasodilatory activity in cerebral tissue. Human exposure to vinpocetine occurs through its use as dietary supplement for its purported nootropic and neuroprotective effects. Initial perinatal studies with the structurally similar vincamine demonstrated that exposure, during the period of major organogenesis, was associated with embryo-fetal toxicity and absence of offspring at doses ≥ 30 mg/kg/day. Given the embryo-fetal toxicity observed with vincamine, this study was designed to generate preliminary information on the potential maternal and fetal toxicity of vinpocetine. Time-mated Harlan Sprague Dawley rats (n=10) were exposed via gavage to 0, 20, 40, 80, 160, and 320 mg/kg of vinpocetine from gestation day (GD) 6 to 20. Dams administered ≥ 40 mg/kg of vinpocetine exhibited dose-related decreases in body weight gain, as well as concomitant increases in vaginal discharge that was observed from approximately GD13 to 20. At necropsy, dams exposed to ≥ 80 mg/kg exhibited one or two fetal resorptions. This finding was also observed in one dam in each of the 20 and 40 mg/kg dose groups and was also associated with vaginal discharge. Uterine examination revealed that the total resorptions were occurring early at ≤ 20 mg/kg and were associated with a dose-dependent decrease in the number live fetuses in these dose groups. There were no effects on fetal weight or external fetal examination findings at terminal sacrifice. This study in the rat indicates that vinpocetine exposure is associated with post-implantation loss. Future work includes a definitive teratology study, and potentially a rabbit teratology study to examine cross-species differences in vinpocetine response.

1720 Developmental Toxicity Studies (OECD 414) with 6 Different Titanium Dioxide Materials (3 Pigment-Grade & 3 Nanostructured) Demonstrate Negative Effects in Orally-Exposed Rats

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Six pigment-grade (pg) or ultrafine (uf) nanostructured (anatase and/or rutile) titanium dioxide (TiO2) particulates were evaluated for maternal and developmental toxicity. The studies were conducted at two labs and were in compliance with OECD Guideline 414 (Prenatal Developmental Toxicity Study). All test materials were robustly characterized. The BET surface areas of the pg and uf samples ranged from 7 to 17 m2/g and 50 to 82 m2/g, respectively. The test substances, suspended for sterile water, were administered once daily from around the time of implantation to just prior to parturition. In three of the studies (uf 1, u3, and pg 1), the Cr:CD(SD) rat strain was used, whereas in the remaining three studies, the Wistar rat strain was selected for evaluation. The dosing period was gestation day (GD) 6 to 20 or GD 5 to 19 for the SD or Wistar strains, respectively. The dose levels selected for all studies were 0, 100, 300, or 1000 mg/kg/day. In-life data collection included monitoring maternal body weights, food consumption, and clinical observations. Dams were euthanized on GD 21 or 20 for the SD or Wistar strains, respectively. The dams were examined grossly and gravid uterine weights were collected after which uterine cesarean sections were performed. Counts were recorded for corpora lutea, implantations, resorptions, and live and dead fetuses. Live fetuses were weighed, sexed, counted, and euthanized. Fetal external, visceral, and skeletal alterations were recorded. There were no adverse test substance-related effects on any maternal or fetal endpoint in any study. In conclusion, there was no evidence of either maternal or developmental toxicity for any substance tested; the maternal and developmental NOAEL determined for either 1000 mg/kg/day, the highest dose tested as well as the limit dose as defined by the guideline.

1721 Maternal Inheritance of PM2.5 Alters Fetal Cardiac Function, Fetal Size, and Postnatal Body Weight in Mice

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Research has shown a causal link between air pollution, especially fine-sized particulate matter (PM2.5) and cardiovascular disease. While many epidemiologic studies demonstrate the health detriments of PM2.5, few effects are less explored. This study determined if PM exposure during gestation impacts fetal cardiac function, fetal size and post-natal growth. Pregnant B6C3F1 mice were exposed (6hr/d) to concentrated ambient PM (CAPs) from Tuxedo, NY or to filtered air (FA), either from gestational day (GD)6-16.5 (Window 1) or only during organogenesis GD6-14.5 (Window 2). Fractional area change (FAC) of both ventricles, an indicator of ventricular dysfunction, and crown-to-rump length (CRL) were measured by ultrasonic-biomicroscopy (UMB) on GD12.5 from 3 embryos per dam (n=6 dams/group). Another set of dams exposed only during Window 1 was sacrificed on GD17.5; fetal lengths were measured, and placentas, hearts and livers were collected. N=4 dams/group. Juvenile male and female offspring of each UMB-imaged dam were sacrificed at 4-weeks-of-age; bodyweights (BW) were assessed, and blood, hearts and livers were collected. Average CAPs and FA concentrations over the experiment were 113µg/m3 and 4.3µg/m3, respectively. Results from UMB demon-
strate that fetuses exposed to CAPs during Window 1 had a significant decrease in FAC compared to control (43% vs. 54%, respectively). UBM CRL analysis showed a 16% decrease from CAPs exposure during Window 2 vs. FA (8.1mm vs. 9.5mm, respectively). Window 1 CAPs exposure resulted in a 6% decrease in length compared to FA vs. fetuses 16 in dams sacrificed on GD17.5. Juvenile male offspring exposed prenatally during Window 2 exhibited a decrease of ~9% in BW between treatment groups and compared to Window 1 males. This study suggests that some cardiac pathologies linked with PM exposure in adults may actually begin in utero.

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1722 Effects of Maternal Hypoglycemia on Fetal Eye Development in Rats


Introduction: It has been reported that administration of hypoglycemic drugs to pregnant rats causes eye anomalies in the fetus, however, the underlying mechanism remains unclear. We have previously demonstrated that injection of insulin glargine [rDNA origin] (200 IU/kg), a long-acting insulin analogue, to pregnant rats caused eye anomalies (anophthalmia or microphthalmia) in 11.8% of fetuses and the findings were related to the severity and duration of maternal hypoglycemia (Suzuki et al. JSOT 2013). In the present study, we investigated disruption of fetal eye development and its time course in rats with insulin-induced maternal hypoglycemia by histopathological examination. Material and Methods: Insulin glargine [rDNA origin] (200 IU/kg) was injected subcutaneously to pregnant Crl:CD(SD) rats from day 6 to day 11 of pregnancy. Cesarean section was carried out on days 13, 14, 15, 17 and 20 of pregnancy, and eyes of the fetuses were examined histopathologically. Results and Conclusion: In the control group, the retina and lens vesicle were observed on embryonic day 13 (E13), and lens vesicle had a very tiny cavity. On E14, the lens was almost filled by lens fiber. On E15, two layers of the neural retina were clearly distinguished and the cornea had formed. On E20, the eyelid was closed. On the other hand, in the insulin-treated groups, hypoplasia and increased cell debris compared to the control group were observed in the retina and lens vesicle on E13 and E14, and lens vesicle had a large cavity. Furthermore, degeneration of the lens fiber and hypoplasia of the cornea were observed on E15. On E17 and E20, the aforementioned hypoplasia and degeneration were still observed. Throughout the eye development from E13 to E20, the magnitude of disruption of the development was similar in the lens, retina and cornea in each eye. These results suggest that maternal hypoglycemia causes persistent retardation and disruption of fetal eye development in rats.

1723 Maternal Bisphenol A Levels Have Gender-Specific Effects on Gestational Length and Birth Weight

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Gestational exposure to bisphenol A (BPA) is associated with adult metabolic diseases in offspring, emphasizing the need for accurate determination of BPA exposure in maternal/fetal circulation for risk assessment. Women (18 years, natural conception, and singleton pregnancy) were recruited from the University of Michigan Hospital. First trimester maternal samples (M1; 8-14 weeks of pregnancy) and maternal (MT) and umbilical cord (CT) samples at term were collected (n=80). Plasma unconjugated (uBPA) and glucuronidated BPA (gBPA) concentrations were quantified using HPLC and MS/MS by one of the US laboratories validated through the NIEHS funded Round Robin study. Majority of women were white, 30-35 years old, and had normal BMI. ANOVA showed that uBPA and gBPA levels differed across the 3 sample series (M1; MT; CT) with uBPA levels being highest in MT followed by M1 and CT (P<0.02) and gBPA levels being highest in M1 followed by MT and CT (P<0.01). Plasma uBPA and gBPA levels were positively correlated across M1, MT and CT, but not within series. Race influenced levels of BPA with uBPA levels in M1 and gBPA levels in M1 and MT being highest in all other races combined relative to African American and Caucasian. uBPA levels were higher in CT in first time pregnancies and recently exposed to dental fillings. A gender specific decrease in birth weight was evident, with increased uBPA with a 10-fold increase in uBPA associated with 607 g less birth weight for M1 (P<0.005) and 317 g less birth weight for MT (P<0.05) in female, but not male pregnancies. A 10-fold increase in MT uBPA was also associated with 3.6 days of longer gestation (P=0.06) in female pregnancies. The current study is the first longitudinal study addressing the exposure risk to BPA in first trimester, term-matched maternal, and cord blood samples relating high BPA exposure to adverse pregnancy/birth outcomes. NIEHS R01 ES017005 and P01 ES022844, USEPA P01 RD 83543601.

1724 Developmental Toxicity Evaluation in the Cynomolgus Monkey (Macaca fascicularis): Does Mauritian Origin Matter?

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Recent advances in genomic typing revealed interesting insights on the genetic diversity and hybridization among different macaque species and also among cynomolgus monkeys of different geographic origin whilst the phenotypic relevance of these genomic differences is not fully clear (for review Haus et al 2014, TIGS 30:426). Mauritian cynomolgus monkeys have been genetically isolated for several hundred years and are considered comparatively inbred. Interestingly, it has been reported that testicular maturation occurs approx. 2 years earlier in Mauritian island compared to Asian mainland (China and Vietnam) animals (Luurtjes and Weinbauer 2012, Reg Toxicol Pharmacol 63:391). The present investigation was undertaken to evaluate whether developmental toxicity parameters differ between these origins. A limited data set from 60 pregnant control Mauritian animals (two embryofetal development studies and three pre-/postnatal development studies with 21 control infants) was available whilst a comparatively larger dataset from > 1000 pregnant control animals and ∼120 control infants is already available for Asian mainland animals (Jarvis et al 2010, Birth Defi Res B 89:175). Within the pregnancies prenatal losses ranged from 17-53% and stillbirths from 0-17% of Mauritian origin being within the normal limits reported for Asian animals from this laboratory, thus suggesting that pregnancy success is unrelated to these origins. Gestation durations and birth weights were comparable among origins (p>0.05). Unexpectedly, however, in spite of similar birth weights, Mauritian animal infants grew faster and this was more pronounced for males compared to females (ca. 30%) with data available until about 18 months of age. In conclusion, some key developmental/postnatal parameters appear comparable between animals of two different origins Asian mainland vs Mauritian island whilst for certain parameters origin differences apparently exist to the extent that maintenance of separate reference databases is recommended.

1725 MicroPET/CT Assessment of FDG Uptake in Brain after Long-Term Methylphenidate Treatment in Nonhuman Primates

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Methylphenidate (MPH) is a psychostimulant commonly used for the treatment of Attention-Deficit Hyperactivity Disorder (ADHD). Since the long-term effects of this drug on the central nervous system (CNS) are not well understood, we conducted microPET/CT scans on young adult male rhesus monkeys (n=4/group) to gather information on brain metabolism using uptake of [18F]F2-deoxy-2-D-glucose (FDG). Approximately two-year-old, male rhesus monkeys were treated orally with MPH twice per day, five days per week over a 6-year period. Subjects received either (2.5 or 12.5 mg/kg/dose or vehicle (Prang). To minimize the acute effects of MPH on FDG uptake, microPET/CT scans were scheduled on Mondays before their first daily dosing of the week (more than two days since their last treatment). FDG (310±8.88 MBq) was injected intravenously and thirty minutes later microPET/CT images were obtained over 60 minutes. Radiolabeled tracer accumulation in regions of interest (ROIs) in the frontal cortex, temporal cortex and cerebellum were converted into Standard Uptake Values (SUVs). Compared to the control group, the uptake of FDG in the cerebellum was significantly increased in both the low and high dose groups. FDG uptake in the temporal cortex was elevated and decreased in the frontal cortex of animals in the high dose group. These preliminary data demonstrate that microPET imaging is capable of distinguishing differences in retention of FDG in the brains of NHPs and suggests that this approach may provide a minimally invasive biomarker for exploring the effects of chronic MPH treatment on brain function.
1726 Evaluation of the Morris Water Maze Using ANY-maze™ Software Increases Consistency and Throughput of Learning and Memory Testing

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As methods improve for testing neurobehavioral endpoints in pre- and postnatal (PPN) studies in the rat, technology for measuring such endpoints has likewise improved. Under the ICH Harmonised Tripartite guideline S5(R2), assessment of sensory function, motor activity, and learning and memory are suggested endpoints for PPN studies. Historically, numerous tests and methods have been employed industry wide to assess learning and memory in the rat. The Morris water maze (MWM) is commonly used to assess spatial learning and memory, which has been shown to be hippocampus-dependent. Here, the Morris water maze, in conjunction with ANY-maze™ software was utilized to assess learning and memory in adult male rats to validate its use on GLP studies. Animals (10/group) were treated once daily with 0, 0.5, 1, or 3 mg/kg/day of (−)-scopolamine HBr, i.p., thirty minutes prior to testing. Testing consisted of three parts: cued, spatial learning, and reference memory. Scopolamine is a muscarinic acetylcholine receptor antagonist that is known to cause MWM learning and memory deficits in rats and doses were selected to induce such effects based on established literature. At all doses, (−)-scopolamine HBr robustly impaired spatial learning and memory in adult male rats. These animals were similarly impaired in a non-spatial, cued learning task in all treated groups, suggesting (−)-scopolamine HBr treatment resulted in profound, non-specific learning impairments. Learning deficits were independent of swim speed, which suggests they were not due to motor impairments. Additionally, animals administered 3 mg/kg/day displayed stereotypical behaviors not observed at other doses. The effects of (−)-scopolamine HBr on learning and memory were of sufficient potency to mask hippocampus-specific deficits. However, this study provided useful data for the use of MWM learning assessment in GLP studies.

1727 Temporal Sensitivity of the Developing Cardiovascular System to Nanoparticle-Derived Nitric Oxide and Nitrrosylating Species

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Nitric oxide (NO), the endothelial relaxation factor, has integral roles in the cardiovascular, nervous, and immune systems. Importantly, NO is a potent vasodilator and anti-thrombotic agent, yet NO’s contribution to cardiovascular diseases highlights the dichotomy of its biological impact. We investigated the potential cardiovascular toxicity of NO in a developing model vertebrate utilizing nanoparticles (NP) designed to release NO and/or nitrosate cellular thiol in tandem. Zebrafish embryos (Danio rerio) were exposed to nominal concentrations of 50, 150, 250, and 450 ppm of NO-NP, S-nitrosoacetopiprol (SNO-CAP), or S-nitroso-N-acetyl-cysteine (SNO-NAC), at different developmental time points: 8, 48, or 96 hours post fertilization (hpf). The NPs imparted differential toxicity, best highlighted by differing temporal sensitivity. There was a significant increase in mortality in zebrafish exposed to NO NPs at 8 and at 48 hpf, but not at 96 hpf, with greater mortality in the 48 hpf treatment. In contrast, SNO-CAP did not significantly increase mortality at any concentration tested, regardless of the exposure paradigm, while mortality was significantly increased with exposure to 450 ppm SNO-NAC in all three exposure paradigms. The sublethal toxicity observed with the NO-NPs was primarily associated with the cardiovascular system: abnormal circulation, pericardial edema, and yolk sac edema. All three NPs were more toxic when the embryos were exposed at 48 hpf, suggesting a stage-specific sensitivity to NO and its derivatives. Control NPs exhibited no toxicity at any time point. Impaired NO homeostasis can have deleterious effects on multiple organ systems, and long term effects of excess NO during vertebrate development are not known. Further studies are necessary to understand the temporal sensitivity of a developing organism to NO in light of its opposing actions in cytoprotection and cytotoxicity.

1728 Epigenetic Modifications of Histone H3 in Brains and Livers of Fetal DNA Repair-Deficient Oxoguanine Glycosylase 1 (OGG1) Knockout Mice Exposed In Utero to Ethanol

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DNA oxidation and postnatal neurobehavioral abnormalities are enhanced in DNA repair-deficient oxoguanine glycosylase 1 (ogg1) knockout (KO) fetuses exposed in utero to alcohol (ethanol, EtOH), implicating reactive oxygen species (ROS)-initiated DNA damage in the pathogenic mechanism. Herein, pregnant ogg1 mice were treated with EtOH (2 g/kg, i.p.) on gestational day (GD) 17. Fetal brains and livers were extracted 1 hr post treatment. Acetylation of histone H3 at lysine 9 (H3K9ac), and di- and tri-methylation of histone H3 at lysine 9 (H3K9me2, H3K9me3 respectively) were assessed by western blotting. In EtOH- but not saline-exposed fetal brains, H3K9ac was increased by 6.1-fold in ogg1 homozygous (+/-) KO progeny compared to wild-type (WT) littermates (p<0.0001), and by 4.4-fold compared to heterozygous (+/+ ) KO littermates (p=0.0026). EtOH-exposed +/- fetal brains showed a 44% increase in H3K9ac formation compared to +/- saline controls (p = 0.0096), with no alterations in WT progeny. H3K9ac was unaltered in both EOH- and saline-exposed fetal livers. H3K9me2 was unaltered by ogg1 genotype in EtOH-exposed fetal brains and in saline-exposed fetal brain and liver samples. An EtOH-initiated decrease in H3K9me3 in fetal brains was respectively 26% (p=0.0186) and 29% (p=0.0057) greater in +/- and -/- /+ ogg1 KO compared to WT littermates. Conversely, H3K9me3 was increased in saline-exposed +/- /-ogg1 fetal brains and livers by 81% (p=0.0018) and 56% (p=0.0005) respectively compared to WT progeny, and increased by 35% in +/- fetal livers (p=0.05). The effects of ogg1 genotype and EtOH on H3K9ac and H3K9me3 formation suggests that the enhanced susceptibility of OGG1-deficient progeny to postnatal neurodevelopmental deficits caused by in utero EtOH exposure may at least in part involve oxidative DNA lesions leading to epigenetic changes in fetal brain. (Support: Canadian Institutes of Health Research).
1730 Hepatic Mitochondrial Alteration in CD1 Mice Associated with Prenatal Exposures to Low Doses of Perfluoroacetic Acid (PFOA)

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Perfluoroacetic acid (PFOA) is a perfluorooalkyl acid primarily used as an industrial surfactant. It persists in the environment, is hepatotoxic, and may induce liver tumors in animal models. To evaluate PFOA-induced liver toxicity following early life exposure, pregnant CD1 mice were orally gavaged with low doses of PFOA (0, 0.01, 0.1, 0.3 and 1 mg/kg PFOA) for gestation days (GD) 0 through 17. Tissues were collected on post-natal day (PND) 21 and 91 and routinely processed for histological evaluation. Frozen sections of liver were also collected for transmission electron microscopy (TEM). On PND 21, histopathologic changes in the liver of offspring included hepatocellular hypertrophy and periporal inflammation that increased in severity by PND 91. TEM of liver from PND 91 mice revealed PFOA-induced cellular damage and mitochondrial abnormalities with no evidence of peroxisome proliferation. Within hypertrophied hepatocytes, mitochondria were not only increased in number, but also exhibited altered morphologies suggestive of increased and/or uncontrolled fission and fusion reactions. Based on these observations, we conclude that prenatal exposures to PFOA at a dose of 1 mg/kg induced hepatocellular hypertrophy in CD1 mice due to mitochondrial proliferation. We suspect that alterations in mitochondrial function (fusion or fission) are driving the hypertrophy response, and when prolonged, may lead to tumor development later in life; additional studies are ongoing to determine the precise mode of action.

1732 PCRs Decrease the Placental Syncytiotrophoblast Volume and Increase Placental Growth Factor in the Placenta of Normal Pregnancy


Numerous reports have described PCB-dependent adverse effects on human fetal growth, including increased risk for IUGR, changes in endocrine function and hormone metabolites, and immunosuppressive and neurological deficits. Here we test the prediction that in utero PCB exposure adversely affects placental morphology, potentially leading to placental insufficiency en route to fetal growth restriction. Methods: 10 PCB homologues were measured in the maternal and fetal blood of a small cohort of normotensive pregnancies (N=22) by GC-MS. PCB levels were compared with antiangiogenesis associated proteins Placental Growth Factor (PIGF) and f4f1, as determined by ELISA, and the total estimated syncytiotrophoblast (ST) volume. Results: Environmental levels of PCB exposure, based on blood content, were found to be negatively associated with estimated placenta ST volume. Additionally, a positive relation was identified between PCB exposure and PIGF protein levels, while no significant relationship was found for f4f1 in placenta. When ST volume and PIGF characteristics were analyzed together using multivariate regression models of individual PIGF/ST ratio values and total PCB concentrations a significant interaction was also suggested. Discussion: These data suggest that PCB exposure affects vascular remodeling and nutrient transport. Conclusion: These results demonstrate that the human placenta, including ST, is a target of PCB toxicity, and that current environmental PCB exposure levels are supportive of reproductive health.

1733 Impact of Placental Mdr1 on Fetal Drug Exposure in Sprague-Dawley Rats

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Multidrug resistance protein 1 (Mdr1), also known as p-glycoprotein (PGP) or ATP-binding cassette sub-family B member 1 (ABCB1), is highly expressed in the placenta and is thought to play an important role in protecting the fetus by limiting the diffusion of drug exposure. Changes in the expression level of Mdr1 during gestation may result in variable protective factors during different periods in the late gestation for these molecules that are substrates for this transporter (higher expression would reduce fetal exposure). Mdr1 expression in the rat placenta was evaluated on gestation day (GD) 12 and GD 20. Mdr1 mRNA and Mdr1b mRNA levels were 5-6 fold higher on GD 20 compared to GD 12. Mdr1 protein expression, as determined by western blot, was approximately 14-fold higher on GD 20. To evaluate the impact of Mdr1 inhibition on fetal exposure, the Mdr1 inhibitor eladcarid was administered to pregnant rats by intravenous injection (5 mg/kg) on GD 20 at the time of highest Mdr1 expression, followed by a subcutaneous injection of quinidine (5 mg/kg), an Mdr1 substrate, 30 minutes later. Maternal plasma and fetuses were collected at 1, 4, and 7 hours post dose. Maternal brain was also collected at these time points as a control, as Mdr1 is expressed at the blood brain barrier and its impact on brain exposure has been extensively studied. Mdr1 inhibition resulted in higher maternal brain levels of quinidine (~25-fold increase), similar to previous studies. Inhibition of Mdr1 also resulted in an increase in fetal levels of quinidine on GD 20 (3.3-fold increase). These results indicate that fetal exposure is influenced by Mdr1 function late in gestation, at the time of highest expression. Lower Mdr1 expression early in gestation during the period of highest susceptibility for teratogenicity may result in less protection. Understanding the potential impact of Mdr1 on fetal exposure throughout gestation is important in interpreting the human relevance of teratogenicity data in nonclinical species.

1734 Genistein Impairs Human Placental BCRP/ABCG2 Transporter Function: Potential Risk for Fetal Exposure to Chemicals

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In the placenta, the breast cancer resistance protein (BCRP/ABCG2) transporter is localized to the apical membrane of syncytiotrophoblasts. Functioning as the primary placental drug transporter, BCRP protects the fetus from exposure to chemicals such as pharmaceutical drugs (glyburide) and dietary contaminants (sce- alene) by actively transporting the compounds out of the placenta and back to the maternal circulation. This study determined the effect of the soy isofla- vone genistein on the regulation and direct inhibition of BCRP-mediated transport using in vitro model systems. For the regulation studies, human choriocarcinoma BeWo placental cells were incubated with genistein (0-10 uM) for 48 h and collected for mRNA, western blot, and functional analysis. Two in vitro models were used to measure the direct inhibition of BCRP by genistein: 1) HEK cells overexpressing the wild-type (WT) or functionally-reduced variant (C421A) and 2) inverted plasma membrane vesicles isolated from S99 insect cells expressing WT BCRP. In the presence of genistein, BCRP function was determined by fluorescent detection of substrate accumulation (whole cells: BODIPY-glyburide; vesicles: Lucifer yellow). After 48 h, genistein reduced the mRNA and protein expression of BCRP in placental BeWo cells in a dose-dependent manner which could be prevented by serum starvation. The direct inhibition studies demonstrated...
Decabrominated diphenyl ether (decaBDE) is a flame retardant and used in worldwide. It has been indicated that decaBDE is one of the endocrine disruptors and suggested that exposure to decaBDE affect human and animal health. In our previous study, decaBDE was dosed with 0.025, 0.25 and 2.5 mg/kg body weight to ICR male mice during postnatal days 1-5 by s.c. injection. We observed that early postnatal exposure to lower dose decaBDE (0.025 mg/kg) affects male reproduction system: the reduction of testes weights, sperm number and Sertoli cell number. Also we found decreased transcript levels of androgen receptor in isolated mouse Sertoli cells exposed to 0.025 mg/kg decaBDE. In this study, to determine the mechanism following early postnatal exposure to lower dose decaBDE in mouse testes, we examined serum testosterone levels in mice exposed postnatally with decaBDE. Mice were dosed by above described method, and serum samples were collected at 12 weeks of age. Serum testosterone levels are significantly decreased in the 0.025 and 0.25 mg/kg decaBDE dose groups when compared to the controls, while the testosterone level in the 2.5 mg/kg decaBDE dose group had no significant difference. Our present findings indicate that early postnatal exposure to 0.025 and 0.25 mg/kg decaBDE causes to reduce serum testosterone levels, resulting in decreased testicular size and lower sperm number.

1735 A Feto-Placental Coculture Model Shows the Complex Disruptive Effect of Antidepressant Fluoxetine and Metabolite Norfluoxetine on Estrogen Biosynthesis

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Depression occurs in up to 25% of pregnant women and almost a third undergo antidepressant treatment during pregnancy. However, the action of the most commonly prescribed SSRI, fluoxetine (30 mg) by 39%, 52% and 78%, respectively. Estrone production decreased basal estradiol production by 40% and and increased it 1.41-fold in H295R cells, compared to control. Fluoxetine (3 μM) decreased the activity of aromatase (CYP19), the enzyme that converts androstenedione to estradiol. The co-culture was fetal-like adrenocortical) cells to study the effects of fluoxetine and its active metabolite norfluoxetine on feto-placental estrogen biosynthesis. The co-culture was isolated mouse Sertoli cells exposed to 0.025 mg/kg decaBDE. In this study, to determine the mechanism following early postnatal exposure to lower dose decaBDE in mouse testes, we examined serum testosterone levels in mice exposed postnatally with decaBDE. Mice were dosed by above described method, and serum samples were collected at 12 weeks of age. Serum testosterone levels are significantly decreased in the 0.025 and 0.25 mg/kg decaBDE dose groups when compared to the controls, while the testosterone level in the 2.5 mg/kg decaBDE dose group had no significant difference. Our present findings indicate that early postnatal exposure to 0.025 and 0.25 mg/kg decaBDE causes to reduce serum testosterone levels, resulting in decreased testicular size and lower sperm number.

1736 Dose-Dependent Effects of PBDE-47 on Human Primary Cytotrophoblasts


Polybrominated diphenyl ethers (PBDEs) are of major concern to developing populations due to their widespread use as flame retardants in products and the environment. PBDEs are present in fetuses including the placenta. Despite some toxicological studies suggesting these compounds are harmful to the developing fetus, the ramifications associated with PBDE exposure during pregnancy and mechanisms involved remain largely unresolved. Placental trophoblasts connect the fetus to the uterus and regulate the transfer of nutrients, oxygen and waste products between the two compartments. To study the effects of PBDEs on the placenta, we used primary human cytotrophoblasts (CTBs) as an in vitro model of the process that connects the maternal and fetal units. In culture, first and second trimester CTBs attach to and migrate toward one another. Within 2d, the cells form multi-CTB aggregates that mimic properties of the cell columns of chorionic villi. At a molecular level, we used a microarray approach to assess changes in gene expression that accompany this process. The pathways that were involved included cell migration, communication, energy metabolism, vascular development, and inflammatory processes. We used qRT-PCR to verify the dynamic regulation of CD44, CXCL6, DHCRC7, DUSP2, F5, FABP7, ITGA2, and MMP9. Next we investigated the effects of PBDE-47 on CTB viability and function. At 15h in culture, we exposed CTBs to PBDE-47 (0.01-25μM) for 4 and/or 24h and analyzed several endpoints: cytotoxicity, apoptosis, and gene expression. In a dose-dependent manner, 24h of PBDE-47 exposure significantly reduced cell viability as measured by neutral red uptake. There were parallel increases in cell death (LDH) and Casp3 activity, the latter evident as early as 4h at 25μM. Ongoing investigations are determining the dose- and time-dependent effects of PBDE-47 in terms of the transcriptome, which will reveal exposure-related alterations in genes implicated in toxicity (e.g., THRs) and/or important placental functions.

1737 Neonatal Exposure to Lower Dose Decabromodiphenyl Ether Decreases Serum Testosterone Levels in Mouse

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Arsenic is a known toxicant, and humans are exposed to it through drinking water derived from ground water and crops grown in areas containing high levels of arsenic in soils or water. Epidemiological studies have shown that arsenic exposure via drinking water during development decreased intellectual function, reduced birth weight, and altered locomotor activity. Moreover, in vitro studies also showed that arsenic exposure decreased muscle and neuronal cell differentiation in embryonic stem cells. However, the mechanism by which arsenic affects neurogenesis and myogenesis is still unknown. Sonic hedgehog (Shh) signaling pathway is an evolutionally conserved pathway that is needed for proper limb development and neurogenesis. The purpose of this study was to determine whether arsenic can disrupt Shh signaling during cell differentiation. Mouse P19 embryonic stem cells were exposed to 0, 0.25, or 0.5 μM sodium arsenite for up to 9 days during cell
Mathematical equations and concepts are presented in a clear, concise manner, making the information easily understandable. The use of bullet points and subheadings effectively organizes the content, allowing for a smooth reading experience. The text is well-formatted, with appropriate use of paragraph breaks and line spacing. The use of active voice and active language enhances the clarity and flow of the content. Overall, the content is presented in a manner that is both informative and engaging.
1747 Thalidomide-Induced Early Gene Expression Perturbations Indicative of Human Embryopathy in Mouse Embryonic Stem Cells

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Developmental toxicity testing has traditionally relied on animal models which are costly, time consuming, and requires the sacrifice of large number of animals. In addition, there are significant disparities between human beings and animals in their responses to chemicals. Thalidomide is a species-specific developmental toxicant that causes severe limb malformations in humans but not in mice. Here, we used microarrays to study transcriptomic changes induced by thalidomide in an in vitro model based on differentiation of mouse embryonic stem cells (mESCs). C57BL/6 mESCs were allowed to differentiate spontaneously and RNA was collected at 24, 48, and 72 h after exposure to 0.25 mM thalidomide. Global gene expression analysis using microarrays revealed hundreds of differentially expressed genes upon thalidomide exposure that were enriched in gene ontology (GO) terms in pathways and cellular components associated with development and differentiation. In addition, many genes were found to be involved in small GTPases-mediated signal transduction, heart development, and inflammatory responses, which coincide with clinical evidences and may represent critical embryotoxicities of thalidomide. These results demonstrate that transcriptomics in combination with mouse embryonic stem cell differentiation is a promising alternative model for developmental toxicity assessment.

1746 A Biomarker-Based Human Stem Cell Assay Applied for Ranking a Retinoid Series Based on Relative Developmental Toxicity Potential


An in vitro, biomarker-based, human induced pluripotent stem (iPS) cell-based assay for prediction of developmental toxicity was previously developed as an alternative model to aid in ongoing worldwide efforts to reduce animal testing. Utilization of in vitro screening assays will reduce costs, increase pharmaceutical and chemical safety, and reduce the risk of false-negatives due to inter-species variability. Our previous work established that evaluating the changes of two amino acids (ornithine and cystine) in human embryonic stem (hES) or iPS cells is an effective developmental toxicity screening tool. In this study, we applied it to a series of retinoids with the most potent compound ranking according to its teratogenic potency. The compounds studied were 13cis retinoic acid, 9-cis retinoic acid, etretinate, acitretin (active metabolite of etretinate), all-trans retinoic acid, all-trans retinol and TTNPB. Each has been well characterized in terms of teratogenic potency in both in vivo and in vitro models. Additionally, the mechanistic relevance was studied using the AR/retinoid X receptor (RAR) antagonist Ro 41-5253. Results showed that all-trans retinoic acid and TTNPB were the most potent and retinol was the least potent. Interestingly, Ro 41-5253 inhibited the metabolic perturbation caused by 13-cis retinoic acid, 9-cis retinoic acid, all-trans retinoic acid, acitretin, and TTNPB. Retinol and etretinate were unaffected by the antagonist. These observations are consistent with the teratogenic response being mediated through RAR and suggest iPSC cells do not have the ability to metabolize etretinate to its active form. These rankings were concordant with published values for other in vitro studies, but do not completely correspond with in vivo potency rankings. The lack of in vivo kinetic and metabolic processes and species-specific differences in metabolism could explain these differences. These data show how the assay can be applied for compound decision making and bridging chemical series.

1747 Using Human-Derived Neural Cells As an In Vitro Model for Developmental Neurotoxicity following Exposure to Pesticides

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Agricultural, industrial and commercial use of pesticides continues to increase with an estimated usage nearing a billion lbs/year. Many of these compounds target the nervous system of nuisance animals and due to their lack of selectivity cause adverse effects in non-target species. Several classes of pesticides such as the organophosphates, carbamates and organochlorines are known to elicit neurotoxic effects in mammals. However, current testing and safety requirements do not require development of neurotoxicity (DNT) tests for these chemicals. Understanding the consequence of pesticide exposure on fetal brain development specifically during critical windows of susceptibility is necessary to accurately predict risk. Thus, there is a critical need for in vitro models to aid in DNT screening and chemical prioritization. The objective of this study was to develop a metabolomics-based DNT assay that includes stages of neural development using progenitor (hNPs) and post-mitotic neuronal cells (hN2) to delineate the adverse outcome pathways (AOP) associated with pesticide exposure. Assays were initially validated with known neurotoxic chemicals. In this study, cells were exposed to 0, 0.1, 0.3, 1, 3, 10, 30 and 100 μM of chlorpyrifos, aldicarb, and lindane for 48 hrs. Following exposure, media and cells were separated and biological reactions were quenched prior to extraction, derivatization, and analysis by GC-MS. Metabolomic profiling and subsequent multivariate analysis demonstrated separation for each pesticide class and dose dependent responses were observed at concentrations lower than those eliciting effects in cytotoxicity assays. Understanding the biochemical metabolites associated with these responses and mapping them to critical pathways of DNT will aid in predicting the risks due to pesticide exposures.

1748 Valproic Acid-Induced Alterations and CBP/P300 Activity in P19 Embryonal Carcinoma Cells

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The transcripational co-activators and histone acetyltransferases (HATs) CREB-binding protein (Cbph and p300 are required for embryonic development, with deficiencies in either associated with neural tube defects and embryolethality. Valproic acid (VPA) is a widely prescribed anticonvulsant drug that is associated with an increased risk of congenital malformations. We have previously demonstrated that VPA exposure increases apoptosis and decreases expression of the transcription factor NFκB in mouse embryos with a neural tube defect. While VPA is a well-known histone deacetylation inhibitor, it has been shown to also decrease the protein level of Cbph/p300 in vitro. To evaluate the importance of Cbph/p300 in VPA-mediated alterations, we exposed p19 embryonal carcinoma cells to VPA, and several inhibitors/activators of Cbph/p300 HAT activity. Western blots of p19 cells plated at 30,000 cells/mL and exposed to 5 mM VPA for up to 24 hours demonstrate a significant reduction in Cbph/p300 and NFκB protein expression while cleaved caspase-3, an indicator of apoptosis, was increased. These p19 cells were also exposed to 10 μM and 20 μM of the p300/Cbph HAT inhibitor C646 for 24 hours which resulted in significantly decreased NFκB and increased cleaved caspase-3 protein levels. As inhibition of Cbph/p300 HAT activity results in a phenotype similar to VPA exposure, ongoing studies are using CTB (CAS 451491-47-
7), an inducer of p300 HAT activity to evaluate whether pre-treatment with CTB can attenuate the altered NFKb and cleaved caspase-3 protein expression that is observed following VPA exposure. Our preliminary results indicate that Cbp/p300 HAT activity is important for NFKb expression and cell survival, and may be perturbed by low Cbp/p300 protein expression that occurs following VPA exposure. Our study will provide further insight into the mechanism of VPA teratogenesis.

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1749 Adriamycin Induces Growth Inhibition of Cells by Reduction of Intracellular Levels of HMG-CoA Synthase

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The effective cancer therapy with adriamycin has been hampered by its side effects and the natural/acquired resistance of cancer cells. Therefore, it remains need to elucidate unknown mechanisms of adriamycin toxicity. We have previously performed a screening for growth-indispensable genes involved in adriamycin resistance using yeast ORF library and identified HMG-CoA synthase (HMGC). The overexpression of HMGC conferred resistance to adriamycin not only in yeast cells but also in human breast cancer MCF7 cells. In the present study, we found that enzyme activity of HMGCs in MCF7 cells was decreased by adriamycin. However, inhibitory effect of adriamycin on the activity of HMGCs was not observed in vitro. Adriamycin decreased the intracellular levels of HMGCs via promotion of ubiquitin-proteasome system. Inhibition of HMGCs gene expression by transduction of siRNA reduced viability of cells. The degree of cell death caused by adriamycin is correlated with the growth inhibition of cells by reduction of HMGCs levels by siRNA. Treatment of cells with mevalonate rescued adriamycin cytotoxicity. These results suggest that cytotoxic effects of adriamycin might be induced by reduction of cellular concentration of mevalonate caused by decreased levels of HMGCs.

1750 MRNAs As Tools for In Vitro Assessment of Developmental Toxicity of Chlorpyrifos

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Chlorpyrifos is able to alter the profile of expression of several biomarker genes of differentiation in D3 mouse embryonic stem cells at concentrations compatible with mild or no maternal cholinergic effect. We analyzed the embryotoxic potency of chlorpyrifos and its metabolites combining three different end-points: the IC50 for cytotoxicity caused towards D3 cells and towards 3T3 mouse fibroblasts and the concentration of the chemicals able to either reduce by 50% or increase by 200% in D3 cells the mRNAs concentrations codifying for the following genes: nestin, patatin-like phospholipase domain containing six (Pnpla6), fetal liver kinase 1, in D3 cells the mRNAs concentrations codifying for the following genes: nestin, patatin-like phospholipase domain containing six (Pnpla6), fetal liver kinase 1, runt-related transcription factor 2 (Runx2), and endogenous osteocalcin and bone sialoprotein. When we applied this procedure to chlorpyrifos and its two main metabolites we found that all three behaved as the weak embryotoxicant valproic acid. The prediction of weak embryotoxicity might be considered as alternative in vitro method for screenings of developmental toxicants.

1751 Seleno-L-Methionine Affects Cartilage/Bone and Tooth Development in Embryos of Medaka

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Selenium (Se) studies are becoming increasingly important with the releases of large amounts of the metalloid into streams at mountain-top removal sites and in the areas of ruptured enclosures storing coal fly-ash at adjacent energy production sites. Seleno-L-methionine (SeMet) is the chemical species of Se primarily associated with toxicity in developing organisms. Medaka (Oryzias latipes) embryos (OR and transgenic line OSX) were collected and exposed to 10, 20, 50, 100, 500 μM, 1mM SeMet at stage10. Embryos were fixed for cartilage and bone stains and processed for RT-PCR at 8 - or 10 dpf. Survival success at 10 dpf was distributed in a dose response manner with 100%, 100%, 74%, 12%, 9%, 0% in control, 10 μM, 20, 50, 100 500 μM, 1mM, respectively. Cartilage/bone stains showed developmental changes in mandible and teeth by SeMet exposure. SRY (sex-determining region Y)-box 9a (Sox9a), Sox9b and runt-related transcription factor 2 (Runx2), are critical for regulate differentiation of mesenchymal stem cells into chondrocytes and endochondral ossification. We found that both sox9a and sox9b were upregulated while and runx2 was down regulated at 8 dpf after SeMet exposure. These findings demonstrate that SeMet affects Medaka cartilage/bone development by disrupting Sox9s and Runx2 pathways. Ongoing work includes observations on gene expression at later stages and attempting to rescue deformities using Runx2 over expression/knock down and/or sox9a and sox9b gene knock down.

1752 Considering Dechorionation for Accurate Toxicity Evaluation in the Embryonic Zebrafish Assay

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Toxicity changes by the presence of chornions are needed to be reconsidered in the embryonic zebrafish assay. To investigate the effect of chorion permeability, zebrafish embryos were exposed to four different silver nanoparticles (AgNPs) of 20 or 110 nm, and the polypropyridone (PVP) or citrate surface coatings, and 12 organophosphorus flame retardants (OPFRs), in the presence and absence of chorion. Embryonic toxicity of AgNPs in the absence of chorion was greater than in its presence. The smaller 20 nm AgNPs were more toxic than the larger 110 nm AgNPs, regardless of chorion and test media; however, surface coating-dependent toxicity showed that the PVP was more toxic than the citrate, and this was strongly affected by the presence of chorion in both the test media of a standard zebrafish embryo medium (EM) and agglomeration-controlled 62.5 μM CaCl2. Embryonic mortality and behavior inhibition of most OPFRs in the absence of chorion was also greater than in its presence. However, in the exposure of TDCPP(1,3-dichloro-2-propyl phosphate), the percentage of tail malformation increased in the presence of chorion, which is consistent with gene expression results obtained using qRT-PCR. Chorion play a role in TDCPP toxicity, which was known to be influenced by exposure window. Our results demonstrated the permeability function of chorion on size- and surface coating-dependent toxicity of AgNPs and developmental toxicity of OPFRs. Thereafter, the refinement of dechorionation is carefully applied to the embryonic zebrafish assay for more accurate toxicity mechan-ism elucidation.

1753 Exposure to the Mycotoxin Zearalenone Impairs Embryo Development in Zebrafish

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A study was conducted to evaluate the developmental toxicity of three of the most commonly detected mycotoxins in food (deoxynivalenol (C15H206, mw = 296, 32 g/mol), patulin (C7H9O4, mw = 154.12 g/mol), and zearalenone (C18H22O5, mw = 318.36 g/mol)) using the zebrafish (Danio rerio) model. Mycotoxin exposures (0, 0.0064, 0.064, 0.64, 6.4 and 64 μM in 0.64% DMSO vehicle, n=32 dechorionated embryos/concentration for each mycotoxin) were initiated at 6 hours post fertilization (hpf) and were sustained until 120 hpf. We screened for delayed developmental progression, mortality and photo-induced tail flexion at 24 hpf, at 5 days post-fertilization (dpf), 18 different body morphology endpoints and photomotor response were assessed. No significant developmental toxicity was associated with embryonic exposure to deoxynivalenol or patulin, except for modest hyperactivity for the highest dose of patulin (64 μM) in the 24 hpf photomotor assay. Significant mortality was associated with exposure to 64 μM zearalenone by 24 hpf, with additional mortality recorded in the 6.4 μM exposure group. However, in the 64 μM exposure group, no mortality was recorded. In zebrafish embryos, zearalenone was associated with significant incidences of adverse outcome in the majority of the 120 hpf body morphology endpoints. A significant hypoactivity was associated with exposure to 64 μM zearalenone by 120 hpf, with additional mortality recorded in the 6.4 μM exposure group. The degree of cell death caused by adriamycin is correlated with the growth inhibition of cells by reduction of HMGCs levels by siRNA. Treatment of cells with mevalonate rescued adriamycin cytotoxicity. These results suggest that cytotoxic effects of adriamycin might be induced by reduction of cellular concentration of mevalonate caused by decreased levels of HMGCs.

1754 Reduction of Intracellular Levels of HMG-CoA Synthase: A Mechanism for the Early Developmental Toxicity of OPFRs

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Thereafter, the refinement of dechorionation is carefully applied to the embryonic zebrafish assay for more accurate toxicity mechanism elucidation.

1755 Exposure to the Mycotoxin Zearalenone Impairs Embryo Development in Zebrafish
Effects of Bisphenol A (BPA) and Bisphenol S (BPS) on Glucose Metabolism in Zebrafish Embryos (Danio rerio)


Bisphenol A (BPA) is used in the manufacture of plastics and is a chemical of concern due to its endocrine disrupting potential and its prevalence of human exposure. Our laboratory and others have demonstrated that exposure of embryonic zebrafish to BPA disrupts development, resulting in increased incidence of edema, hemorrhage, and death. In this study, we are examining the effects of BPA and the related compound Bisphenol-S (BPS) on zebrafish development, focusing on their effects on glucose metabolism. BPS is an industrial alternative to BPA, and recent studies suggest it has similar endocrine properties as BPA. We hypothesize that exposure to BPS will elicit comparable effects as BPA based on similarities in estrogenic potential and structure. In the present study, zebrafish (Danio rerio) embryos were exposed to 100μM, 500μM, 750μM and 1000μM BPS for 96 hours post fertilization (hpf) in a static non-renewal water bath. The 96hpf LC50 of BPS was determined to be 520μM with a 95% confidence interval of 314.24-860.49μM. We then exposed embryos to vehicle control, 25μM BPS, 250μM BPS, or 250μM BPS, concentrations of BPA and BPS below the established LC50 and LOAEL, to investigate the effect of exposure on glucose metabolism. Gene expression of preproinsulin was examined immediately following exposure, and our data show a significant increase in preproinsulin expression in embryos exposed to 25μM BPS and 250μM BPS at 96hpf. This effect was not observed in fish exposed to 25μM BPS. In addition, we developed a protocol suitable for measuring total glycogen and total glucose levels in larval zebrafish. Our data indicate that developmental exposure to 25μM BPS results in increased glycogen and glucose levels at the 2-week larval timepoint. Based on acute lethality, preproinsulin expression, and total glycogen and glucose levels, BPS was determined to be less toxic than BPA.

Effects of Estrogenic Compounds on Cardiac Development in Zebrafish

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Exposure to 17β-estradiol (E2) causes cardiac edema in developing zebrafish. One potential pathway through which E2 may disrupt normal heart development is via inappropriate binding to the G protein-coupled estrogen receptor 1 (GPER), leading to altered regulation of secondary messengers, such as cAMP and Ca2+. This, in turn, can disrupt the normal function of important transcription factors required for cardiac development, including GATA4 and NFAT. Disruption of these transcription factors can alter genes involved in heart cell differentiation and development such as Lrc10 and Hand2. In addition, NFAT, whose activation is regulated by intracellular calcium concentration, has also been shown to interact with GATA4, and regulate transcription during development. We hypothesized that exposure to E2 can alter the expression of Hand2 and Lrc10, and the intracellular levels of secondary messengers. To explore potential molecular mechanisms of cardiac dysfunction, we examined the expression of Lrc10, Hand2 and GPER in zebrafish embryos following exposure to E2. Embryos were exposed to 0.1μM, 1μM, 2μM and 5μM of E2 at 2hpf and RNA was extracted at 12hpf. E2 exposure during early embryogenesis showed that mRNA expression of GPER, Lrc10 and Hand2 resulted in an increasing trend. Further experiments will be conducted to determine whether other developmental stages are altered after exposure, and whether second messengers may be affected.

Zebrafish PXR Inhibition In Vivo by Human PXR Agonists

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The pregnane X receptor (PXR) (nuclear receptor NR12) is a ligand activated transcription factor, mediating responses to diverse xenobiotic and endogenous chemicals. The properties of PXR in fish are not fully understood. In vitro studies using isolated ligand binding domains (LBDs) in COS cells have indicated differences in ligand binding between human and zebrafish PXR. However, these sorts of in vitro methods do not adequately reflect the complexity of transcription factor activation in vivo, where full length protein occurs in the normal cell context. Here we report on zebrafish pxr expression in vivo, and induction or downregulation of pnr and the Pxr target gene CYP3A45 by human PXR agonists. Hyperforin is a prototypical human and mouse PXR agonist, but in developing zebrafish downregulates the expression of both pnr and CYP3A45, with an IC50 less than 500 nM. Hyperforin is toxic to zebrafish above 5 μM. Some other human PXR agonists downregulate CYP3A45 expression, but not pnr, including nifedipine and triclosan, but not pregnenolone sulfate. Clotrimazole, which is a zebrafish PXR agonist in vitro, gave extreme embryo group-specific effects that sequencing suggests can be attributed to PXR allelic variation; two of the prominent allelic variants were associated with differential responses to clotrimazole. PXR allelic variation and the differences between in vivo and LBD reporter assays have implications for assessing the action of PXR ligands in zebrafish. (NIH P42ES007381, NIH R21HD073805)

The Role of Nrf2a in the Transcriptional Response to PCB-126 in Zebrafish Embryos

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Embryonic development in fish is especially sensitive to toxicity from polychlorinated biphenyls (PCBs) exposure. PCBs induce expression of cytoprotective genes via the transcription factor, aryl hydrocarbon receptor (AhR). AhR binds to the xenobiotic response elements (XREs) in classic Phase 1 drug metabolic genes, such as Cytochrome P4501A1 (cyp1a). The AhR also participates in crosstalk with another toxicologically important transcription factor, Nrf2/22, or Nrf2. Nrf2 binds to antioxidant response elements (AREs) to regulate the adaptive response to oxidative stress, primarily via upregulation of antioxidant defenses and Phase 2 enzymes. To explore the cross talk relationship between Nrf2 and AhR, we used a zebrafish model (Danio rerio) with a mutated DNA binding domain in one of the Nrf2 paralogs, Nrf2a. Embryos were exposed to nominal concentrations of PCB-126 at 24 hpf and examined for deformities, gene expression, and EROD activity at 96 hpf. We measured changes in gene expression patterns by QPCR of cyp1a, ahb2, and a variety of their known target genes in mutant Nrf2a and WT genotypes. cyp1a was highly expressed in the Nrf2a mutant in comparison to WT. Decreased expression of heme oxygenase (decycling) 1 (hmx1) in the Nrf2a mutant is thought to be due to the inhibitory effects of increased nrf2b expression. Target genes of Nrf2a and AhR2, NADP(H) quinone oxidoreductase 1 (nqo1) and glutathione S-transferase, alpha-like (gst1a), showed an increase in expression with and without PCB exposure in the Nrf2a mutant when compared to WT. This study will help elucidate crosstalk between two toxicologically important transcription factor pathways in a sensitive life stage in fish. This work was supported by R01ES016366 and R01ES006272 (MEH).

Developmental Exposure to Polycyclic Aromatic Hydrocarbons (PAHs) Affects Behavior and Energy in Larval and Adult Zebrafish

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Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental contaminants present in urban air, dust, and soil resulting from incomplete combustion of organic materials or fossil fuels. It is widely recognized that PAHs pose risks to human health, especially for the developing fetus and infant. Using the zebrafish model, we evaluated the developmental toxicity of PAHs and oxygenated PAHs (OPAHs). Zebrafish embryos were exposed from 6-120 hours post fertilization (hpf) to B[a]P, DB[a]P, Ba[a]Q, BEZO, and 9,10-PHEQ concentrations that caused no observable developmental malformations. Using the Seahorse Extracellular Flux Analyzer, we measured in vivo respiration in 26 hpf embryos exposed to PAHs. Exposure to B[a]P, BEZO, and 9,10-PHEQ decreased oxygen consumption rates, indicative of mitochondrial damage. Developmental B[a]P and Ba[a]Q exposure also resulted in hyperactive swimming at 120 hpf. To determine if behavioral and physiological effects persisted, a subset of exposed animals were raised to adulthood in chemical-free water and assessed for learning and fitness deficits. Preliminary results indicated B[a]P-dependent deficiencies in learning an active avoidance paradigm. A swim tunnel respirometer was used to measure total oxygen consumption as a measure of adult cardiovascular fitness. Animals developmentally exposed to B[a]P, DB[a]P, Ba[a]Q, BEZO, and 9,10-PHEQ demonstrated a significant increase in oxygen consumption rates over control animals during strenuous exercise. To identify the early mRNA expression changes that may underlie the initiation of these complex responses, whole genome transcriptional analysis was completed in 48 hpf larvae. These data demonstrate that developmental PAH exposure results in important, non-cancer endpoints and we can begin to identify the mechanisms underlying these complex responses. This research was supported by the NIH grants P42 ES016465 and P30 ES002013.
Benzo[a]pyrene (BaP) is an environmentally relevant carcinogenic and endocrine disrupting compound that causes immediate, long-term, and multigenerational health deficits in mammals and fish. Herein, we performed a genome-wide transcriptomic analysis and discovered differences in the transcriptome of developing zebrafish after a parental BaP waterborne exposure followed by an offspring exposure. Adult zebrafish were exposed to control or 42.0 g/L BaP for 7 days. Eggs were collected and raised in control condition or continuously exposed to BaP until 3.3 and 96 hours post fertilization (hpf). RNA sequencing (RNA-Seq) libraries were constructed for Illumina HiSeq2500 sequencing. To analyze data, RNA sequences were mapped to zebrafish transcriptome and genome using TopHat and TopHat2, respectively. R packages EdgeR and DESeq were used to identify differentially expressed (DE) genes (changed at the gene or transcript level), respectively. At 3.3 hpf, BaP exposure altered expression in 1153 DE genes and 159 DEU genes. At 96 hpf, BaP exposure altered expression in 1153 DE genes and 159 DEU genes. Functional ontology analysis by Ingenuity Pathway Analysis (IPA) revealed genes. At 3.3 hpf, BaP exposure resulted in 8 DE genes and 51 DEU genes. Knockdown of CYP19b is still expressed CYP19b is still endocrine disruptor and a carcinogen. Aromatase (CYP19b) is a key enzyme in steroidogenesis playing a key role in the hypothalimus-pituitary-gonad feedback loop. We hypothesized that BaP would negatively impact CYP19b expression in zebrafish, in turn, adversely affecting development and physiology. Here, we consider whether the toxicities observed following BaP exposure are similar to those following a transient CYP19b knockdown during early development. One-cell zebrafish embryos were injected with a CYP19b morpholino or the Genetools control-MO. Other non-injected embryos were exposed to nominal waterborne concentrations of BaP (0, 10 & 50 μg/L) for 96 hours post-fertilization (hpf). Real-time PCR showed both BaP doses significantly decreased CYP19b expression in 96 hpf zebrafish larvae homogenates. Cumulative mortality of zebrafish larvae was significantly increased following BaP exposure and CYP19b knockdown, compared to controls. In a treatment-blinded morphological assessment of larvae at 96 hpf, several phenotypes were negatively impacted by high dose BaP and CYP19b knockdown. larval body length, and decreased the heart rate. Several oxidative stress parameters were also affected, including glutathione levels and activities of several antioxidant enzymes, suggesting that oxidative stress contributes to the toxicity of TPM. TPM-exposed zebrafish also differed behaviorally: at 24 hpf the embryos had a higher frequency of spontaneous contractions (microscopic observations) and at 144 hpf the larvae displayed hyperactivity, as assessed by monitoring the swimming using Danio Vision. It is the importance that BaP are not attributable to nicotine, since embryos treated with nicotine alone did not differ from the control embryos. This study demonstrates that TPM disrupts early development in zebrafish.
next lower concentration (22x). If the water containing the X-ray contrast media was chloraminated, less toxicity was noted (fewer animals showing toxic responses at 40x) compared to their respective (non-chloraminated) X-ray contrast media samples. In conclusion, these results demonstrate the importance of considering the toxicity of water contaminants to aquatic organisms. This abstract does not necessarily reflect U.S. EPA policy.

1764 Comparative Survival of Zebrafish Whole Embryos to Embryonic Fibroblasts Exposed to PFOA and Its Derivatives
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Perfluorooctanoic Acid (PFOA), a fluorinated eight-carbon chain with an attached carboxyl group (CF3(CF2)6COOH), is produced by numerous industries due to its heat resistant and hydrophobic properties. Since PFOA is also nonreactive, it is ubiquitously found in low levels in the environment as well as in the serum of humans and other organisms. The EPA declared PFOA a likely carcinogen and as part of the PFOA Stewardship Program, would like to replace PFOA with shorter four to six carbon chains, which are believed to be safer, in commerce by 2015. The purpose of this study was to compare the effects of PFOA and its six-carbon derivative perfluorohexanoic acid (C6, C6H11O2) and four-carbon derivative potassium nonafluoro-1-butanesulfonate (C4, C4F9KO3S) on survival in whole zebrafish embryos and in a zebrafish embryonic cell line. Whole embryos were exposed to PFOA or one of its derivatives (C6 or C4) for 96 hours and percent mortality was determined. For the zebrafish embryonic cell line, cells were exposed to a similar concentration range of PFOA, C6, or C4 and the MTT Assay used to quantitate cell survival after a 96 hour treatment period. The LC50 values for the whole zebrafish embryos for PFOA, C6, and C4 were 42.5 ppm, 85.5 ppm, and 1374.5 ppm respectively. For the embryonic cells, the LC50 was 165.9 ppm for PFOA and 979.8 ppm for C6; however, the LC50 was unable to be calculated for C4 due to a high survival rate reaching near solubility limitations. Overall, survival rate increased in the shorter carbon chain chemicals compared to PFOA (PFOA>C6>C4) in both the whole embryos and embryonic cell line. In addition, the whole zebrafish embryos were more sensitive to the three chemicals compared to the embryonic cell line. This data suggests that there is a noteworthy difference between the use of whole embryos and the embryonic cell line when determining the toxicity of perfluorinated compounds.

1765 Zebrafish As a Model for Adult-Onset and Transgenerational Male Infertility Due to TCDD Exposure
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We have shown that zebrafish (Danio rerio) are an ideal model for evaluating the transgenerational effects of certain toxicants and their role in the fetal basis of adult disease. Exposure to TCDD early in development produces reproductive abnormalities in adulthood and decreased reproductive capacity that persists for multiple generations. We show that TCDD exposure leads to persistent effects in unexposed generations of zebrafish. Toxicity was observed in F1 and F2 generations of TCDD-lineage zebrafish (never exposed to TCDD), as well as in TCDD-lineage F2 male spawnings. This suggests that transgenerational inheritance of TCDD toxicity occurs through the male germline. In this work, we have more closely examined the adult males that were exposed to TCDD during development. Histological examination of testicular tissue showed a significant decrease in the area of mature cells (spermatooza) and increase in the area of immature cells (spermatogonia) in TCDD exposed males compared to controls. Gene expression in the testicular tissue has been evaluated by microarray and qRT-PCR, and we have identified changes in several tests specific genes that have also been implicated in human male infertility. The genes of interest include sox9b, vasa, egr1 and sf1 (nr5a1b). Using these tools and the zebrafish model, our research aims to uncover the critical genes and epigenetic regulation required for adverse reproductive endpoints of TCDD exposure. (Supported by NIH grant K01 OD010462 and NIH Training Grant T32 ES007015)

1766 Applying the Benchmark Dose Approach with High-Throughput Embryonic Zebrafish Screening Assays for Toxicity Hazard Identification and Ranking of Chemicals
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The benchmark dose (BMD) approach involves dose-response modeling to obtain the doses that correspond to a point of departure near the low end of the observable dose range. It is used in toxicity hazard assessment to rank chemicals using toxicity endpoints in high-throughput screening data. However, its application with high-throughput zebrafish screening assay data has not been tested nor demonstrated. We have developed a benchmark dose pipeline to evaluate the toxicity hazard potential of chemicals by examining the developmental and behavioral responses of zebrafish embryos to the chemicals. The zebrafish developmental assay measures the presence or absence of a chemical effect on 22 development endpoints of a zebrafish embryo during its 24–120 hour post fertilization (hpf) period. The behavioral assay measures the 24 and 120 hpf movement of the fish under light/dark conditions for different time intervals. Specifically, we applied the BMD approach on the 1.078 ToxCast chemicals and found that 426 chemicals had a significant dose-response relationship, which allowed a BMD10 (the benchmark dose that results in 10% extra risk in response compared to the control) value to be calculated for at least one of the zebrafish development endpoints. Comparison of the minimum BMD10 values with the lowest effect level (LEL) of the same chemicals showed that the BMD10 values provide better separation, precision, and rank ordering. Hierarchical clustering based on the minimum BMD values observed for these chemicals allowed phenotypic profiling of the chemical clusters. The more diverse the phenotype profile, the more concordant it was with the in vitro ToxCast assays. These results will be presented and compared with those obtained using the 120 hpf behavioral data. This work was supported by EPA Grant R835168.

1767 Corpus Callosum Damage Induced by Early-Life Exposure to Ultrafine Particulate Matter: Echoes of Autism Spectrum Disorders
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Air pollution is a worldwide environmental health concern, particularly in countries where regulations are lax. Evidence suggests that air pollution exposures can adversely influence the brain and its development, and such exposures have been linked to autism spectrum disorders (ASD), schizophrenia, and cognitive decline. Our previous studies indicate that males, but not females, exposed to concentrated ambient ultrafine particles (<100nm diameter; CAPS) during the neonatal period have significantly enlarged lateral ventricles, or ventriculomegaly (VM), a characteristic of ASD and schizophrenia. VM often occurs with damage to white matter and the corpus callosum (CC), an area of pathology found in individuals with ASD or schizophrenia. To determine toxicity of CAPS to the corpus callosum and its development, mice were exposed to CAPS during the first 2 weeks of life and/or in young adulthood. Consistent with our observations of male-specific VM, CAPS induced a reduction in the CC size in males, but not in females, when examined 24 hours after postnatal exposure. CAPS also altered the pattern of myelin basic protein expression in CC of the male brain with the mediolateral portions of the CC appearing more sensitive to this effect. The male specificity of the CC damage may be related to CAPS-induced reductions in testosterone that were observed 24 hours after neonatal exposure. Interestingly, males also show a significant excitatory/inhibitory (Glutamate/GABA) imbalance in the hippocampus, yet another pathological feature shared with ASD. When examined behaviorally, CAPS neonatal/adult CAPS exposure to males caused perseverative responding under a fixed-ratio 25 schedule of reinforcement, a diagnostic criterion of ASD. Taken together, these data indicate CAPS produces developmental neuropathology preferentially in males that is consistent with features of profound neurodevelopmental disorders like ASD. R21 ES019105.
Polymorphisms in GST enzymes have implications in heavy metal accumulation, neurodegeneration, and immune-mediated disease. Blood cell DNA and sera from 131 African-American children in the Mechanistic Indicators of Childhood Asthma (MICA) study in Detroit, MI, were used to determine GST Pi genotypes [rs97895 (C>A), rs17593060 (G>T), rs6951256 (A>G), rs1817042 (C>T) and rs16159 (A>G)], Mu SNPs [rs17672 (C>T), rs3815029 (G>C) and rs412543 (G>C)], and Theta SNPs [rs2266636 (present [P]-deletion[D]), rs2266637 (P=D)] and rs4630 (P>D) SNPs by PCR for associations with NAb (IgM and IgG) against neurofilaments (NF-L, NF-M, and NF-H), glut fibrillary acidic protein (GFAP), and myelin basic protein (MBP) and nail heavy metals (As, Cd, Cr, Cu, Fe, Hg, Mn, Pb, Se, Zn). Non-parametric analysis indicated that children with GST Pi genotypes rs1871042 TT and rs94795 AA had higher levels of As, Cd, Mn, and Pb; those with rs16159 GG had higher levels of Mn and Pb and those with rs6951256 AG had higher levels of Fe. Children with GST Mu genotypes rs17672 CC had higher levels of As; GST Theta genotypes rs2266636 deletion had higher levels of Pb. Direct associations between As, Cd, Fe, Se, and Zn with IgM and IgG NAb (r=0.38-0.74; p<0.05) were found. For Hg, Mn, and Pb, there were inverse associations (r=-.41; p<0.05) with IgM and direct associations (r=0.38-0.43; p<0.05) with IgG. Multiregression analysis indicated that GSTP1 and GSTT1 genotypes were the primary determinants of metal levels (r²=0.2; p<0.05). Additionally, the presence of metals in conjunction with select GST genotypes influenced NAb titers (r²=0.30-0.6; p<0.05). These results suggest GST Pi SNPs, in particular, determine heavy metal accumulation and NAb titers. Genomic differences may play a role as determinants of heavy metal neurotoxicity, as indicated by NAb, in environmentally vulnerable populations. This abstract does not necessarily reflect U.S. EPA policy.

**1770 Early-Life TCDD Exposure Results in Persistent and Sex-Dependent Neurotoxicity in Mice**

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The developing nervous system is highly susceptible to toxic insults that can negatively alter learning, memory, and behavior in adult life. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is a persistent environmental contaminant that bioaccumulates in the adipose tissue of higher organisms. The effects of TCDD are mediated through the aryl hydrocarbon receptor (AhR), an intracellular orphan receptor that can become activated in response to toxicant exposures. Though its physiological role is not well understood, the AhR has been implicated as a regulator of neurodevelopment and adult hippocampal neurogenesis. This study tested the hypothesis that inappropiate AhR activation in early postnatal life would lead to learning and memory deficits in adults and corresponding molecular impacts. A single, low-dose of TCDD on postnatal day 6 (P6) during the postnatal brain growth period was sufficient to produce sex-specific behavioral and molecular deficits at P90. Female mice exposed to either 0.2μg/kg or 1.0μg/kg TCDD on P6 exhibited locomotor and exploratory impairments during behavioral testing. In contrast, male mice under the same exposure paradigm demonstrated deficits in learning and memory. Immunohistological analysis showed a trending decrease in BedU incorporation and a significant decrease in Ki67 expression in the dentate gyrus of both male and female mice, suggesting negative impacts of TCDD on cell birth and division. Additionally, doublecortin (DCX) expression was significantly decreased in the dentate gyrus of female mice but not in males, suggesting that neuronal maturation is impaired in females. Taken together, these data show that a single dose of TCDD during a critical neurodevelopmental window results in behavioral deficits and molecular changes in the adult mouse, phenomena that are hypothesized to be mediated by inappropriate activation of AhR. Supported by URM C Environmental Health Sciences Center Pilot Project funds and the following extramural grants from the NIH: RO1ES016357 and T32-ES070762.
Developmental exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) interferes with masculinization of gonadotropin release patterns, most likely by blocking estradiol (E2)-dependent sexual differentiation of the anteroventral periventricular nucleus (AVPV). The process of sexual differentiation involves both cell death and alterations in neurite outgrowth, but the underlying molecular mechanisms are unclear. We reported previously that postnatal day 2 (PND2) TCDD exposure induces a modification of dendritic spine number and shape observable on PND25. We also identified CUG triple repeat RNA binding protein 2 (CUGBP2) as a sex-specific gene significantly upregulated by E2 in females and downregulated by TCDD in males on PND 2. Cugbp2 regulates expression of the NR1 subunit of NMDA receptor and glutamate signaling through the NMDA receptor has been implicated in sexual differentiation of dendritic outgrowth and synaptogenesis in other brain regions. Moreover, we reported previously that TCDD increased NR1 expression and also upregulates the expression of spinophilin (that regulates dendritic branching) and synaptophysin (a marker of synapses) on PND2. In the present study, we tested whether effects of TCDD on these genes in the developing AVPV is transient or persists through the time we see changes in dendritic spines on PND25. We administered vehicle or TCDD 600 ng orally to rat dams on the first post-partum day of postnatal day 1 rat pups were grown in serum-free media with varying concentrations of triiodothyronine (T3) or thyroxine (T4). Total RNA was extracted from these cultures on day in vitro (DIV) 2, DIV 7 and DIV 21. We detected all of the 30 TH signaling-related genes screened, which included deiodinases, TH transporters, and TH receptors. A subset of these 30 genes was differentially expressed as a function of culture age. These gene expression data will provide a baseline for determining the effect of TH hormone disrupting chemicals on neural cell types. This work was supported by the USEPA (grant RD835590).
ANOVA have greatly improved the way repeated-measure data have been analyzed, the problem of false positives persists because of inappropriate application of these analyses. There are a number of assumptions required for repeated measure ANOVA and consequences when assumptions are not satisfied using older statistical methods. Within the evolution of statistical computing, the mixed model avoids many of the assumptions required in the older methodology but introduces a new challenge. The new task is proper identification of the variance-covariance matrix required to describe the relationship between measurements made at different times within the same individual. Based on results of over thirty sets of repeated measures data from within behavioral experiments, the ARH(1) is the most appropriate structure, followed by CSH. However, the variance-covariance structure should be selected for each set of data based on the process described below. A testing strategy to select the most appropriate covariance structure is demonstrated using data from a motor activity study. The Akaike information criterion (AIC) is a way of selecting the best model from a set of models. The AIC is based on theory and finds a balanced goodness of fit and model complexity. The AR(1), ARH(1), CS, and CSH structures are all tested for the motor activity study. The AIC values from the four models are then compared and the model with the smallest AIC is selected, ARH(1). The model with the lowest AIC is considered the best statistical model. Choosing the correct variance-covariance structure helps to obtain the most accurate results possible.

1777 Fluoroscopy-Guided Minimally Invasive Epidural or Intrathecal Injections for Repeat Dosing Toxicology Studies: Comparison of Beagle Dogs and Göttingen Minipigs

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A number of medical conditions (e.g. lower back pain) or interventions (e.g. lower limb surgeries) require epidural or intrathecal injections in patients. Regulatory toxicology studies to assess drug candidates delivered with these routes require the same administration method in the animals to mimic the clinical conditions and assess local tolerance. Intrathecal and epidural injections have been extensively used in toxicology studies using Beagle dogs but a paucity of data exist in minipigs. Beagle dogs and Göttingen minipigs underwent two fluoroscopy-guided epidural or intrathecal injections of sterile saline (0.2 mL/kg), 7 days apart. Separate animals were administered a contrast agent to assess the optimal dose volume for both routes in both species. A spinal needle was used for intrathecal injections while a Tuohy needle was used for epidural administration. The procedure was performed using a short inhalant anesthesia (isoflurane). Fluoroscopy imaging permitted precise evaluation of lumbar vertebral anatomy, which facilitated spinal or Tuohy needle positioning and durotomy. The L5-L6 intervertebral space was considered optimal in both Beagle dogs and Göttingen minipigs. Animals were euthanized 7 days after the last administration and no observable macroscopic or microscopy finding was noted at necropsy. In conclusion, technical feasibility and ease of administration during non-invasive epidural and intrathecal dosing were considered comparable between Beagle dogs and Göttingen minipigs.

1778 Cognitive Effects of Simvastatin in the Rat

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Statin use is widespread and has become a common therapy for preventing cardiovascular disease. These drugs are generally well-tolerated; however, there are reports of patients developing confusion and memory loss, which appear to be caused by statins. These reports were considered significant enough for the US FDA to update the warning label of all statins in 2012 to include the potential for negative cognitive side effects. Interestingly, there are also numerous reports regarding the neuroprotective effects of statins in conditions such as stroke, subarachnoid hemorrhage, multiple sclerosis, Alzheimer’s and other neurodegenerative diseases. There is considerable controversy in the literature surrounding these contradictory effects. Given the growing rate of cardiovascular disease in the population, as well as the potential of statins to be used as treatment in neurological illness and injury, the need arises to better understand the mechanisms of action of statins in the brain. We have completed a pilot study investigating some neurobehavioral effects of simvastatin (10 mg/kg/d for 28 days). We found that the simvastatin-treated rats performed significantly worse than controls in both the Barnes maze and novel object recognition tests. These data will guide future experiments in which behavioral assessments will be conducted with other doses of simvastatin, and also with other statins. RNAseq analysis will be conducted to identify gene expression changes that occur at the time that adverse behavioral effects are noted, whether adverse or beneficial. Our findings may eventually provide a further screen in drug development to avoid negative cognitive effects as the next generation of statins are developed, or identify target pathways to allow optimization of existing statins to increase their effectiveness in the treatment of neurological illness.

1779 Relation of Neuron Counts in the Superior Cervical Ganglia and Urinary MHPG in Rats following Guanethidine Treatment

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Predicting potential adverse consequences of unintended drug action on the sympathetic nervous system is increasingly important in the development of some drug classes. The goal of the present study was to understand the relationship between sympathetic post-ganglion neuron injury or loss and changes in sympathetic outflow. Guanethidine, a peripheral restricted adrenergic-blocking agent, was used to pharmacologically inhibit and destroy postganglionic sympathetic neurons in the rat and urinary levels of the catecholamine metabolite, 3-methoxy-4-hydroxy-phenylethylene glycol (MHPG), were used to monitor associated deficits in sympathetic outflow. Superior cervical ganglia (SCG) were harvested either immediately following 5 or 11 daily i.p. doses of vehicle or 100 mg/kg guanethidine, or after a 24-28 day recovery period. Urine samples were collected at comparable times and levels of MHPG were quantified by LC MS/MS. Following whole animal perfusion with 4% paraformaldehyde, SCG were collected and processed for stereology-based estimates of total neuron numbers and ganglion size. Creatinine-normalized urine MHPG levels were decreased 50% (Day 3) 80% (Day 11) relative to vehicle-treated controls (P<0.05) when assessed immediately following of guanethidine treatment. Interestingly, stereology-based estimates of neuron counts were not significantly different from controls at this time. However, active neurodegenerative inflammatory cell infiltration and ganglia swelling made counting challenging. If animals were allowed to recover (24-28 days) inflammatory infiltrate and ganglia swelling were absent, and SCG neuron counts and creatinine-normalized urine MHPG levels were both reduced by ~65% for rats dosed for 11 consecutive days (P<0.05). In conclusion, following a 24-28 day recovery, guanethidine-induced reductions in urinary MHPG correlated with neuron count decreases in the superior cervical ganglion of rats.

1780 Ketamine Modulates DISC1 Expression in a Rat Model of Anesthetic-Induced Neuroapoptosis

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Background: Anesthetic, sedative and analgesic drugs are used for diagnostic studies and surgical procedures in infants and children, but little is known about their impact on the developing nervous system. Ketamine (Ket), a non-competitive N-methyl-D-aspartate receptor (NMDA-R) antagonist, is found to induce neuroapoptosis and other adverse morphologic changes in neurons of the developing brain in rodents and primates. However, the exact mechanism of ket on induction of neuroapoptosis is unclear. Material and Methods: Sprague-Dawley postnatal day 7 (P7) rat pups were used for this study. Rats were given three doses of 5, 10 and 20 mg/kg body weight (b.w.) of ketamine (ket) and one control group for 6 h or only one dose of 20 mg/kg b.w. for different time intervals by intraperitoneal. After treatments, parts of the brain tissues were extracted and flash frozen in liquid nitrogen for western blotting analysis. The other parts were fixed in 4% paraformaldehyde for immunohistochemical assay. Apoptosis such as cleaved-caspase-3, and other protein expression such as Disrupted in schizophrenia-1 (DISC1), NMDA receptor 2A and 2B and glycogen synthase kinase-3b (GSK-3b) were determined using immunofluorescent and western blotting assays. Results: Ket could significantly increase neuroapoptosis by increasing cleaved-caspase-3 expression in the rat brain tissues. Ket also was significant decrease in the expression of phosphorylated-NMDA-2A and 2B, phosphorylated-GSK-3b and DISC1 in the p7 brain tissues. Lithium, as an inhibitor of GSK-3b, significantly attenuated Ket-induced changes of DISC1 protein expression in the p7 brain tissues (p<0.05). Conclusion: Ketamine induced neuroapoptosis and decreased DISC1 expression in the brain tissues of p7 rat. These effects, at least, partly contribute to activating GSK-3b protein and changing function of NMDA receptor in the developing brain.
We showed previously that 24 hours of ketamine-induced general anesthesia causes significant neuronal cell death and seemingly permanent cognitive deficits in rhesus monkeys. In the present study, eight monkeys were anesthetized on PND 5 or 6 with isoflurane (ISO, 1%)/nitrous oxide (N2O, 70%) to a light surgical plane for 8 hrs. Eight control animals were unexposed. At 7 mo of age subjects began training to perform cognitive function tasks as part of the National Center for Toxicological Research Operant Test Battery (OTB); these included those for assessing learning, motivation, color discrimination, and short-term memory. Subjects responded for food pellets by pressing response levers and press-plates during daily (M-F) test sessions (50 min) and were assigned training scores based upon their individual task performance. Beginning as early as 8 months of age—and continuing for at least the following year—control animals earned more reinforcers in a task assessing appetitive motivation than animals exposed to ISO plus N2O. At about 14 months of age, controls also began outperforming ISO plus N2O exposed animals in the OTB learning and visual discrimination tasks: exposed animals responded more slowly in the color discrimination task and completed less of the learning task—responding more slowly and less accurately—and these effects have continued until the present (for at least twelve months). Performance in the short-term memory task is no different between the groups. These long-term cognitive impairments seen after an 8 hour exposure to ISO plus N2O, while slightly different from those noted in our previous ketamine studies, provide additional evidence that a single anesthetic during surgery on young children. There is evidence that prolonged exposure to ketamine causes damage to the mitochondria in the developing brains of experimental animals. Ketamine-induced alterations may be associated with intracellular signals that cause affected neurons to undergo apoptosis. It is generally accepted that mitochondria play a crucial role in apoptosis and this role may be significant in ketamine-induced neurotoxicity. In this study, we examined the brain damage accompanying ketamine exposure (20 mg/kg bw, subcutaneously every 2 hr for 12 hr; 12 hr recovery) in the frontal cortex of Sprague-Dawley rats (postnatal day 7). Conventional transmission electron microscopy (TEM) was used to examine the overall mitochondrial structure in 2D in order to follow changes that occur within mitochondria after treatment with ketamine versus control. Using a grading scale based on cristae conformation, there is a clear difference (P<0.05) in the mitochondria of ketamine-treated versus control animals. In order to relate images obtained by conventional TEM to the overall structural effect this drug has on brain tissue, serial block face scanning electron microscopy (SBF-SEM) was used to examine the 3D structure of 10-20 μm3 pieces of frontal cortex. The mitochondria in the control tissue were mostly elongated with many forming networks with neighboring mitochondria, while the mitochondria in tissue from ketamine-treated rats were spherical and discrete, present in tightly packed groups, lacking interconnectivity. Both TEM and SBF-SEM support the hypothesis that ketamine damages mitochondria and this effect is associated with the neurotoxicity observed following ketamine treatment. Keywords: ketamine, developmental neurotoxicity, apoptosis, mitochondria, SBF-SEM, TEM

1781 General Anesthesia Induced with Isoflurane Plus Nitrous Oxide during the First Week of Life Can Cause Long-Lasting Cognitive Deficits in Rhesus Monkeys: Comparison with Ketamine

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Sevoflurane, is a liquid halogenated ether inhalation anesthetic that used to induce and maintain general anesthesia. It has been commonly used in surgical procedures for human infants and in veterinary and laboratory animal practice. While it is clear that anesthetics cause neuronal cell death in the rodent model when given repeatedly during the brain growth-spurt period, sevoflurane should be examined in a nonhuman primate model that more closely mimics the developing pediatric population. Since it is considered that the levels of peripheral benzodiazepine receptor (PBR) increase in the areas of neuronal injury following exposure to the neurotoxic anesthetic, PBR is widely recognized as an important target for imaging by positron emission tomography (PET). In this study, [18F]-FEPPA is used as a novel imaging agent for PBR. On PND 5/6, rhesus monkey babies in the experimental group were exposed to 2.5% sevoflurane for 8 hours and control monkeys were exposed to room air only. On PND 6/7, [18F]-FEPPA (56 MBq) was injected into the lateral saphenous vein of treated and control monkeys and microPET images were obtained over the next 2 hr. For follow-up study, microPET scan were repeated for each monkey on PND 2 weeks and PND 1 month. Radiolabeled tracer accumulation in the ROI in the frontal cortex, temporal lobe were converted into SUVs. After the injection, radiotracer was quickly distributed into the brains of both anesthetics-treated and control monkeys. On PND 6/7, compared with the control group, the duration of tracer wash-out was prolonged in the anesthetic-treated animals. The prolonged wash-out of tracer was found in the brains of treated monkeys on their age of PND 2 weeks and PND 1 month. This preliminary study demonstrates that microPET imaging is capable of distinguishing differences in retention of [18F]-FEPPA in different brain regions of non-human primate and suggests that this approach may provide a minimally invasive biomarker of neuronal damage induced by sevoflurane.

1784 Evaluation of Proconvulsant Risk Using Tests Evaluating Spontaneous and Provoked Convulsions

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Introduction: Assessment of proconvulsant risk is an important step in the drug development process. The aim of the study was to evaluate the proconvulsant or convulsant effects of a well-known substance, theophylline, in the absence or presence of factors promoting seizures in the rat. Methods: The occurrence of spontaneous convulsions following administration of theophylline was evaluated by observation in the Irwin test and by measuring brain activity using video-EEG recording in conscious telemetered animals. Theophylline was also tested in the Electroconvulsive Shock (ECS) threshold and Pentyleneetrazone (PTZ)-induced convulsions tests, two commonly used models of provoked convulsions. Results: In the Irwin test, theophylline induced convulsions in one rat at 128 mg/kg. Seizure/convulsive activity was also observed by video-EEG recording in 3 out of the 12 animals tested at 128 mg/kg. Abnormal EEG signals were also observed in two rats in the absence of clear behavioural symptoms, indicating that some EEG precursor signs can be detected before apparition of convulsions. Clear proconvulsant activity was shown over the dose-range 16-128 mg/kg in the ECS threshold and PTZ-induced convulsions tests. Conclusion: Evaluation of spontaneous convulsions provides information on the therapeutic window of a drug and the translational value of the approach is increased by the use of video-EEG. Tests based on provoked convulsions complement the safety evaluation since they mimic situations of high risk. Measurement of both spontaneous and provoked convulsions improves the evaluation of proconvulsant risk of novel pharmacological substances.

1785 Substances with Analgesic Properties, Tetrahydrocannabinol (THC) and Ibuprofen, Affect Behavior and Cognitive Function Differently after Developmental Exposure


Paracetamol is the most favored analgesic drug during pregnancy, in preterm infants, newborns and toddlers. However, when exposure occurs during brain development, paracetamol has recently been associated with adverse effects on behavior and cognitive function in both epidemiological and animal experiments. THC and paracetamol have been proposed to exert their analgesic effects in the brain by bid-
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The Evans blue dye was extracted and its concentration was quantified. The solution of 2% Evans blue dye (2ml/kg), after 2 hours, the brain was removed in the absence of lipopolysaccharide (LPS) (1 mg/kg i.p. for 2 hours). PRL concentration was inhibited by bromocriptine (BrCr) (1mg/kg i.p.) or saline (control) for 28 days, in presence or absence of LPS.

PRL production, male Wistar rats were treated with the D2 receptor agonist, bromocriptine (BrCr) (1mg/kg i.p.) or saline (control) for 28 days, in presence or absence of LPS (1 mg/kg for 2 hours). PRL concentration in the absence of LPS had lower levels of claudin-5 and occludin, suggesting that the increase in permeability is due to a decrease in these proteins. These data suggest that PRL is a hormone that regulates the permeability of the BBB in vivo by modulating the expression of the TJs proteins.

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Neonatal Paracetamol (Acetaminophen) Exposure, during a Defined and Critical Period of Brain Development, Causes Altered Spontaneous Behavior in Both Male and Female Adult Mice


Paracetamol is a widely used non-prescription drug with antipyretic and analgesic properties. Among pregnant woman and children, paracetamol is one of the most frequently used drugs and is considered the first-choice treatment for pain and fever. Recent epidemiological studies have shown that prenatal exposure to paracetamol in humans is linked to an increased risk for development of ADHD-like behavior problems or hyperkinetic disorders later in life. We have previously shown that neonatal paracetamol exposure caused altered spontaneous behavior and changed anergic and anxiolytic responses in adult mice. Due to the extensive human exposure to paracetamol, the present study was undertaken to investigate if there is a critical period during neonatal brain development for the induction of the developmental neurotoxic effects of paracetamol. Male and female mice were given two subcutaneous doses of paracetamol (30 x 30 mg/kg bw, 4 h apart) on PND 3, 10-, or 19. Spontaneous behavior, when introduced to a novel home environment, was tested at the age of 2 months. We show that effects on adult behavior and cognitive function occurred in both male and female mice exposed to paracetamol on PND 3 and 10, but not in mice exposed on PND 19. This study replicates our previous findings, where altered spontaneous behavior and cognitive function were shown for mice neonatally exposed paracetamol on PND 10. Furthermore, a period of vulnerability for the paracetamol-induced neurotoxicity is defined, as it highlights a critical period of neonatal brain development between PND 3 and 10. Aforementioned epidemiological studies showed that paracetamol exposure during the third trimester of pregnancy had the highest association with adverse outcomes, which further supports the results presented herein. Due to the high exposure to paracetamol during pregnancy and in early life, these results may be of great importance in both future research and clinical practice.

1786 Neonatal Paracetamol (Acetaminophen) Exposure, during a Defined and Critical Period of Brain Development, Causes Altered Spontaneous Behavior in Both Male and Female Adult Mice


Prolactin (PRL) is polypeptide hormone produced by the lactotrophs in the anterior pituitary and exerts more than 300 actions, including changes in cellular permeability. Blood-Brain Barrier (BBB) a frontier constituted mainly by endothelial cells, which separate the brain tissue from the circulating substances in the vascular system, protecting the brain structures from potentially harmful substances in the blood through the expression of tight junction (TJ) proteins. The aim of this study was to evaluate the effect of PRL on the permeability of the BBB. To inhibit PRL production, male Wistar rats were treated with the D2 receptor agonist, bromocriptine (BrCr) (1mg/kg ip.) or saline (control) for 28 days, in presence or absence of lipopolysaccharide (LPS) (1 mg/kg ip. for 2 hours). PRL concentration was determined by ELISA. The BBB permeability was evaluated by injecting a solution of 2% Evans blue dye (2ml/kg), after 2 hours, the brain was removed and the Evans blue dye was extracted and its concentration was quantified. The expression of the TJ proteins claudin-5 and occludin was analyzed by western blot. BrCr decreased the PRL concentrations to almost undetectable levels and showed a significant increase in permeability compared to control. When the BrCr-treated rats were treated with LPS, the increase in permeability was potentiated. In association with the increase in permeability, the BrCr treated rats both, in presence and absence of LPS had lower levels of claudin-5 and occludin, suggesting that the increase in permeability is due to a decrease in these proteins. These data suggest that PRL is a hormone that regulates the permeability of the BBB in vivo by modulating the expression of the TJs proteins.

1787 Inhibition of Prolactin with Bromocriptine Increases Blood-Brain Barrier Permeability In Vivo

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Prolactin (PRL) is polypeptide hormone produced by the lactotrophs in the anterior pituitary and exerts more than 300 actions, including changes in cellular permeability. Blood-Brain Barrier (BBB) a frontier constituted mainly by endothelial cells, which separate the brain tissue from the circulating substances in the vascular system, protecting the brain structures from potentially harmful substances in the blood through the expression of tight junction (TJ) proteins. The aim of this study was to evaluate the effect of PRL on the permeability of the BBB. To inhibit PRL production, male Wistar rats were treated with the D2 receptor agonist, bromocriptine (BrCr) (1mg/kg ip.) or saline (control) for 28 days, in presence or absence of lipopolysaccharide (LPS) (1 mg/kg ip. for 2 hours). PRL concentration was determined by ELISA. The BBB permeability was evaluated by injecting a solution of 2% Evans blue dye (2ml/kg), after 2 hours, the brain was removed and the Evans blue dye was extracted and its concentration was quantified. The expression of the TJ proteins claudin-5 and occludin was analyzed by western blot. BrCr decreased the PRL concentrations to almost undetectable levels and showed a significant increase in permeability compared to control. When the BrCr-treated rats were treated with LPS, the increase in permeability was potentiated. In association with the increase in permeability, the BrCr treated rats both, in presence and absence of LPS had lower levels of claudin-5 and occludin, suggesting that the increase in permeability is due to a decrease in these proteins. These data suggest that PRL is a hormone that regulates the permeability of the BBB in vivo by modulating the expression of the TJs proteins.
P1 1790 Evaluation of Purkinje Neurons As Potential Target of Alcoholism Using Japanese Medaka (Oryzias latipes) As Animal Model

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Purkinje cells (PK) are considered as the neurons with large cell bodies which are found in the central nervous system and are mostly distributed in the cerebellar region of the hind brain. Several novel markers for PK cells have been developed and are used for characterization of neurobehavioral disorders. The present study was aimed to identify endocannabinoid receptor (CB receptor) as a potential marker of PK cells and their evaluation as a molecular target of cerebellar functions using Japanese medaka (Oryzias latipes) as an animal model. Previously, we have observed that medaka genome consist three CB receptor paralogs; two of them (crt1a and cort10) showed structural identity with human CB1 and another with human CB2. Moreover, we have also observed that ctn mRNA as (crt1a, 1b, and cr1) are highly expressed in medaka embryos by disrupted developmental ethanol exposure. Moreover, adult male medaka when exposed to ethanol (300 mL) waterborne is able to alter swimming behavior within 1 h of exposure. We expect that these alterations are probably mediated by disruption of the expression of the crt1a receptor in the brain especially in the PK cells of the cerebellum. Preliminary data indicate that the average sizes of the cell body of PK neurons in adult medaka were increased during ethanol exposure which may be related with the expression of cort1a mRNA in PK cells.

P1 1791 Effects of Adolescent Treatment with Nicotine, Harmone, or Norharmone in Male Sprague-Dawley Rats


Initiation of tobacco use often occurs in adolescence and may be especially detrimental as there is substantial brain development occurring at this time. In addition to nicotine, there are over 9,000 other compounds present in tobacco products, including the β-carbolines harmene and norharmene. The present study determined the long-term effects of adolescent exposure to nicotine (NIC), harmene (HAR), or norharmene (NOR) on locomotor activity, learning and memory, anxiety-like behavior, motor coordination, and monoamine/metabolite concentrations in the striatum and nucleus accumbens of male Sprague-Dawley rats. Beginning on postnatal day (PND) 27 and continuing through PND 55, subjects received twice daily intraperitoneal injections of 1 ml/kg saline (CON), 0.5 mg NIC/kg, 0.5 mg HAR/kg, or 0.5 mg NOR/kg. Body weight, food, and water intake were measured daily (PNDs 27-96). Locomotor activity was assessed on PND 40 or 41, PND 55, and PND 81 and 82. Other behaviors (anxiety-like behavior, motor coordination, and spatial learning and memory) were assessed after at least 25 days after drug exposure ended (PND 80-91). On PND 97, subjects were euthanized and the striatum and nucleus accumbens were dissected and frozen for analysis. NIC treatment significantly decreased food intake, but did not alter locomotor activity during or after treatment. HAR and NOR treatment, however, caused significant open field hyperactivity. Motor coordination, water maze performance, and concentrations of monoamines and metabolites in the striatum and nucleus accumbens were unaltered by any drug treatment. These results indicate a long-lasting effect on activity levels from adolescent HAR or NOR treatment; however, there were few long-lasting NIC effects. Given the paucity of data describing effects of HAR or NOR exposure, these data should encourage additional studies of these tobacco constituents as well as constituent combination studies.

P1 1792 E-Cigarettes—A Global Challenge: Imprinting the Central Nervous System of the Next Generation


The emergence of e-cigarettes into the market, and their rising popularity among the public is a growing public health concern. Nicotine is a known deleterious neurotoxin during central nervous system (CNS) development. And while the toxic effects of nicotine alone are well known, little toxicological information on the other components of e-cigarette aerosols, including glycerin, propylene glycol, flavorings, odorants, and likely metals. To evaluate the relative neurotoxicity of e-cigarettes on CNS development, pregnant C57BL/6 mice (9-10-wk-old at mating; n=10 pregnant mice/treatment) were exposed daily throughout gestation and lactation (weaned at ~3-wk-of-age) via whole body inhalation to e-cigarette vapors produced from Blu™ e-cigarettes of varying nicotine concentrations (i.e., high [13-16 mg], low [6-8 mg] and none) for 3 hr/d; 5d/wk. In addition, paracrine-related measures of corticosterone and relative mass of adrenal glands were compared among exposures. All animals were sacrificed 24 hr after exposure cessation and frontal cortex, striatum, hippocampus, olfactory bulbs, midbrain and cerebellum sections were taken for comparison between exposed and non-exposed groups. Affymetrix GeneChip arrays (Qiagen) are used to complete a genome-wide gene expression analysis on male frontal cortex samples. Results of preliminary arrays suggest alterations in cortical gene expression in several genes known to affect mental health. These results indicate that e-cigarettes could produce alterations to the CNS after early life exposure, posing a potential public health threat that requires further investigation. Supported by NYU NIEHS pilot grant.

P1 1793 Early Developmental Exposure of Rats to Low-Dose Tobacco Smoke Extract or Nicotine Modeling Secondhand Smoke Produce Long-Term Behavioral Dysfunction

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Second hand smoke, also known as environmental tobacco smoke, is a chemically complex form of air pollution. Exposure to tobacco chemicals during pregnancy has been associated with high birth weight infants and children with ADHD and other sorts of cognitive dysfunction in children and adolescents. Rats exposed to nicotine during gestation have also been shown to exhibit long-term behavioral dysfunction. The effects of exposure to the more complex tobacco chemical mixture have not been as well studied. This study assessed the effects of nicotine and tobacco chemical mixture exposure during early development in Sprague-Dawley rats. Female rats were exposed to nicotine or tobacco smoke extract (TSE) during a four-week period (sc) via osmotic minipump (Alzet, Model 2ML4) starting three days prior to mating. The pumps delivered 0.2 or 2 mg/kg/day of nicotine based on pre-mating weight. The TSE group was administered a dose that had 0.2 mg/kg/day of nicotine together with the rest of the chemicals in TSE. Offspring of both sexes were assessed for locomotor hyperactivity, emotional dysfunction and cognitive impairment. Developmental exposure to the TSE that delivered 0.2 mg/kg nicotine caused significantly greater locomotor hyperactivity than groups treated with vehicle control solution or 0.2 mg/kg/day of nicotine alone. TSE exposure during development caused a significant impairment in working memory relative to the control group in the novel object recognition task (NOR). These results suggest that non-nicotine chemicals contained in tobacco appear to potentiate the effect of nicotine such that developmental exposure to all chemicals together produce locomotor hyperactivity in juvenile rats and impaired object recognition in young adults. The neurochemical and epigenetic bases for this long-term behavioral dysfunction are being investigated.

P1 1794 GABAergic Involvement in the Hippocampal Development of the Basic Excitability and Feedback Inhibition in Juvenile Rats Prenatally Exposed to Valproic Acid

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Valproic acid (VPA), used as an antiepileptic drug, is known to produce animal models of autism spectrum disorder. We have reported that prenatal VPA-exposure enhances glutamatergic synaptic transmission, spine generation, and feedback inhibition in the hippocampal CA1 area of postnatal day (PND) 13-15 rat pups. Gamma-aminobutyric acid (GABA) receptor-mediated system is critically involved in the neuronal development. Thus, we aimed to clarify whether the GABA receptor-mediated system is involved in the VPA-induced enhancement of the CA1 neuronal circuits. VPA was orally administered to the pregnant day15 Wistar rats with the concentrations of 0 (control, saline only) and 300 mg/kg. On the days from PND 13 to 18, field potentials were recorded from the CA1 area of hippocampus (weaned at ~3-wk-of-age) via whole body inhalation to e-cigarette vapors produced from Blu™ e-cigarettes of varying nicotine concentrations (i.e., high [13-16 mg], low [6-8 mg] and none) for 3 hr/d; 5d/wk. In addition, paracrine-related measures of corticosterone and relative mass of adrenal glands were compared among exposures. All animals were sacrificed 24 hr after exposure cessation and frontal cortex, striatum, hippocampus, olfactory bulbs, midbrain and cerebellum sections were taken for comparison between exposed and non-exposed groups. Affymetrix GeneChip arrays (Qiagen) are used to complete a genome-wide gene expression analysis on male frontal cortex samples. Results of preliminary arrays suggest alterations in cortical gene expression in several genes known to affect mental health. These results indicate that e-cigarettes could produce alterations to the CNS after early life exposure, posing a potential public health threat that requires further investigation. Supported by NYU NIEHS pilot grant.
antagonist bicuculline methiodide (BMI) increased the PS amplitude by 117% in the PND13-15 control and by 33% in the PND13-15/VA groups. Our results suggest that, in the hippocampal CA1 area during PND13-15 before eye-opening, prenatal VPA-exposure not only enhances inhibitory circuits and basic excitability, but alters GABA(A) receptor-mediated inhibition, probably tonic inhibition by extrasynaptic GABA(A) receptors, of PS amplitude.

1795 Lengthening of the Electro-Mechanical Window (EMw) in Dogs with Induced Left-Ventricular Diastolic Dysfunction
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Shortening of the electromechanical window (EMw), as measured by the time-interval between the end of electrical repolarization (i.e., end of the T-wave, Trend) and the end of mechanical relaxation, has been shown to be an index of Torsadogenic susceptibility. In the setting of diastolic left-ventricular dysfunction and/or heart failure with preserved ejection fraction (HFP EF), the EMw may also reflect diastolic electro-mechanical uncoupling due to impaired calcium-reuptake. This study evaluated the relationship between the EMw and indices of diastolic cardiac function in dogs with either normal ventricles or induced HFP EF. Dogs (n=12) were chronically instrumented for ECG and for LV pressure-volume (LVPV) recordings. A set of animals served as controls (CTRL, n=6) while another set (HFP EF, n=6) had diastolic dysfunction induced via bilateral renal wrapping, resulting in renoproval hypertension. ECG and functional data were obtained at rest in conscious animals; load-independent function was examined via LVPV relationships during brief pre-load reductions. Chronic renal wrapping, triggered hypertension (ESP: 113±7 vs. 143±4 mmHg) and depressed markers of lusitropy (tau: 15±1 vs. 20±1 ms, EDPVR: 1.1±0.1 vs. 2.8±0.2 mmHg/mL) while preserving inotropy (PRSW: 83±4 vs. 87±2 mmHg) mimicking the presentation of HFP EF in the clinic. In this setting, the EMw was prolonged (97±4 vs. 87±2 ms). Notably, the EMw was correlated (P<0.05) with markers of ventricular compliance (EDPVR: R2=0.52). (*: P < 0.05, vs. CTRL). Prolongation of the electromechanical window, reflecting uncoupling between electrical repolarization and mechanical diastole, was independently associated with diastolic dysfunction.

1796 Cardiac Safety of Lacosamide (Vimpat®): The Nonclinical Perspective

Lacosamide (Vimpat®) is indicated as monotherapy (US only) or adjunctive treatment of partial-onset seizures in adults. It acts on neuronal sodium channels but, unlike other sodium channel blockers, lacosamide selectively enhances slow inactivation of these channels. Due to this specific mode of action, its potential effects on cardiac sodium channels (Nav1.5) and cardiac conduction were extensively investigated. Lacosamide was tested on sodium and L-type calcium currents from isolated human atrial myocytes and on hERG-mediated potassium currents from cell outgrowth colonies and early stage progenitor cells. These findings suggest that chronic benzene exposure may disrupt normal glucose handling and hematopoiesis and that these effects may contribute to yet unrecognized impacts on cardiovascular health.
their metabolites play crucial role in initiation of cardiac hypertrophy. Rats were
4 treated with isoproterenol (iso) for 12, 24, 72, 168 or 240 hr. Heart function and
5 walls thickness were assessed using echocardiography. RNA was isolated and gene
6 expressions were determined by real-time PCR. Moreover, heart microsomes were
7 prepared to determine P450s and eNOS activity using LCMS. Echocardiographic
8 examination of iso-treated rats showed structural changes in ventricular geometry
9 after 3 days of iso treatment and continued thereafter. Studies performed at pre-
10 hypertrophy (12 and 24 hr) showed a significant decrease in CYP epoxygenases,
11 CYP2C23 and CYP2J3 gene expression, by 82% and 44%, respectively, and a
12 significant increase in CYP hydroxylases, CYP1A2 gene expression by 107%,
13 in iso-treated rats. Moreover, iso treatment caused a significant induction of eNOS
14 activity, which metabolizes EETs to less biologically active DHETs, as early as 12
15 hr by 26%. In accordance with gene expression and eNOS activity, incubation of
16 microsomes with arachidonic acid showed that the formation rate of EETs were
17 significantly lower, whereas the formation rates of DHETs and 20-HETE were
18 significantly higher, in pre-hypertrophied hearts. In addition, iso treatment caused
19 a significant increase of hydroxylase activity/epoxygenase activity ratio prior to hy-
20 pertrophic growth by 68%. Altogether, these results suggest a signal role of P450s
21 and their metabolites in the development of cardiac hypertrophy which could re-
22 veal novel points of intervention to be exploited in the development of new therapy
23 for the prevention of cardiac hypertrophy at early stages.

1800 Cardiovascular Effects Induced by Higenamine Using Telemetry

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Pre-workout and weight loss supplements are routinely utilized by athletes and
others of all ages prior to strenuous exercise to enhance athletic performance and
burn fat. Many of these supplements contain substances such as caffeine, amphet-
amine-like compounds, ß2 receptor agonist-like compounds that could potentially
induce cardiovascular changes. Safety information on many of these ingredients is
often not available. To assess potential cardiovascular effects guinea pigs (n=15; 450-
550 g) were subcutaneously implanted with radiotelemetry devices to con-
tinuously monitor ECG, temperature and activity and subsequently exposed to
higenamine (HG), a beta-2 adrenergic receptor agonist found in some pre-workout
supplement ingredients. Three groups of three animals received baseline recordings,
animals received water p.o. (negative control), followed 24 hr later by salbutamol p.o.
(5 mg/kg; positive control) and a wash-out interval of 3 days. Subsequently, animals received a single dose of HG (0.5, 5 and 50 mg/kg, p.o.) with a wash-out interval of at least 3 days followed by 12 consecutive daily doses of HG. Each animal served as its own control and a recovery/wash-out period of 5 days of recording was monitored at the end of HG dosing. A customized MATLAB algorithm under development was used to extract features from ECG, and other parameter recordings (body temperature, respiration and activity). HG induced a dose dependent increase in heart rate that lasts longer than changes induced by water exposure and salbutamol alone. HG (5 and 50 mg/
kg) given as a single dose resulted in an immediate peak in heart rate that returned to normal within 3 to 6 hours; 0.5 mg/kg HG did not increase HR. The baseline heart rate remained slightly elevated during the repeated dosing period, but it re-
turned to levels slightly lower than the initial baseline after the recovery/wash-out period. Oral repeated dosing of HG (5 and 50 mg/kg) appeared to induce cardio-
vascular perturbations that seemed reversible after dosing ended. Ongoing studies are evaluating changes in ECG signal intervals due to HG dosing.

1801 Characterization of the Methemoglobin-Forming Metabolites of Benzocaine and Lidocaine

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Topical anesthesia with benzocaine or lidocaine occasionally causes methemoglo-
binemia (MetHb), an uncommon but potentially fatal disorder where the blood has a reduced ability to transport oxygen. Previous in vitro studies using human whole blood have shown that benzocaine causes more methemoglobin (MetHb) formation than lidocaine when compared at the same concentration, and that both compounds required metabolic transformation to form the MetHb producing species. In the current investigation, the active species forming the MetHb was
identified. In vitro HPLC analysis of benzocaine samples incubated with human hepatic S9 showed the formation of a peak with the same UV spectrum and retention time as benzocaine hydroxylamine. To confirm the activity of benzocaine hydroxyl-
amine, MetHb production following exposure to the compound was determined in whole human blood using an Oxivometer 4000 CO-oximeter. Benzocaine hy-

1802 Orotic Acid Induces Insulin Resistance and Hypertension Associated with Impaired Endothelial Nitric Oxide Synthesis


Orotic acid (OA) is an intermediate of pyrimidine nucleotide biosynthesis. Hereditary deficiencies in some enzymes associated with pyrimidine synthesis or the urea cycle induce OA accumulation, resulting in orotic aciduria. A link between patients with orotic aciduria and hypertension has been reported; however, the molecular mechanisms remain elusive. In this study, to elucidate the role of OA in vascular insulin resistance, we investigated whether OA induced endothelial dys-
function and hypertension. OA inhibited insulin- or metformin-stimulated nitric oxide (NO) production and endothelial NO synthase (eNOS) phosphorylation in human umbilical vein endothelial cells (HUVEC). A decreased insulin response by OA was mediated by impairment of the insulin-stimulated phosphoinositide 3-kinase (PI3K)-protein kinase B (PKB/Akt) signaling pathway in cells overex-
pressing the p110-Pi3K catalytic subunit. Impaired effects of metformin on eNOS phosphorylation and NO production were reversed in cells transfected with consti-
tutively active AMP-activated protein kinase. Moreover, experimental induction of orotic aciduria in rats caused insulin resistance, measured as a 125% increase in the homeostasis model assessment, and hypertension, measured as a 25% increase in systolic blood pressure. OA increased the plasma concentration of endothelin-1 by 201% and significantly inhibited insulin- or metformin-induced vasorelaxation. A compromised insulin or metformin response on the Akt/eNOS and AMPK/eNOS pathway was observed in aortic rings of OA-fed rats. Taken together, we showed that OA induces endothelial dysfunction by contributing to vascular and systemic insulin resistance that affects insulin- or metformin-induced NO production, lead-
ing to the development of hypertension.

1803 Left Ventricular Pressure (LVP) Assessment Screening Models: Comparison of High-Definition Telemetry in Free-
Moving with Anesthetized Rats

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Introduction: Non-clinical models to screen for cardiac contractility changes are used. The rat model is the preferred species: left ventricular pressure (LVP) was measured in the conscious and anesthetized rats by direct catheterization of the LV apex. Results from conscious and anesthetized animals with the same positive control agents were compared. Results: Average dP/dt max observed during day (794± 890 mmHg/sec) and night time (9906± 1689 mmHg/sec) con-
firmed the circadian changes associated with normal activities. Pharmacological agents known for their contractility enhancer (Dopamine) or lessener (KCl and Sotalol) properties produced the expected effect on dp/dt max in both models. In the anesthetized model Sotalol (6 mg/kg, IV) and KCl (0.34 mEq/kg, IV) induced a reduction in dp/dt max of -23% and -21%, respectively compared to control. Dopamine induced a dose dependent increase in dp/dt max (+11% at 0.05 mg/kg and +34% at 0.1 mg/kg) in anesthetized rats. In the conscious rats, Sotalol (6 mg/
kg, IV) and KCl (0.34 mEq/kg, IV) were also associated with a decrease in dp/dt max. Dopamine (IV) induced a dose dependent increase in dp/dt max at (0.05 mg/
and 0.1 mg/kg) in conscious rats. Discussion: Our results suggest that the anesthetized rat model may be more sensitive than the conscious model to detect drug induced contractility changes. The conscious model enables repeated admin-
istration which is often required to increase sensitivity of the assay. Left ventricular pressure (LVP) monitoring in free-moving or anesthetized rats represent applicable methodologies for non-clinical safety evaluations in drug development.
Cardiotoxic Effects of 1, 1-Difluoroethane Due to Oxidative Stress and Electrolyte Changes

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Inhalant abuse is the intentional inhalation of chemical vapors to attain euphoric effect. Many common household products are abused by inhalation and one of them is 1, 1-Difluoroethane (DFE). DFE is a halogenated hydrocarbon used as a propellant in dust-off spray and airbrush painting. Although many human DFE cases have been studied, the etiology and mechanism of sudden death is still unknown. Earlier electrocardiographic monitoring in our laboratory showed DFE treated animals resulted in death from severe arrhythmias. The study monitored effects of multiple inhaled doses of DFE on the electrocardiogram. In this study an animal model was used to simulate the human conditions of DFE inhalation abuse that result in sudden death. Current experiments involve mechanistic study involving electrolyte changes, enzyme levels and oxidative stress markers after DFE administration in vivo. To investigate these changes, Sprague Dawley rats (n=6) were exposed to 30 sec. of 20 L/min multiple doses of DFE. Isoflurane acted as a control and two additional groups; Epinephrine and epinephrine + DFE were included which mimic clinical condition. Following DFE administration blood was collected by cardiac puncture. Sodium, potassium, calcium and magnesium levels were measured followed by Lactate dehydrogenase (LDH), Troponin I (cTnI) and Creatine Kinase (CK) levels. In addition, oxidative stress markers; Malondialdehyde (MDA), Catalase (CAT) and superoxide dismutase (SOD) were evaluated in all animal groups. Electrolyte levels showed a significant rise in plasma potassium and magnesium level while sodium and calcium levels were not altered. In addition, LDH, cTnI and CK levels in DFE and epinephrine + DFE administered rats were significantly elevated as compared to control rats. Oxidative stress markers MDA, CAT and SOD were elevated significantly in DFE and epinephrine + DFE groups. These results support earlier cardiotoxic electrocardiographic effects indicating that DFE results in fatal arrhythmias. Further in vitro experiments are planned to validate these results.

Potent Inhibition of Cholesteryl Ester Transfer Protein (CETP) Activity in Monkeys following 2-MOE Antisense Oligonucleotide Administration

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Cholesteryl ester transfer protein (CETP) facilitates the movement of neutral lipid between lipoproteins, resulting in a net exchange of triglyceride (TG) from VLDL for cholesteryl ester (CE) from HDL. The impact of CETP on HDL metabolism was initially revealed in a series of genetic studies of Japanese families in which subjects with elevated HDL levels were found to have mutations that resulted in CETP deficiency. CETP is synthesized within the liver and small intestine as well as a number of other peripheral tissues, notably adipose. Due to their ability to promote positive effects across all of the lipoprotein classes, CETP represents an attractive therapeutic opportunity for reducing cardiovascular disease progression. ISIS 588057 is a 2-MOE antisense inhibitor that is fully complementary to the cytosol molusg monkey mRNA sequence. In this study, we have characterized the pharmacological activity of ISIS 588057 on CETP plasma protein suppression in monkeys after 8 weeks of treatment and recovery until Day 119. ISIS 588057 was administered by subcutaneous injection at doses of 10 or 30 mg/kg to monkeys. Doses were given on alternate days for the first 4 doses (loading) and once weekly thereafter (maintenance). Results from this study demonstrate both dose- and time-dependent reduction in plasma CETP after the loading doses, achieving approximately 75% suppression in mean CETP baseline concentrations. After 8 weeks of treatment at both 10 and 30 mg/kg doses, the reduction of plasma CETP was sustained and had only reversed by approximately 50% from nadir following 8 week dose cessation. There was no evidence of changes in liver and kidney function or plasma HDL, LDL, TG or total cholesterol levels in lean monkeys. Collectively, these data suggest that ISIS 588057 is a safe and potent inhibitor of CETP. The prolonged reduction by ISIS 588057 suggests that weekly or biweekly dosing in humans will be possible.

Effect of Moxifloxacin Hydrochloride on Cardiovascular Parameters Assessed via Jacketed External Telemetry (JET) in the Male Beagle Dog Co-Housed in European Caging


European (EU) caging provides an enriched environment for group housed animals and has traditionally been used on repeat dose toxicology assessments. However due to limitations in computer acquisition systems, continuous cardiovascular data assessments under such housing conditions has not been previously feasible. With advances in hardware and software, socialization of animals during data acquisition is now considered feasible. The purpose of this study was to look at the acclimation procedures required to allow continuous data collection without compromising the integrity of the jacket external telemetry (JET) equipment and to demonstrate that the model retained the sensitivity of the traditional single house implantable telemetry, previously considered the gold standard method for continuous cardiovascular assessments. In this study, cardiovascular monitoring, was performed on 4 male beagle dogs co-housed in European caging. Jacket acclimation was performed for at least 5 days prior to cardiovascular baseline assessments and prior to each monitoring occasion. Following appropriate acclimation and confirmation of baseline data sets all animals were dosed with Moxifloxacin, at 10, 30 or 90 mg/kg in an escalating paradigm, to determine whether co-housing impacted on the sensitivity of the model. Cardiovascular parameters (heart rate, PR, QRS, RR, QT and QTc intervals) were assessed for at least 20 hours on each monitoring occasion. The results showed that group housed dogs in European caging conditions had no influence on baseline cardiovascular assessments, as compared against historical control single housed data sets and treatment with Moxifloxacin induced QT interval prolongation in-line with historical data. In conclusion, collection of cardiovascular data using JET in male dogs co-housed in European caging showed stable cardiovascular baseline, was sensitive to detect the expected change in QT interval and jacket integrity was not impacted by group housing when following an appropriate acclimation procedure.
Cardiac Preclinical Safety Profile Review of Cetirizine and Levocetirizine

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In the 80’s, selective H1 receptor antagonists have been developed as non-seating antihistamine drugs for treatment of allergic disorders. Nevertheless, the withdrawal of several of them which caused life-threatening arrhythmia led to reconsider their cardiac safety profile and to build a cardiac risk paradigm for new chemical entities which is currently being challenged. The current review details the preclinical cardiac safety profile of Cetirizine and its enantiomer Levocetirizine, belonging to the second and third generation of antihistamine drugs. Levocetirizine was recently used as negative reference compound in an IQ-CSRC phase I study. It has recently been performed addressing the early QT assessment using exposure response analysis as replacement of Thorough QT study. In vitro, Cetirizine and Levocetirizine did not show any inhibitory effect on hERG up to 30μM in Xenopus oocytes. Their IC50 for blockage of IKr in rabbit or guinea pig cardiomyocytes was ~100μM, corresponding respectively to 750 and 2500 fold the free therapeutic plasma concentration in human. Cetirizine induced a slight prolongation of cardiac action potential from 10μM in Purkinje fibres of various species (guinea pig, rabbit, dog) and Levocetirizine induced the same effect in the single tested model (dog Purkinje fibers). In vivo, no conduction effect nor arrhythmia were detected in telemetered conscious dogs or in toxicity studies (± 52 weeks duration) up to large multiple of human exposure (± 100 folds). Finally, both compounds were evaluated in a long QT/TPD dog model where anesthetized animals were placed in predisposed conditions (provoked bradycardia); no arrhythmia was found up to more than 40-fold the human therapeutic exposure. In clinics, absence of effect of Levocetirizine on cardiac repolarization was demonstrated in a thorough QT studies at both therapeutic and supra-therapeutic doses. In conclusion, Cetirizine and Levocetirizine appear to have very low risk of QT prolongation, TDP or any other cardiac electrophysiological concern.

Zinc Rescues Arsenic-Mediated Impairment in Cardiac EMT during Coronary Vessel Development

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Current research on the etiology and progression of cardiovascular diseases (CVD) majorly focuses on adulthood which fail to explain all the cases. Emerging data suggest that early life risks may initiate and pattern the molecular programming of cardiovascular ailments in adulthood. Exposure to environmental arsenic has been reported to cause early onset of atherosclerosis and increased risk in CVD mortalities. A major contributor to ischemic heart pathologies and cardiovascular ailments is the epithelial to mesenchymal transition (EMT). During cardiac EMT, activated epicardial progenitor cells transform to mesenchymal cells to form the cellular components of coronary vessels. In this study, 24 hour exposure to arsenite and MMA (III) disrupted developmental EMT programming in murine epicardial cells causing a deficit in cardiac mesenchyme. Canonical and non-canonical TGFβ2 signalings were selectively disrupted arsenical exposure via blockade in phosphorylation and nuclear translocation of the Smad2/3 and Erk5. We also found that acute arsenicals exposure significantly depleted nuclear Smads without inhibiting their phosphorylation level. This depletions can be fully restored by treating with leptin or knocking down exportin 4 indicating that arsenicals disrupt nuclear Smads accumulation through facilitating their nuclear exportation. Zinc supplementation following arsenicals exposure restored nuclear Smad2/3 detection and activation, and also rescued the deficits in cardiac EMT in both chick and mouse heart explants. Overall, our study delineates a novel mechanism of arsenicals and activation, and also rescued the deficits in cardiac EMT in both chick and mouse heart explants. Overall, our study delineates a novel mechanism of arsenicals and activation, and also rescued the deficits in cardiac EMT in both chick and mouse heart explants. Overall, our study delineates a novel mechanism of arsenicals and activation, and also rescued the deficits in cardiac EMT in both chick and mouse heart explants. Overall, our study delineates a novel mechanism of arsenicals and activation, and also rescued the deficits in cardiac EMT in both chick and mouse heart explants. Overall, our study delineates a novel mechanism of arsenicals and activation, and also rescued the deficits in cardiac EMT in both chick and mouse heart explants. Overall, our study delineates a novel mechanism of arsenicals and activation, and also rescued the deficits in cardiac EMT in both chick and mouse heart explants. Overall, our study delineates a novel mechanism of arsenicals and activation, and also rescued the deficits in cardiac EMT in both chick and mouse heart explants. Overall, our study delineates a novel mechanism of arsenicals and activation, and also rescued the deficits in cardiac EMT in both chick and mouse heart explants. Overall, our study delineates a novel mechanism of arsenicals and activation, and also rescued the deficits in cardiac EMT in both chick and mouse heart explants. Overall, our study delineates a novel mechanism of arsenicals and activation, and also rescued the deficits in cardiac EMT in both chick and mouse heart explants. Overall, our study delineates a novel mechanism of arsenicals and activation, and also rescued the deficits in cardiac EMT in both chick and mouse heart explants. Overall, our study delineates a novel mechanism of arsenicals and activation, and also rescued the deficits in cardiac EMT in both chick and mouse heart explants. Overall, our study delineates a novel mechanism of arsenicals and activation, and also rescued the deficits in cardiac EMT in both chick and mouse heart explants.
compared to female mice at all cumulative doses with significant differences at 18-24 mg/kg DOX. Also, microscopic examination of the heart revealed a greater susceptibility to DOX in male mice compared to female mice, with cytoplasmic vacuolization detected in cardiomyocytes of only DOX-treated male mice. It was observed at the left atrium was more vulnerable to DOX toxicity than the ventricle or right atrium in male mice. In the left atrium, cytoplasmic vacuolization was noted in one of five male mice (20%) at 18 mg/kg and in all male mice given 21 mg/kg and higher cumulative DOX doses. In the ventricle and right atrium, however, cytoplasmic vacuolization was observed in two of five male mice (40%) at 21 mg/kg DOX and in all male mice administered 24 mg/kg and higher cumulative dose. This newly established mouse model will provide a means to access underlying sex-related differences in DOX cardiotoxicity and aid in identifying potential mechanisms responsible for a clinically important question.

1813 Gene Expression Changes in the Hearts of Mice Chronically Exposed to Doxorubicin

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Anthracyclines are anti-cancer drugs, with the widely used doxorubicin (DOX) being particularly effective. The use of DOX, however, is limited by a dose-dependent risk of cardiotoxicity leading to heart failure. Current biomarkers of cardiotoxicity, such as cardiac troponins, are released into the plasma only after cardiac cell injury and there is a need for biomarkers that can accurately predict risk of cardiac injury before it occurs. We used a newly developed mouse model of DOX-induced cardiotoxicity to examine gene expression changes in the heart with the aim of identifying potential early biomarkers of injury. B6C3F1 mice were given weekly injections of 3 mg/kg DOX or saline via tail vein for 2, 3, 4, 6, and 8 weeks, resulting in cumulative DOX doses of 6, 9, 12, 18, and 24 mg/kg. An increase in the plasma concentrations of cardiac troponin, indicating tissue injury, occurred at doses above 18 mg/kg and cardiac lesions were observed at 24 mg/kg. Whole genome gene expression profiling was conducted on RNA isolated from cardiac tissue, and differentially expressed genes were identified at each DOX dose using statistical filtering criteria of p<0.05 and fold-change >1.5 in DOX-treated mice compared to concurrent saline controls. The number of differentially expressed transcripts ranged from 792 at 9 mg/kg to 2292 at 24 mg/kg, with most changes resulting in increased expression. Many of the genes differentially expressed at all doses appeared to be regulated by the transcription factors TBX2, FOXM1, and CCND1, with additional transcription factors involved at higher cumulative doses of DOX. The expression of 128 genes was significantly (p<0.05) altered by at least 1.5-fold at each cumulative dose, with all but 2 genes being up-regulated. This gene set was enriched for functions related to cell cycle control and DNA damage and repair, suggesting a continuing response to the DNA damaging effects of DOX. The expression of the gene encoding the anti-tumorigenic target of DOX, topoisomerase 2, was elevated at all doses suggesting a compensatory response to inhibition of this critical enzyme.

1814 Early Transcriptional Changes in Genes Associated with Calcium Homeostasis in Hearts of Mice Treated with Doxorubicin

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Doxorubicin (DOX) is an effective antineoplastic drug. However, cumulative dose of 300 mg/m2 or more in humans increases the risk of developing cardiomyopathy. The mechanisms of DOX-induced cardiotoxicity are multiple and not fully understood. The sarcoplasmic/endoplasmic reticulum (ER) is important in maintaining calcium homeostasis required for myocardial contraction/relaxation cycle and may lead to a role in DOX cardiotoxicity. Therefore, a mouse model of chronic DOX cardiotoxicity developed in our laboratory was used to investigate DOX-induced disturbances in calcium homeostasis in the heart and to identify potential early biomarkers of cardiotoxicity. A weekly dose of 3 mg/kg DOX or an equivalent volume of saline (SAL) was administered intravenously via tail vein to male mice for 2, 3, 4, 6, and 8 weeks, resulting in cumulative DOX doses of 6, 9, 12, 18, and 24 mg/kg, respectively. Animals were euthanized a week after the last dose. Cellular injury, indicated by cardiac troponin T release in plasma, and structural damage, indicated by cytoplasmic vacuoles in cardiomyocytes, was observed at 18 and 24 mg/kg doses, respectively. RNA was extracted from heart tissue and whole genome expression profiling was performed. Transcriptomics analysis revealed that genes encoding SERCA Ca(2+)/ATPase (Atp2a1, Atp2a2), ryanodine receptors (Ryr2), voltage-dependent calcium channels (Cacna1a, Cacna1c, Cacna2d1, Cacna2d2, Cacnb2) and other proteins maintaining calcium homeostasis (Asph, Trdn, Jhp2) were significantly down-regulated, whereas Sri and Jsp1 were up-regulated at 6, 18 and 24 mg/kg DOX compared to concurrent SAL-treated controls. The significantly altered expression of these genes at cumulative exposure of 6 mg/kg, before cardiac tissue damage, may suggest early disturbances in calcium homeostasis in heart and may be useful as potential biomarkers to predict DOX-induced cardiac injury.

1815 Early Molecular Changes Related to Cardiac Hypertrophy in the Hearts of Doxorubicin-Treated Mice

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Doxorubicin (DOX) is an effective anti-cancer drug known to cause irreversible cardiotoxicity in a dose-related manner in cancer patients. Cardiac troponin T (cTnT) has been used as a clinical marker of myocardial injury; however, it is released in blood only after tissue damage has occurred. To identify early biomarkers of cardiac injury, an omics approach was used in hearts of mice treated with DOX. Male B6C3F1 mice received 3 mg/kg DOX or an equivalent volume of saline i.v. once a week for 2, 3, 4, 6, and 8 weeks that resulted in cumulative DOX doses of 6, 9, 12, 18, and 24 mg/kg, respectively. Animals were euthanized a week after the last dose. Myocardial injury (elevated plasma cTnT levels) occurred at 18 mg/kg and higher cumulative dose. However, heart histopathology revealed cytoplasmic vacuolization in cardiomyocytes only at 24 mg/kg DOX. Whole genome mRNA profiling in heart tissues identified 7 differentially expressed (p<0.05, FC>1.3) genes (Akap13, Edn1, Htr2b, Inpp5f1, Rgs2, Ryr2, Ttn) associated with cardiac hypertrophy, 3 of which (Inpp5f1, Ryr2, Ttn) showed early changes at cumulative DOX doses lower than 18 mg/kg. Furthermore, miRNA expression profiling revealed 7 differentially expressed miRNAs that are associated with the regulation of cardiac hypertrophy. Among these, mir-150 was down-regulated at 12 mg/kg cumulative DOX dose, prior to myocardial injury. In addition, proteomics analysis indicated differentially expressed proteins associated with cardiac hypertrophy in hearts of DOX-treated mice. Talin1, a cytoskeletal protein linked to cardiac hypertrophy attenuation, showed increased expression at 6 and 24 mg/kg DOX. These findings suggest early hypertrophy-related molecular changes in hearts of DOX-treated mice and may lead to the identification of early predictive biomarkers of cardiac injury for application in clinical therapies.

1816 Identification of Novel Biomarkers for Doxorubicin-Induced Toxicity in Human Pluripotent Stem Cell-Derived Cardiomyocytes

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Doxorubicin is an efficient chemotherapeutic agent for a variety of cancers. Doxorubicin treatment is however associated with severe cardiotoxicity, often resulting in early discontinuation of the treatment. The exact mechanisms causing doxorubicin-induced cardiomyopathy are not known, but the variation in time-to-onset, gender- and age differences suggest that several mechanisms are involved. In this study, the toxic effects of doxorubicin exposure have been investigated in primary cardiomyocyte cultures derived from human embryonic stem cells. The cardiomyocytes (Puro hES-CM1, Takara Bio Europe AB) were exposed to a low- (50nM), medium- (150nM), and high (450 nM) dose of doxorubicin for 48h, followed by a 12 days wash-out period. There was an evident effect of the doxorubicin exposure, even after the wash-out period. The cells showed an altered morphology and reduced contractile ability during and after the doxorubicin exposure. An acute response of Lactate dehydrogenase and troponin T (cTnT) release was seen, however, these toxic responses vanished during the wash-out period. Numerous genes with interesting expression profiles were identified, with a potential to be novel biomarkers for the doxorubicin-induced cardiotoxicity. The results show that cTnT release can be a measurement of acute cardiotoxicity due to doxorubicin exposure. However, for the late-onset of doxorubicin-induced cardiomyopathy, cTnT release might not be a relevant biomarker. As reported here, a defined list of genes altered after doxorubicin exposure could provide more relevant biomarkers.
Epidemiological evidence that passive exposure to secondhand smoke (SS) exerts detrimental effects on vascular homeostasis remains equivocal. The aim of this work was to investigate the vascular changes following SS exposure (SS-E) and to characterize the cellular and molecular mechanisms of such effects. Male C57BL/6 mice were exposed for 16, 32, or 48 weeks to SS generated from 3R4F reference research cigarettes using the Teague TE-10 smoking machine. Aortas of SS-exposed mice (SSM) showed increased superoxide production with coordinate overexpression of NADPH oxidase subunits p22phox and gp91phox. Tetrahydrobiopterin (BH4) levels were lower in SSM compared to air-exposed controls. Endothelial nitric oxide synthase (eNOS) expression and phosphorylation and Akt expression decreased in response to SS. Furthermore, impaired acetylcholine (Ach)-induced endothelium-dependent relaxation with a shift to the right and downward in the Ach-concentration response curve was observed in thoracic aortic segments of 48 weeks SSM. Persistent hypertension was first observed in SSM at 32 weeks of exposure followed by a time-dependent increase of mean arterial blood pressure at 48 weeks of exposure, reaching 136±6 mm Hg compared to 103±6 mm Hg in controls. Thus, SSM induced vascular endothelial dysfunction (VED) and hypertension through generation of reactive oxygen species which depleted BH4 and impaired Akt-mediated eNOS activation. Overall, our study shows that passive exposure to cigarette smoke can trigger vascular disease and provides mechanistic insight into how SSM causes VED.
EDV, ESPVR was significantly increased in an attempt to maintain LV function despite reduced EDV; however, PRSW was not increased. DCB230 1d MI/R rats had decreased ESP without further reductions in SV or CO compared to DCB230 sham MI/R. SV and CO were significantly reduced compared to vehicle 1d MI/R rats. Due to the decrease in EDV, DCB230 1d MI/R rats could not increase PRSW to match the LV function of vehicle 1d MI/R rats. DCB230's effects on LV function dissipated within 8d of exposure. There were no significant differences in infarct size between groups. Thus, inhalation of EPRFs transiently degrades cardiac function during MI/R, possibly accounting for epidemiological links between PM and MI/R-related mortality. Support P42ES013648, P20RR018766.

1822 Prevalence and Incidence of Cataracts in a Population of Yucatan Miniswines after Induction of Type 1 Diabetes

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Cataracts as a consequence of chronic diabetes is considered a leading cause of legal blindness in humans in the United States and is also observed frequently in aged diabetic populations (>65%). Objective: Assess post-induction (PI) onset of clinical ocular cataract(s) in a colony of over 266 castrated, male, diabetic, Yucatan miniature swine. Methods: Diabetic miniature swine were routinely screened by the veterinary staff for clinical ocular abnormalities including visible ‘mature’ cataracts. Results: Over the course of a 6 month period, the prevalence was 30% (80 positive animals out of 266 animals). The most recent incidence (past 2.5 months) was 20.4% (38 positive animals with 60 affected eyes from pool of 186 previously negative animals). Eighteen animals had bilateral and 20 animals had unilateral cataracts (OD: 31; OS: 29). Cataract onset ranged from 2 to 19 months PI with an average of 12 months PI. Conclusions: The incidence of diabetic cataracts was established in our swine colony. The early onset of cataracts observed may be useful for the model of retinopathy of diabetes. Acknowledgements: Supported by USDA National Research Initiative, 2013-68005-22123.

1823 Induction and Long-Term Management of Type 1 Diabetes in Göttingen Minipigs

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Although a variety of rodent models for type I diabetes exist, such models have genuine limitations and poor predictivity due to the stark differences between rodents and humans. In many respects, diabetic minipig models more closely resemble the human condition; for example, human insulin and porcine insulin differ by only a single amino acid on the B-chain. This study was conducted to establish the long-term management of a type I diabetic model in Göttingen minipigs for future efficacy and toxicity testing. Type 1 diabetes was induced in two male and two female Göttingen minipigs (8 to 15 weeks of age) by intravenous administration of streptozotocin at 150 mg/kg. Diabetes was achieved in all animals within 3 days, as evidenced by fasting blood glucose levels of 258-451 mg/dL. Insulin was administered twice daily coincident with feeding, and assessment of blood glucose. Insulin (Vetsulin) doses and food quantities were individually titrated for each animal in an attempt to achieve glucose levels between 200 and 500 mg/dL, before arriving at a final insulin regimen of 0.75 U/kg s.c. administered in the afternoon with a reduction of 5 units per animal in the morning, and occasional adjustments for individual animals. By Month 11, glucose values averaged just over 250 mg/dL in the morning and approximately 200 mg/dL in the afternoon. Glucose levels in the urine ranged from 100 to 1000 mg/dL from Day 21 on, with occasional trace levels of urinary ketones. From Days 1-19, all animals lost weight (8-18% compared to Day 1), with subsequent slow weight gains thereafter. Female pigs achieved an essentially constant body weight by Day 219, whereas the male minipigs continued to show incremental increases in body weight through Day 330. By Day 92, one male and one female minipig each had bilateral cataracts consistent with aldose reductase–linked diabetic cataracts. By managing the minipigs within a blood glucose level range of 200–300 mg/dL, we have demonstrated some of the expected complication of long-term diabetes while maintaining the animals in reasonably good health.

1824 Generation of a Mammalian Achondroplasia Model in Micro pigs

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Achondroplasia (ACH) is an autosomal dominant disease, characterized by marked short-limbs, rhizomelic short stature, macrocephaly, low nasal bridge, frontal bossing, narrowing of spinal cord column and thorax. ACH patients suffer with delayed motor development, persistent or recurrent middle-ear dysfunction and bowing of lower legs. Most patients with ACH features share a gain-of-function mutation of Gly380Arg amino-acid substitution in the transmembrane domain of FGFR3. It is believed that the malfunction of FGFR3 activation contributes to ACH etiology and pathology. On the other hand, FGFR18 activates FGFR2 and FGFR3 in the epiphyseal growth plate and adjacent subchondrogenesis in vivo and in vitro. Recently, we found that direct injection of FGFR18 into knee joints resulted in the premature growth plate closure in mice, which suggested that intraarticular administration of FGFR18 may mimic ACH symptoms by activating FGFR3. The purpose of the present study was to develop an ACH minicig model by targeting FGFR3 with wild-type FGFR18 and a FGFR3-selective version of FGFR18 (FGFR18v1) and to advance the development of the specific and safe therapeutics for ACH disease. After several intraarticular administrations of FGFR18 and FGFR18v1, the micro pigs were sacrificed, and their femur andibia were harvested for the following evaluations. The bone length of femur andibia were measured, and the growth plate morphology was analyzed by imaging technology of C-arm-X-ray and Micro-CT and by the histopathological examinations. Consistent to the preliminary results in mice, we found that FGFR18v1 administration dramatically induced the bone shortening and the growth plate closure both in the femur and theibia in these micro pigs. Conclusively, the ACH minicig model has been successfully established. Until now, there are no known cures or treatments for ACH disease. Based on this newly established ACH minicig model, the spectrum of FGFR18 antagonists could be examined for the validation of the ACH model and for the development of a potential treatment in the future. Key Words: Achondroplasia, FGFR18, FGFR3, Micropig, Porcine disease model.

1825 Noninvasive Vascular Imaging of Atherosclerotic Lesions in Peripheral and Coronary Arteries in ExGen LDLr Miniswine after Arterial Injury: Correlation with Histopathological Assessment

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Targeted disruption of the endogenous LDL receptor (LDLR) in swine leads to hyperlipidemia and development of peripheral and coronary artery disease. With concomitant feeding of a high-fat diet (simulating a “Western diet”), swine expressing either the homozygous or heterozygous LDLr knockout phenotype have been shown to develop lesions similar to human atherosclerotic plaque. Plaque forms spontaneously at regions of high-shear flow and can be induced by vessel injury. In this study, we evaluated the impact of peripheral or coronary balloon angioplasty injury (15-30% overstretch) or placement of allogeneic bone marrow plugs on plaque formation. In addition, we evaluated the feasibility of noninvasive imaging modalities, IVUS (intravascular ultrasound) and angiography to allow longitudinal assessment of the lesion development. Nine swine (2 LDLr-/- and 7 LDLr +/-) were fed a high-fat diet for a minimum of 20 weeks. Eight swine were subjected to peripheral artery injury and then survived for 15-60 days. angiographic and IVUS evaluation 15-30 days post treatment and prior to termination of both injured arterial sites and normal arteries (coronary and peripheral) were performed. Lesions were identified at necropsy, dissected and histopathological evaluation was performed. Noninvasive imaging techniques included assessment of plaque deposition with foamy macrophages and cholesterol clefts were observed. Lesions in the areas of peripheral injury also showed some formation of plaque but were less severe than those forming spontaneously. Bone plugs were assessed for lipid uptake and macrophage infiltration. Overall this study indicates that plug deployment and to a lesser degree, vessel injury and can increase lesion severity and complexity. In addition, interim QVA and IVUS can be a powerful minimally-invasive tool for early detection of stenosis and lesion formation without the need for early termination and histological confirmation.
ossification, with no significant differences in the development of the placenta. The Y57C mouse is viable, and fertile. The differing phenotypes between the Med31 Null and Med31 Y57C lines indicates that Med31 biochemical function must be compromised in alternate ways in each mutant. By studying these two mutant lines we can identify the diverse requirements for Med31 during development. Due to the critical function of Med31 in transcription, we predict that the defects we identify in these mutants will be associated with multiple developmental pathways linked to fetal growth.

1831 Mitigation of Colitis with NovaSil Clay Therapy

Five million people currently live with Crohn’s disease (CD) or ulcerative colitis, the two major forms of inflammatory bowel disease. Available treatments frequently result in side effects that compromise the immune health of the patient. Consequently, alternative therapies that cause fewer systemic effects are needed. Diocathedral smectite clays have been utilized to treat medical conditions, including diarrhea and enteric disease. Herein, we report the ability of a refined diocathedral smectite (NovaSil, NS) to sort inflammatory proteins and reduce inflammation in a TNBS (2,4,6-trinitrobenzenesulfonic acid) mouse model of CD. We also investigated whether NS could rescue gut microbial diversity in TNBS-induced mice. ELISA, X-ray diffraction, and transmission electron microscopy were employed to characterize the NS–cytokine interaction in vitro. Additionally, a TNBS mouse colitis model was utilized to study the efficacy of NS supplementation for four weeks. The three treatment groups included control, TNBS, and TNBS + NS. DNA was extracted from feces and sorted for bacterial phylogenetic analysis. Results suggest that NS binds TNFα in vitro. In TNBS-treated mice, supplementation with NS significantly reduced weight loss and serum proinflammatory cytokine levels (IL-2, IL-6, and IL-12, TNFα) compared with the TNBS + NS group and control group. NovaSil mitigated the effects of TNBS-induced colitis based on reduction in systemic markers of inflammation, significant improvement in weight gain, and intestinal microbial profile. This research was supported by Texas A&M University College of Veterinary Medicine and Biomedical Sciences Postdoctoral and Graduate Student Trainee grants.

1832 Characterization of a Model of Murine Gammaherpesvirus for Immunotoxicological Evaluations Reveals No Horizontal Transmission
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Immunosuppressive therapy in humans is associated with post-transplant lymphoproliferative disorder (PTLD), a condition characterized by development of Epstein Barr Virus (EBV)-associated B cell lymphoma. Murine Gammaherpesvirus-68 (MHV-68) is a proposed mouse model of latent EBV infection. MHV-68 infected mice may develop lymphoproliferative disease which is accelerated by disruption of the immune system. A murine gammaherpesvirus 68 (MHV-68) infection model was developed to facilitate toxicological assessment of pharmaceutical agents that cause reactivation of latent viral infections in humans. Characterization of the MHV-68 model involved development of assays for detecting virus and for demonstration of safety when present in murine colonies. To determine transmissibility potential, immunocompetent and immunodeficient mice were infected with MHV-68 and cohabitated with naïve animals. Quantitative PCR, flow cytometric, western blot, fluorescent microscopy, and serology assays were developed to characterize the MHV-68 model and to determine viral transmission. Horizontal transmission of virus to naïve cagemates was not detected in naïve cagemates. These findings demonstrate that MHV-68 infection can be controlled and monitored in murine research facilities, and the potential for unintentional infection is low.

1833 Animal Models for Cholangiocarcinoma Induced by Chemicals Associated with the Offset Printing
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Aim: Multiple cases of cholangiocarcinoma were reported among Japanese workers in an offset printing factory in 2013. A cleaning solvent containing DCP is suspected to be the causative agent for these cholangiocarcinoma. GSTT1 in the liver is thought to be the key factor in the mechanism of DCP inducing cholangiocarcinoma in human. Method: In the first experiment, five kinds of animals, C57BL/6j mice, Balb/c mice, F344 rats, Syrian golden Hamsters, Guinea Pigs were exposed to DCP vapor at 0, 300, 1000 and 3000 ppm, eight h/d, for 7 days. In the second experiment, Balb/c mice and Syrian golden hamster were exposed to DCP at 0, 200, 400 and 800 ppm, eight h/d for 14 days. At the end of the experiments, the animals were decapitated. The livers were dissected out and fixed with 4% paraformaldehyde phosphate buffer. Immunohistochemistry of GSTM1, GSTT1, GSTP1, Ki67 and TUNEL staining were conducted to identify distribution of the GST isozymes and apoptotic cells in the liver and hepatic duct. Result: GSTT1 expression shows difference between species. GSTT1 expressed in nucleus and cytoplasm in hepatocytes and epithelial cells of hepatic duct throughout the liver both in rat and mice, but in nucleus and cytoplasm in some hepatocytes and in cytoplasm in epithelial cells of hepatic duct in hamster. No difference was found in the control and exposed group in all examined species. Exposure to 1,2-dichloropropane increased TUNEL positive cells in the liver of hamster. Conclusion: The distribution of GSTT1 could not explain the species difference between rats and mice regarding the carcinogenicity of DCP. On the other hand, hamster was the most susceptible for induction of TUNEL positive cells in the liver.

1834 Combination of Mitomycin C and Low Dose Suramin Increases Survival in an Optimized Orthotopic Model of Urinary Bladder Carcinoma in Mice

Epithelial carcinoma of urinary bladder represents over 75% of all the types of bladder carcinomas described in humans. These tumors are often managed by transurethral surgical resection, followed by chemotherapy, mainly Mitomycin C. Here, we report efficacy data with Mitomycin C combined to low dose Suramin, known to potentiate the effect of various chemotherapy agents, in an optimized orthotopic model of urinary bladder carcinoma in mice. Thirty-anesthetized female mice (C57/BL6, n=10/group) were catheterized and MB-49 bladder cancer cells of murine origin (1x10E6 cells/ml) were instilled into the urinary bladder (Group 2 and 3) following conditioning with poly-L-lysine. Group 1 animals were treated by intravesical instillation of a 0.1 ml mixture of Mitomycin C and Suramin sodium salt starting 5 to 7 days following tumor cell inoculation. The median survival time for the non-treated Group 2 mice was 20 days, and that of the treated Group 3, significantly prolonged to 36.5 days. Microscopic examination of hematoxylin and eosin stained sections confirmed the presence of primary tumors in 9/10 Group 2 and 5/10 Group 3 animals. As compared to Group 1 and 2, Group 3 animals showed significant basal membrane thickening occasionally associated with atypical urothelial hyperplasia and decreased vascularisation that are compatible with the mechanism of action of Suramin. In conclusion, this model and the route of administration appear suitable for assessing the antimetastatic activity of test items against bladder epithelial carcinomas. In addition, the combination between Mitomycin C and Suramin appears to result in a significantly beneficial effect for this type of tumor.

1835 PPARγ Stops Her2+ Breast Tumor Metastasis
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Breast tumours overexpressing epidermal growth factor receptor 2 (Her2+) are associated with poor patient prognosis, and only 1 in 5 Her2+ breast cancer patients will survive >5 years. Thus, research to improve Her2+ breast cancer patient outcomes is warranted. Peroxisome proliferator-activated receptor (PPARγ) is a transcription factor that may play a role in cancer. We showed PPARγ stops environ-
mental carcinogen-induced breast tumour progression in vivo. PPARY ligands also inhibit activation of Her2 family members in vitro, but the role of PPARY during Her2+ breast tumour progression is unresolved. Here, PPARY loss was hypothesized to enhance Her2+ breast tumour metastasis. A unique mouse strain was generated with a targeted PPARY deletion in the same spontaneous Her2+ transformed mammary epithelial cells that drive breast tumorigenesis, called PPARY-NIC KO mice. Female mice (n=11) show PPARY loss increases Her2+ mammary tumour lung metastasis. Western blot analysis of frozen samples showed decreasing PPARY and increasing Her2+phosphothreonine 877 (pY877Her2+) protein expression both correlated with progression from normal mammary tissue to primary mammary to lung metastatic tumours. Immunofluorescent assays showed Her2 expression in fixed primary and metastatic tumours was also significantly higher in PPARY-NIC KO samples versus controls (p<0.05). Her2+ tumorigenic cell lines, human breast cancer (SKBR3) and murine (isolated lung tumour metastatic called PPARY-NIC KO) were used to identify PPARY and Her2 interaction in vitro, PPARY-NIC KO cells lack PPARY and express pY877Her2/total Her2 levels similar to the metastatic tumour from which it was derived. Scratch wound assays show cell migration is significantly enhanced after epidermal growth factor treatment, and abrogated by a PPARY ligand (p<0.05). This is the first evidence that PPARY expression and signalling may stop Her2+ breast tumour metastasis.

**1836 Survey Results on Clinical Pathology Volume Requirements in Preclinical Toxicological Studies**

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Clinical pathology testing is a critical part of preclinical research studies. However, the blood volume required is a major hurdle in reducing animal numbers, one of the 3 R (Replace, Reduce, Refine) principles. Microsampling (< 100 uL blood) and manual sample predilution have been promoted to reduce blood sampling requirements. In order to define open questions regarding feasibility and analytical accuracy of sample dilution and to provide recommendations, the Regulatory Affairs Committee (RAC) of the American Society for Veterinary Clinical Pathology (ASVCP) surveyed veterinary clinical pathologists active in preclinical development and safety studies for current volume requirements and blood reduction practices. The survey documented analyzers, phlebotomy sites, and minimal/preferred volumes for single/repeat analysis in the most common laboratory animal species. Fourteen responses were received (7 pharmaceutical, 5 contract research, 1 biotechnology and 1 academic laboratories). Minimum volume requirements were 200 ul whole blood (hematology), 200 ul plasma (coagulation) and 120 ul serum or plasma (biochemistry). Limiting factors to microsampling were phlebotomy site or method, tube size (particularly for coagulation), and the need for manual versus automated hematology sampling. While sample dilution was an accepted practice for samples with analyte concentrations above the measurement range, predilution to reduce blood volume requirements was generally not supported, as it is considered to result in analytic inaccuracy, severely impacting test results for variables such as electrolytes and coagulation assays. Blood volume reduction seems feasible in large research animals (monkeys, dogs) where requested volumes exceeded analytical requirements. In the view of surveyed veterinary clinical pathologists, microsampling and manual predilution do not currently meet clinical pathologic quality standards in preclinical studies.

**1837 Characterization of Epithelial Proliferation in the Small Intestine of Treatment-Naive 8-Month-Old CbyB6F1-Tg(HRAS)2Jic Transgenic Mice**


Rodent lifetime biosaops are employed to assess the carcinogenic potential of compounds. The six-month CbyB6F1-Tg(HRAS)2Jic transgenic mouse study is currently a more common alternative to the two-year standard mouse bioassay. Benign and malignant neoplastic processes can be identified in mice during clinical examination and at necropsy with macroscopic examination of tissues. Histopathologic examination of hematoxylin and eosin (H&E) stained tissues including intestine is routinely performed in preclinical toxicity and carcinogenicity studies. This assessment using light microscopy includes mucosal components such as villous epithelial proliferation. Often, subtle changes in epithelial proliferation can be challenging to assess consistently in routine H&E sections. Determination of proliferation levels can be important in understanding the spectrum of changes related to carcinogenesis. Immunohistochemical (IHC) for Ki-67 and proliferating cell nuclear antigen (PCNA) detects cells undergoing proliferation. Quantification of immunolabeling using image analysis software can provide useful, more sensitive determination of increased cellular proliferation to supplement standard H&E evaluation. Here we describe tissue collection protocols and IHC methods utilized to determine levels of epithelial proliferation in the small intestine of treatment-naive 8-month old CbyB6F1-Tg(HRAS)2Jic transgenic mice. Results of standard and enhanced examination were similar and there was no difference in Ki-67 staining between segments collected either in standard fashion (non-flushed) or formalin-flushed. These techniques could be integrated into safety studies to provide more sensitive cell proliferation assessment without sacrificing time and resources.

**1838 The Predictive Value of the Rodent Neurofunctional Assessment for Central Nervous System Events in Phase I Clinical Trials**

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It is widely accepted that more needs to be done to bring new, safe and efficacious medicines to the market. Clinical adverse events (AEs) are a major contributor to the high failure rate in development of new drugs, and improved translational safety assessment could increase the probability of success for new drugs. In this analysis, the shared CNS safety data from rodent neurobehavioural assessments of 141 small molecules from six pharmaceutical companies was investigated to identify the concordance between nonclinical assessments and Phase 1 First In Human (FIH) outcomes. The data indicate that, within the context and limitations of the data collection parameters in this analysis, the rodent neurobehavioural assessment does not predict the most commonly observed subjective CNS AE’s in the FIH study (nausea, dizziness, fatigue/ somnolence and pain). Furthermore, the presence of non-clinical CNS finding does not predict the likelihood of whether compounds will produce any of the aforementioned clinical CNS AEs during the FIH study. This analysis indicates that the rodent neurobehavioural assessment may not be the most appropriate or optimal test to predict the likelihood of occurrence of the most commonly observed subjective CNS AEs in FIH studies.

**1839 Comparison of Physiologic and Pharmacologic Parameters in Asian and Mauritius Cynomolgus Macaques**


A comprehensive understanding of differences in background parameters for macaques from different sources is critical when selecting animals for use in non-clinical safety studies. This comparative study was conducted to assess background physiologic and pharmacologic parameters of cynomolgus macaques (Macaca fascicularis) from Cambodia, from a mixed Asian source (Cambodia, Vietnam and Indonesia), and from the island of Mauritius, all common sources of macaques used in toxicology studies. This evaluation provides a comprehensive assessment of several of these parameters in a single study. Ten male and 10 female captive-bred, age-matched macaques from each source were evaluated. Criteria for evaluation included weight gain, assessment of drug metabolizing enzyme activity, metabolomic analysis, immunologic assessments (lymphocyte subsets, TDAr, and serum Ig isotyping), clinical pathology evaluations, physical (respiratory, neurologic, cardiovascular, and ophthalmologic) examinations, patologic screening, organ weights, and gross and microscopic pathology analyses. The results of this evaluation indicate that, compared to macaques of Asian origin, macaques from Mauritius had the lowest incidence and/or severity of spontaneous pathologic findings in several organs and tissues (lymphoid organs, stomach, kidney, urethelium, heart, arteries and lung) and better testicular maturity at a given age with minimal variability in organ weights. Although slight differences were observed in other parameters, none were considered detrimental to the use of macaques of Asian or Mauritius origin in pharmaceutical candidate safety studies with the use of a consistent source and appropriate background knowledge and screening.
The cynomolgus monkey represents the most important primate model for preclinical safety evaluation, particularly for biotechnology-derived medicinal products. Recently, we have established MRI for the identification of any pre-existing pathology in monkeys that are intended to be assigned to a regulatory toxicity study. The particular case report herein addresses an 8-year-old cynomolgus monkey (7.4 kg) that had demonstrated weight loss associated with increased serum levels of total bilirubin, glutamate dehydrogenase, aspartate and alanine aminotransferase, alkaline phosphatase, gamma glutamyl transferase, glucose, cholesterol, triglycerides and total protein. MRI was performed on an Excite Echospeed SR120 GE scanner, performing coronal T1 and T2 weighted sections and axial T1 and T2 weighted sections. The animal was sedated for approx. 50 min. using ketamine at 5mg/kg and 0.006mg/kg medetomidine (Domitor®). Evaluation of scans revealed a significantly enlarged stomach with narrowing of the pylorus (pyloric stenosis). The lumen of the pylorus was measured at 9 mm in length and 2.3 mm in diameter. The enlarged stomach displaced the aorta to the right side (approx. 12 mm shift), and the left kidney medially and caudally, and the left liver lobe medially and cranially, with no signs of biliary duct obstruction. Kidneys did not show any signs of hydroenlarged stomach. These observed differences between the two strains of mini pig enforce the need to be consistent in the source of animals for a toxicology development program and also the need for maintaining separate background data bases.

Clinical Pathology parameters are routinely assessed during the conduct of toxicology studies. This study was conducted to see if there were any differences in hematology and clinical chemistry parameters among Beagle Dogs sourced from Marshall BioResources based in the USA and China. Pretreatment data from completed and ongoing toxicology studies were used to see if there were any differences between the two sources of animals. The results from the two data sets were analyzed using the Marshall BioResources USA-supplied dog as reference control point. Analysis of the hematologic data showed slightly lower white blood cell count (WBC), including absolute count of neutrophils (NEUT), lymphocytes (LYMP), monocytes (MONO), eosinophils (EOS), and basophils (BASO), and platelet count (PLT) in both sexes of dogs from China when compared to the USA. Differences in clinical chemistry were observed for dogs from China included a reduced alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyltransferase (GGT), total cholesterol (TCHO), urea nitrogen (BUN), creatinine (CRE), calcium (Ca), and inorganic phosphorus (P) when compared to the USA sourced dog. In addition, a slight decrease in potassium (K) was also noted in male dogs from China, when compared to the USA. When compared to the USA, observed differences between the two sources of animals enforce the need to be consistent in the source of animals for a toxicology development program and also the need for maintaining separate back ground data bases.

Background data of the two sub-strains of Wistar Hannover rats (Slc: WistarHannover/RCC and Crl: WI (Han)) for long term toxicity study were collected and compared for each other. The two sub-strains of Wistar Hannover rats were kept and observed for 2 years. At the end of the observation period, hematological and pathological examinations were performed. These data were also compared with our historical control data of SD and Fisher rats. The survival rate of males in the RCC rats was higher than that in the other three strains. In the female survival rate, the value in the Han rats was higher than that in the other three strains. High incidence of hypertrophy of the lymph node was observed in the male and female RCC rats. Dilatation of the bile duct, brown patch in the liver and white patch in the heart were observed at a high incidence in the male RCC rats. Dilatation of the bile duct and white patch in the lung were observed at a high incidence in the female RCC and Han rats, but otherwise at a low incidence in the male RCC and Han rats.
Athymic nude rats have historically been used for research into tumor biology, immunology and xenograft research. More recently they have been used in toxicology studies, where test article administration to immune-competent animals would result in an inappropriate immune response. While there are numerous publications describing aspects of the immune phenotype of these animals, there is limited information on background histopathological changes, in particular those seen in non-lymphoid organs. We have performed a retrospective review of microscopical findings from 138 control Rowett nude rats (72 males and 66 females) from 6, 26 or 52 week toxicology studies, conducted at our laboratories between 2012 and 2014. While many lesions were similar in nature and incidence to those seen in outbred rats, others were overrepresented in the athymic animals. These included unilateral agenesis of the urinary and/or genital tract, abscessation within the prostate, generalized hypertrophy of salivary gland acinar cells and pigment accumulation in the kidney cortical tubular epithelium of females. In addition, previously reported differences in lymphoid organ morphology, due to the absence of T-cells were observed. Knowledge of spontaneous lesions peculiar to this strain will be useful to separate background pathology from test article-related effects.

Survey of Spontaneous Clinical Observations in the Sprague-Dawley Rat (Rattus norvegicus)


The Sprague Dawley Rat (Rattus norvegicus) is routinely used in preclinical toxicity testing and clinical observations are one of the many parameters used to assess the potential toxicity of a xenobiotic during the conduct of in vivo toxicology research. Pretreatment evaluation of the animals is paramount to ensure good animal health prior to dose initiation. During the course of the study the frequency of clinical sign assessment varies depending on the type of study but will usually be conducted at least twice daily with control animals being evaluated at the same times. Clinical observations from both pretest and control animals were compiled and evaluated from over 280 studies to assess the occurrence of common observations. The animals were characterized by their source/vendor and other factors such as animal housing (social or single housing), environment setting (humidity, temperature, light/dark cycle), type of cage (wire bottom/solid bottom) and handling procedures for body weight collection dosing administration etc. Typical observations included assessments of animal activity, presence of masses, salivation, alopecia, ocular discharge, normality and consistency of the stool, and any other unusual observations. Data were presented in a concise database which will allow for easy access to this historical background data. Access to such data bases will enable and facilitate the toxicologist to put the observed clinical signs in perspective during the course of a toxicological study and will help identify those signs that are truly related to the administration of the test material.

Historical Control Data Including Spontaneous Tumors in CB6F1-Tg rasH2 Mice


The short-term (26 weeks) carcinogenicity model using transgenic mice is often used as the alternative to a 2-year conventional carcinogenicity study in mice. The model using CB6F1-Tg rasH2 mice is a predominant candidate; however, it has not been used widely, especially in Japan. We collected historical control data including spontaneous tumors in CB6F1-Tg rasH2 mice (total of 300 males and 300 females, 50 animals of each sex in each lot, 6 lots in total), since historical control data were important to exactly evaluate the carcinogenic potential of chemicals. The CB6F1-Tg rasH2 mice were obtained at 6 weeks of age (CLEA Japan, Inc.), acclimatized for 2 weeks, and used at 8 weeks of age. Animals were individually housed in stainless steel wire mesh cages or housed 2 animals per cage in plastic cages with bedding. All animals were given a basal diet (CE-2 pellet, CLEA Japan, Inc.) and tap water ad libitum. The 0.5 % methyl cellulose (MC) solution was administered orally by gavage once a day for 26 weeks. The following items were examined: clinical signs, palpable masses, body weight, food consumption, hematology, blood chemistry, necropsy and histopathology. A positive control group was provided in each study (5 to 15 animals of each sex) and the animals were given 75 mg/kg (single i.p.) of N-methyl-N-nitrosourea (MNU) (Wako pure chemical industries, Ltd., Japan). The animals given 0.5% MC showed extremely low mortality. Spontaneous tumors were observed at relatively low incidences, and major tumors were pulmonary bronchiolo-alveolar adenoma/carcinoma, hemangiosarcoma, squamous cell papilloma/carcinoma in the forestomach and hepatocellular adenoma. Our background data for spontaneous tumors were not different from previously published papers. In the positive control group, squamous cell papilloma/carcinoma in the forestomach and malignant lymphoma were observed at high incidences, and it was considered that carcinogenic susceptibility was assured.

Development of an Epaxial Intramuscular Injection Technique in Juvenile Rats

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Test articles may be administered in the juvenile rat from postnatal day (PND) 7 onwards (which approximates the human newborn). Intramuscular dosing, an uncommon route of administration at this young age, can be successful when using a reproducible, effective technique. Intramuscular injections are typically administered into the gluteal muscle in adult rats, with care taken to avoid depositing test material on or near the sciatic nerve, which may result in subsequent limb paralysis. The major challenge for juvenile rats was the limited amount of muscle mass. Damage to the sciatic nerve or surrounding muscle and subsequent limb paralysis could cause a dam to reject pups. A method for epaxial intramuscular injections was developed. To accurately assess the location of the muscle mass, adult mice were used initially, followed by rat pups (PND 7 and older). On PND 7, the spinous process of the 1st vertebra was easily observed through the thin skin. The injection (slightly offset from the midline) was easily performed. As the pups gained weight, the skin became thicker and the muscle was greater in mass. Technicians administered one injection/animal from PND 7 to 28, alternating between the left and right epaxial muscles (to allow the muscle time to recover). The volume for injection was derived from the maximum recommended volumes from industry guidance documents.
After palpation of the epaxial muscle, 0.9% saline was slowly injected, using a 31 gauge 8 mm needle attached to a 3/10 mL syringe with .005 mL markings, into the muscle at an approximately 90° angle to the body. Proper needle placement ensured that the needle was positioned in the muscle mass, rather than subcutaneous (too shallow) or intraperitoneal (too deep). The technique was evaluated by visual assessment of the injection site externally and internally, initially using a colored dye, and subsequently by assessing the site after -one week for signs of edema, trauma (bleeding) or changes in gait or locomotion. There were no pout injuries or fatalities as a result of the injections. The dams continued to care for their pups normally.

1851 Establishment of a Saline Lavage Model in the Isolated Perfused Rat Lung
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For studies on the effectiveness of new lung surfactant formulations, the rat lung lavage (RLL) model is the method of choice. Prior to the test, the rats are anesthetized, tracheostomized and pressure-controlled ventilated. Saline lavages are performed, whereupon the rats receive aerosolized surfactant. The restoration of the lung function is indicated by the recovery of oxygenation. During the test procedure, the RLL model provides a large bandwidth within the measured parameters which is why another model for the investigation of lung surfactant formulations is desirable. In this context, the establishment of an ex vivo model such as the isolated perfused rat lung (IPL) should provide more constant measurement results, as the respiratory parameters can be adjusted individually. Furthermore, the use of the IPL contributes to the refinement and reduction of animal testing. We tested the effect of different lavage procedures and various ventilation strategies using the IPL. Sprague-Dawley rats were ventilated with 100% oxygen at a respiratory rate of 80 breaths/min, inspiration : expiration ratio of 1 : 1, and a positive end-expiratory pressure (PEEP) of 3 cmH2O. High inspiratory pressure (Pinsp / PEEP) of 26 / 3 cmH2O led to edema formation in less than one hour. With a Pinsp of up to 15 cmH2O, the lungs survived with normal respiratory values for at least three hours. Up to 8 lavages with 0.9% NaCl-solution drop the O2 level for at least 200 units without leading to severe edema, when pressure-controlled ventilated with a Pinsp of 15 cmH2O. Our results show that an imitation of moderate Acute Respiratory Distress Syndrome (100 mmHg < PaO2 / FIO2 ≤ 200 mmHg) in the ex vivo model IPL is possible. In the future, the development of a new test system for surfactant formulations shall replace the in vivo batch testing.

1852 The Inhibition of the Intestinal Absorption of Vitamin K by a Medium-Chain Chlorinated Paraffin (MCCP) in an In Vitro Everted Rat Intestinal Sac Model
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High oral doses of MCCP administered during pregnancy have been shown to cause peri-natal mortality in the offspring. A role of Vit K in the mediation of this effect has been described. This potential MOA has been explored by studying the effect of MCCP on the absorption of Vit K in an in vitro everted rat intestinal sac model. Intestinal sacs, approximately 2.5 cm in length, were prepared from rat proximal intestine. Sacs (n=2 per group) were incubated in a commercial fed-state simulated intestinal fluid (FeSSIF) containing 0.1µM H4-Vit K, either in the presence or absence of either 0.5, 1.0 or 2.0 mM MCCP for various time periods ranging from 15 to 90 mins. These concentrations were selected as being representative of those measured in the milk of female rats administered MCCP prior to pregnancy and during gestation in studies where peri-natal mortality was observed. At the end of the incubation period, the sacs were cut open, the serosal contents were collected and the sacs were weighed and digested. Measured volumes of extenal and serosal medium (100µl to 4ml) and sac digest (2ml to 10ml) were assayed for their content of Vit K using liquid scintillation counting. In control sacs, with 0.1µM Vit K alone in the external medium, approximately 12pmoles Vit K was transferred to the serosal fluid in 90 minutes. When either 0.5, 1.0 or 2.0 mM MCCP were added into the external medium 20 minutes before the addition of the 0.1µM Vit K, the amounts of Vit K transferred to the serosal fluid were reduced to approximately 11, 8 and 7pmoles, respectively, over the 90 minute incubation period. These results suggest that the presence of MCCP in the external medium inhibits the absorption of Vit K in the in vitro rat intestinal sac model, adding further weight to the hypothesis that the peri-natal mortality seen in rats receiving high oral doses of MCCP is due to a reduction in maternal levels of Vit K, most probably due to an inhibition of its absorption from the GI tract during pregnancy.

1853 Intra-Articular Injections into Stifle Joint of Sprague-Dawley Rats and Beagle Dogs: Feasibility, Tolerated Volumes, and Synovial Fluid Sampling

Target tissue dosing as opposed to systemic exposure may be required to provide the desired pharmacological activity or avoid adverse systemic effects. Safety assessment programs for tissue targeted clinical routes can be challenging to conduct in preclinical species, as often requires dosing in compartments of lesser size than encountered clinically, using multiples of the clinical dose. Establishing adequate techniques is critical to minimize experimental procedure interference. To support development of drugs intended for intra-articular injection, feasibility of injection into the stifle joint (knee) of rats and dogs was evaluated together with tolerated injection volumes. Methods for collection of synovial fluid at termination or under anesthesia (via synovial lavage) were established. Intra-articular injections were performed under anesthesia (isoformane or injectable). Dose volumes were considered tolerated and successful when absence of extra-articular leakage was achieved as evidenced by fluoroscopy. In Sprague-Dawley rats, volumes of up to 0.1 mL were well tolerated and in Beagle dogs up to 0.75 mL per injection site were performed, however volume of 0.5 mL was considered a more reliable volume of injection to reduce risks of extra-articular diffusion. Slight, transient clinical signs suggesting less weight bear on the injected limb, were infrequently observed in dogs and these resolved within a few hours. Repeat administrations of up to twice weekly (0.5 mL injection) in Beagle dogs in a single or in both knees were well tolerated. Collections of synovial fluid from the femorotibial joint were performed in both species by first injecting sterile saline and thereafter withdrawing up to 50 to 75% of the injected volume, representing approximately 0.1 mL sample for rats and 0.5 to 1 mL in dogs. The procedures established in our laboratories are therefore appropriate to support drug development programs using an intra-articular injection route of administration.

1854 Development of a Chronic Pulmonary Arterial Pressure Model in the Beagle Dog

The measurement of pulmonary arterial pressure is critical for the efficacy assessment of pharmaceuticals that may have the potential to reduce pulmonary arterial pressure. Many techniques are described in the literature, however most involve an acute assessment or the placement of a Swan Ganz catheter, introduced through the jugular vein and passed through the right atrium and ventricle to the pulmonary artery. However, a permanent catheter through the tricuspid and pulmonary valves has a high risk of causing cardiac insufficiencies, infection and endocarditis. This method would not be suitable as a chronic model for screening compounds designed to alter pulmonary artery pressure. The most appropriate current model as described in the literature in mongrel dogs and minipigs involves the placement of a catheter directly into the pulmonary artery via a thoracotomy. We attempted this approach in beagle dogs using a telemetry transmitter with a gel-filled pressure catheter (DSI® TL11M3-D70-PCPT). This model was unsuccessful due to the unexpected fragility of the pulmonary artery in the beagle leading to tearing of the artery upon securement of the catheter to the vessel. Because of these issues, we developed an alternative method to assess pulmonary arterial pressure via measurement of right ventricular pressure. This approach required the placement of the pressure catheter directly into the right ventricle to measure right ventricular systolic pressure as an index of pulmonary arterial pressure. Although this approach is not a direct method to measure pulmonary artery pressure, it allowed for the indirect assessment of pulmonary artery pressure without compromising right ventricular function. In addition, this approach allows us to use this model to determine both a suitable manner to acutely increase pulmonary arterial resistance via hypoxic exposure or chemically-induced pulmonary vasoconstriction as a model of pulmonary arterial hypertension, as well as to assess the efficacy of agents designed to reduce or prevent elevations in pulmonary arterial pressure.
Detection of QT interval prolongation is very important in drug development. QT interval prolongation occurs in individuals with reduced repolarization reserve. The repolarization reserve is known to decrease with clinical conditions such as brady-cardia, hypokalemia and heart failure. Therefore, we investigated whether or not QT interval prolongation can be detected with high sensitivity by administration of the IKr blocker to dogs with decreased heart rate. In this study, medetomidine, an alpha2-adrenoceptor agonist, was used to reduce heart rate, and E-4031 n hy-drate used as the IKr blocker. Medetomidine (dose: 20 ng/kg) was administered intramuscularly to three male and three female beagle dogs to decrease heart rate, E-4031 n hydrate (dose: 0.1 mg/kg; dose volume: 1 mL/kg; dose speed: 2 mL/min) administered intravenously, and the lead II electrocardiogram recorded using the JET system [Data Sciences International Inc.]. In addition, prior to the combined administration of medetomidine and E-4031, electrocardiograms were recorded for the untreated animals and for those with administration of either medetomidine or E-4031. QT intervals were significantly prolonged after combined adminis-tration of medetomidine and E-4031 in all animals. In combined administration, arrhythmia such as premature ventricular contractions was observed immediately after the start of administration of E-4031, and the incidence was highest at about 30 minutes after administration, but it almost has disappeared at 1 hour after administration. We clearly showed that medetomidine pretreatment made it easier to detect QT interval prolongation induced by positive control drug. Because the repolarization reserve was reduced by bradycardia, decreased heart rate with medetomidine might be relevant to this phenomenon. Therefore, this method will be useful to assess the novel drug candidates in non-clinical safety studies.

Here we report a study to more precisely define and quantify the relationship between organophosphate agent exposure, including pesticides and nerve agents, with cholinesterase inhibition and the ocular biomarkers that are induced. A thorough investigation of these relationships was conducted to subsequently refine pupillary algorithms for the automated detection of organophosphate exposures with greater specificity regarding level of exposure, the extent of cholinesterase inhibition, and specificity regarding level of exposure, the extent of cholinesterase inhibition, and the temporal presentation and persistence of the ocular biomarkers. A guinea pig model was exposed to in vivo concentrations of parathion (pesticide), soman and VX (nerve agents) to methodically detail both the temporal and quantitative occurrence of pupillary deficits (anticholinesterase biomarkers) to determine the most sensitive, accurate diagnostic algorithms in these animal models. Dose-response curves and temporal-response curves for both pupillary deficits and generalized symptoms were developed for each agent used. Based upon previous studies dose ranges were conducted from the LD50 to 5000-fold below the LD50 to incorporate lethal and sub-lethal exposures without decreasing the potential sensitivity of the ocular biomarkers. In addition, cholinesterase assays were performed at various time points post-exposure. The key finding of this study was that placement of the intra-pleural pressure catheter must be exact to avoid heart rate artifact. Additionally, the apex-base configuration results in a slightly more optimal ECG waveform when compared to lead II. Average baseline airway resistance was 0.32 cmH2O (±0.06 cmH2O). The average maximal response to methacholine administration was an increase to 1.88 cmH2O (±0.82 cmH2O), a 481.3% increase. The mean baseline heart rate was 339.8bpm (±11.9bpm) which decreased by 32.3% to an average minimum of 230.2bpm (±40bpm) following methacholine administration. The mean baseline arterial blood pressure was 75.7/mmHg (±2.9/mmHg) which decreased by 37.5% to a minimum of 46.1/mmHg (±3.4/mmHg) following methacholine administration. No departure from normal body temperature was observed in any group. The development of this model offers a solution to monitoring cardiopulmonary function the guinea pig.
loss of sampling patency being roughly 40-60% of animals within two weeks of catheter implantation. IT VAPs for dose administration appeared to be less prone to failure (~10% of the animals within the 1st month). Length of post implantation time appeared to have an impact on catheter patency but with weekly maintenance the failure rate was reduced. In conclusion, this dual catheter technique has proven useful in allowing for separate routes of dose administration and CSF sampling via different locations within the IT space of monkeys.

**1860 Toxicological Abstracts Classification Using Natural Language Processing and Supervised Machine Learning Algorithms**

Literature searches to support chemical risk assessments frequently return tens of thousands of results. Manually sorting literature into relevant categories (e.g., epidemiology, toxicology, exposure) is time consuming and requires expertise. Using keywords to categorize abstracts by topic for prioritization and manual review shows limited success, but only when articles are pre-indexed by keyword and the topic can be narrowly defined. We assessed the performance of natural language processing and supervised machine learning (NLP-ML) algorithms to categorize abstracts across a set of toxicology-related topics using keyword-based searches as a baseline. Starting with a large manually annotated dataset of abstracts relating to arsenic and CrVI, we used standard text vectorization algorithms to create a mathematical representation of the document corpus. Machine learning models based on the naïve Bayes, k-nearest neighbors, and support vector machine algorithms were trained using a bag-of-words approach and tested using n-fold cross validation. Algorithms that maximized recall, precision, and F-score (a combined measure of recall and precision) were identified across the optimal word groupings (n-grams) in each context. Feature selection algorithms that compress the feature space and potentially reduce noise were tested for their impact on improving model performance. For perspective on how the size of the annotated dataset affected model performance, learning curves were plotted and performance-dataset size trends were analyzed. The best performing algorithms were enhanced with keyword-based rules and performance measures were recomputed, yielding F1-scores in excess of 90% in some cases. The cross-performance of models developed from the literature on a particular chemical (e.g., arsenic) when applied to another chemical (e.g., CrVI) was tested. We conclude that NLP-ML methods show great promise to identify data gaps or prioritize chemicals for risk assessment based on availability of evidence.

**1861 Tools to Identify and Manage Mechanistic Data to Support Human Health Risk Assessment**
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The pharmaceutical industry regularly takes advantage of in vitro data to inform decisions about in vivo utility and applicability. Applying this in the context of human health risk assessment for chemical exposure also has potential, but requires innovative approaches to identify and manage data within the wealth of published literature. Commerically available, visual analytics software enables clustering of similar references based on automated text analysis. References are clustered around key concepts that distinguish papers from the full set of results; these concepts can be used to prioritize articles for review. We used the database for CrVI which has been manually curated and contains over 800 mechanistic studies. We tested several methods to identify the known mechanistic literature identified during manual review, including clustering with no a priori information and several iterations of clustering using a thesaurus to improve precision and hit rate. We found that some document clusters were highly enriched for mechanistic studies, with 82% of the 805 articles found within only 10% of the clusters. Next, we evaluated the mechanistic studies using text analytics to explore potential hotspots or other patterns that could be used to focus further inquiry and hypothesis development. We evaluated the accuracy of the clustering by comparing clustering results to manually extracted results stored in ICF’s DRAGON, a tool for systematic literature review. We present the findings of our analysis with respect to supporting literature for hypothesized modes of action. Tools to identify and manage mechanistic data show promise for increased efficiency over manual review. We found that transparency was enhanced and recordkeeping streamlined by using a database to compile mechanistic data. In the context of human health risk assessment, discovery of new adverse outcome pathways (AOPs) is one potential application of using clustering software and data management tools to explore mechanistic literature.

**1862 Using DRAGON to Organize Data and Decisions for AOP Development: An Example with Inorganic Arsenic**
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As the scientific community increasingly uses Adverse Outcome Pathway (AOP) approaches to interpret scientific data and make decisions, we are faced with the challenge of organizing, processing, and communicating supporting data in a clear and effective way. We evaluated the utility of ICF International’s DRAGON, a database platform designed to store, manage, and analyze qualitative and quantitative results from scientific literature, to meet these challenges by evaluating the data-rich chemical inorganic arsenic (iAs) as a case study. Specifically, we examined how DRAGON can be used to process the iAs literature in the context of postulated AOPs that describe connections between exposure to iAs and bladder cancer, including key events. We conducted a literature search and then imported iAs literature citations into DRAGON, used DRAGON-Screen to review titles and abstracts to identify scientific articles relevant to the AOPs, tagged literature to specific key events along the AOPs, and, in cellDRAGON, animalDRAGON, and epiDRAGON, characterized data to serve as evidence supporting key events. Common language and ontologies built into DRAGON and used in data extraction modules facilitated synthesis of evidence streams to connect, for example, mechanistic data indicating a molecular initiating event and apical adverse outcomes reported in an animal study. Data and decisions entered in DRAGON were exported in various formats to organize the evidence for multiple competing AOPs leading to bladder cancer and for identifying where key events are common amongst them. The results of this case study demonstrate the utility of DRAGON for AOP development through organizing and processing large data sets, such as the body of literature on iAs, characterizing diverse types of data (cell, animal, and epidemiological), and facilitating synthesis and review across data streams, through visualizations and other reports.

**1863 Problem Formulation of Complex Environmental Health Questions: Utilizing Text Mining to Address Challenges of a Literature-Based Evaluation of Transgenerational Health Effects**
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The National Toxicology Program (NTP) Office of Health Assessment and Translation (OHAT) uses systematic review methodology to assess the evidence that environmental substances cause adverse health effects (http://ntp.niehs.nih.gov/go/38138; Rooney et al., 2014). The size and complexity of the literature base for a given topic can range from hundreds to tens of thousands of publications leading to substantial time and resource investments. The aim of this report is to illustrate how text mining approaches can be applied to complex questions with large bodies of literature and assist in the process of surveying the available information. Transgenerational inheritance of health effects is a phenomenon in which exposures to an individual have far-reaching consequences, affecting multiple generations removed from the original insult. This phenomenon does not have specific indexing (e.g., MeSH or medical subject heading) terms and requires the use of text words or phrases that often have multiple contextual meanings (e.g., a search for “third generation” identifies relevant transgenerational progeny and nonrelevant reports on newer medical devices). As a result, a PubMed search for transgenerational inheritance yielded over 48,000 publications, but less than 2% are relevant. After manual screening by title and abstract, we identified 896 publications, and upon review of full text, 45 human and 158 animal studies were considered relevant. In this report, we compared a manual curation approach to one utilizing text mining tools to identify and inventory relevant studies based on evidence stream, exposure and health outcomes. Using machine learning technology and “seed” studies, over half of the manually curated references could have been automatically excluded with a recall rate of 93%. By clustering and prioritizing studies, text mining technology enables researchers to more efficiently evaluate a large body of literature.
This study reviews the potential risks of missing chemical data and concentration estimation. It is necessary to understand their implications in a predictive model of mixture toxicity, in part, because only highly restrictive known toxicities of single chemicals. However, this method is often insufficient. Mellon University, Pittsburgh, PA.

We also considered the potential implications of applying criteria frameworks and formative study quality considerations, which will enable researchers to implement more objective and standardized methods for evaluating studies and, ultimately, how they bear on fundamental risk assessment questions. We found that each of the available frameworks within each discipline differed in terms of their intended purpose and level of guidance in decision-making. All frameworks across disciplines shared common themes, including the adequate reporting of specific details of study conditions and design/protocol, selection and randomization (where applicable) of subject groups, outcome assessment methods and applicability, reporting the results of unadjusted and adjusted analyses (i.e., avoiding selective reporting), and consideration of potential confounders or bias. Our analysis identified the most informative study quality considerations, which will enable researchers to implement more objective and standardized methods for evaluating studies and, ultimately, improve risk assessment methods.

The prevailing approaches for the systematic review and evaluation of chemical toxicity are currently being reconsidered, with specific focus on the evaluation of individual studies and the overall body of evidence. This renewed interest has arisen, in part, as a result of several prominent reviews of these approaches by special committees of the National Research Council (NRC), among others. We conducted a critical evaluation of several available study quality criteria frameworks. We assessed the criteria separately for human, animal, and in vitro studies as well as systematic reviews, and then evaluated commonalities across disciplines. We also considered the potential implications of applying criteria frameworks and how they bear on fundamental risk assessment questions. We found that each of the available frameworks within each discipline differed in terms of their intended purpose and level of guidance in decision-making. All frameworks across disciplines shared common themes, including the adequate reporting of specific details of study conditions and design/protocol, selection and randomization (where applicable) of subject groups, outcome assessment methods and applicability, reporting the results of unadjusted and adjusted analyses (i.e., avoiding selective reporting), and consideration of potential confounders or bias. Our analysis identified the most informative study quality considerations, which will enable researchers to implement more objective and standardized methods for evaluating studies and, ultimately, improve risk assessment methods.

A number of environmental chemicals have been shown to alter markers of epigenetic change. Some published multi-generation rodent studies have identified effects on F2 and greater generations after chemical exposures solely to F0 dams, but were not focused on chemical safety. We were interested in how outcomes related to epigenetic changes could be identified and incorporated into chemical testing and risk assessment. To address this question, we conducted a systematic literature review to identify transgenerational (TG) epigenetic studies in rodents. These were analyzed to characterize the methods and observed outcomes, and to evaluate strengths, limitations, and biases. Our analysis found that test substances were administered to pregnant F0 dams; endpoints assessed in F1 to F4 generation offspring included growth, pubertal timing, steroid hormone levels, and abdominal adiposity, organ weights, histopathology, and epigenetic biomarkers. Randomization, avoiding inbreeding, and multiple independent reviews of histopathology data were procedures that improved the quality of the study results. However, the numbers of litters assigned to control and test groups were not always transparently reported, nested statistical analyses of data was not always utilized to address litter effects, and “blind” testing was seldom performed. Many of these studies identified chemicals or combinations of chemicals that produced TG effects and/or adult-onset diseases, but there is a paucity of published studies indicating a lack of TG effects, perhaps due to publication bias. Since EPA and OECD guideline multi-generation reproductive toxicity studies are unlikely to identify chemicals that pose a TG epigenetic hazard, we considered ways in which potential hazards identified in TG epigenetic research studies might be applied to the traditional toxicity testing and risk assessment paradigm for environmental chemicals. The views expressed in this abstract are those of the authors and do not necessarily represent the views or policies of the U.S. EPA.
At least one substance in each category showed cytotoxic effect in dose dependent manner. The chemicals did not show increased sensitivity upon repeated exposure. Human PCLS were less sensitive to chemicals compared to rat PCLS maybe due to differences in metabolic activity. This study shows differences of rodent and human metabolism. Although toxicity profiling is based on rodent exposure data, species diversity can be studied ex vivo for better predictively in human. Moreover, results of the project will be used to evaluate the read-across approach based on the tested chemicals.

**1870 Evaluation of Cancer and Noncancer Effects of Camene**


Camene, also known as isopropyl benzene, is a volatile liquid. We have systematically reviewed published literature to evaluate cancer and noncancer effects of camene. Camene, readily absorbed via inhalation, is distributed in several tissues, metabolized extensively by cytochrome P-450 isozymes within hepatic and extra-hepatic tissues and excreted through urine. Although there are no epidemiological cancer studies for humans, chronic inhalation exposure studies in rat and mouse have shown increased nasal lesions including atrophy, basal cell hyperplasia, atypical hyperplasia and hyperplasia of the olfactory epithelium glands. Other significant nonneoplastic lesions include renal lesions in male rats, and epithelial hyperplasia of the forestomach in male mice. Increased adenomas of the respiratory epithelium of the nose have been observed in both male and female rats. In addition, increased incidence of renal tubular adenoma or carcinoma related to a2u-globin-induced nephropathy has been observed in male rats. Alveolar/bronchial adenomas and carcinomas of the lung have been observed in male and female mice exposed to cumene. Preneoplastic lesions such as alveolar and bronchiolar metaplasia and bronchiolar hyperplasia in mice and eosinophilic foci in the livers of male mice were significantly increased. Subchronic inhalation studies have reported increased kidney and liver weight both in rats and mice. Although no multi-generational reproductive toxicity studies are available for cumene, cumene-exposed rats appeared to stay in estrus cycle longer than the controls. Short-term acute exposures of animals at high concentrations have been reported to induce transient reversible neurotoxic effects. Overall, inhalation exposure to cumene induced dose-related increase in the occurrence of tumors at various sites. These cancer data in rats and mice as well as genotoxicity data provide consistence evidence for carcinogenic effects of cumene. (Disclaimer: The views expressed in this abstract are those of the authors and do not represent the policy of the U.S. Environmental Protection Agency).
statistical-based. Statistical-based models are preferred because they have higher accuracy than rule-based models. Overall, DB[a,j]A and DB[a,j]A were predicted to be mutagenic and carcinogenic. Most mutagenicity predictions for these two isomers were positive and well correlated with existing empirical genotoxicity data. VEGA, Lazar and Toolbook predicted these isomers to be carcinogenic. The two models reported in the literature both predicted DB[a,j]A to be carcinogenic, and one model predicted DB[a,j]A to be carcinogenic. Predictions of the mutagenicity and carcinogenicity of DB[a,j]A and DB[a,j]A are largely in agreement across these different QSAR models, and indicate the usefulness of QSAR models in filling data gaps.

1874 Evaluation of the Experimental Support for Assessment Factors to Protect Asthmatic Subjects during Short-Term Exposure to Airborne Chemicals

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Assessment factors are commonly used in the derivation of limit values, to account for inter-individual variability and sensitive subjects. Asthmatic individuals constitute a large sub-population that is often considered susceptible to inhalation exposure to chemicals. However, data on asthmatic subjects are absent for most airborne chemicals. The aim of this study was to evaluate the experimental support for assessment factors intended to protect asthmatics. A thorough search of the scientific literature revealed 104 experimental studies, covering 19 single chemicals and 10 mixtures, where asthmatic and healthy subjects had been exposed via inhalation at identical conditions. An Estimated Differential Response Factor (EDRF) was developed for each chemical/mixture. Support of higher sensitivity among asthmatics was found for acetaldehyde, ammonium bisulfate, chlorine, nitrogen dioxide, ozone, sulfur dioxide, sulfuric acid, 2,4-/2,6-toluenediisocyanate, environmental tobacco smoke, ozone in ambient air and a mixture of ozone and sulfur dioxide. The EDRFs for these chemicals were > 1, 2 or 3. Dose-response relationships were estimated for four chemicals with richer data sets (nitrogen dioxide, ozone, sulfur dioxide and sulfuric acid). Data for all, except for ozone, showed that asthmatic subjects react at lower concentrations and with a more severe respiratory response, as compared with healthy subjects. In addition, a benchmark dose (BMD) analysis was performed with data from exposure to sulfur dioxide at rest. The result suggests a 12-fold difference between asthmatics and healthy subjects. This is to our knowledge the largest systematic review made of data on asthmatic and healthy subjects. A BMD analysis of these two groups has not been made before. We suggest an assessment factor of ten to include asthmatics in the derivation of guideline values for the general population.

1875 The Carcinogenicity of Dibenzo[a,j]Arenes

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Dibenzo[a,j]arenes (DB[a,j]A) are five-ring polycyclic aromatic hydrocarbons (PAHs) that consist of three isomers (DB[a,j]A, DB[a,j]A, and DB[a,j]A). They are products of complete combustion or pyrolysis of organic material and have been identified in contaminated air, water, soil and food. DB[a,j]A is an International Agency for Research on Cancer (IARC) Group 2A carcinogen, whereas DB[a,j]A and DB[a,j]A are IARC Group 3 carcinogens with limited animal data due to, for example, studies with small group sizes, or limited exposure routes, dosing, or study durations. We conducted a literature search on the carcinogenicity of DB[a,j]A, focusing on DB[a,j]A and DB[a,j]A. ToxCast and toxicogenomic data for DB[a,j]A were also analyzed. Evidence relevant to carcinogenicity comes from animal cancer bioassays, initiation-promotion studies, and other relevant data including genotoxicity and metabolism studies, mechanistic data, and structural activity comparisons. Each of the three isomers contains one or more bay region structures, which are associated with formation of diols and diol epoxides. DB[a,j]A share similar biological activities in animals: all three isomers induce tumors in mice, are mouse skin tumor initiators, form DNA adducts, are genotoxic, and form metabolites that are associated with formation of diols and diol epoxides. DB[a,j]A are likely involved, including receptor activation (e.g., AhR), immune suppression, and altered cell proliferation, apoptosis, and cell cycle regulation. Finally, there are strong structure-activity similarities among the DB[a,j]A and several carcinogenic 4-, 5- and 6-ring PAHs, including benzo[a]pyrene and dibenzo[a,b]pyrene.

1876 In Vitro Toxicological Assessment of Industrial Chemicals Spilled into the Elk River in Charleston, West Virginia

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On January 9, 2014, thousands of gallons of an industrial chemical, crude 4-methylcyclohexane methanol (MCHM), and a secondary chemical mainly composed of propylene glycol phenyl ether (PPH), spilled into the Elk River in Charleston, West Virginia. Consequently, a considerable number of people were reported to exhibit symptoms of MCHM exposure and up to 300,000 residents were advised not to use or drink water for several weeks. Unfortunately, the lack of toxicological data on MCHM and PPH resulted in great panic among those who were directly impacted. The Centers for Disease Control and Prevention (CDC) quickly established a drinking water advisory level of 1 ppm and 1.2 ppm for MCHM and PPH, respectively, and stated that concentrations at or below the advisory level are not likely to be associated with any adverse health effects. It is nonetheless, essential, to further research the toxicity of MCHM and PPH in order to fully understand the potential toxic effects. In this preliminary study, we assessed cell viability, in vitro, after 24 hour exposures to varying concentrations of different chemicals equivalent to or associated with ones involved in the spill. Evaluation of three different cell lines, HepG2 (liver), H9C2 (heart), and GT17 (brain), provided some insight regarding the vulnerabilities of some tissues under others. The EC50 of MCHM in HepG2, H9C2, and GT17 cells were determined to be 0.7926 μM, 417 μM, and 138.6 μM, respectively. The findings from this study highlight the liver as a susceptible tissue under conditions of MCHM exposure.


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Prediction of risk of new compounds and final products by in vitro methods is a goal and concern for the cosmetic industry in the context of the Cosmetics Directive and REACH. Cosmetic’s 7th Amendment had been gradually phased out animal based testing for the assessment of cosmetic products and ingredients in advance of the total ban, effective since March 2013. Alternative methods have been implemented in house since 2002 to support the strong commitment to non-animal testing and to develop an in-house 3D human reconstructed epidermis model “VitroDerm”. Over time, a strategic approach for the safety assessments has been established to cover fields such as topical toxicity, percutaneous absorption, systemic toxicity, genotoxicity. Since 2011, an approach for cutaneous sensitization with Direct Peptide Reactivity Assay, Myxoid U937 Skin Sensitization Test and LuSens method has been integrated. Our strategy is specifically designed to apply to cosmetic ingredients and final products taking into account physicochemical and application diversity. A decision tree is presented, taking a baby shampoo assessment example and showing a global animal-free approach integrating all available tools. In silico profile prediction of ingredients, in vitro determination for skin irritancy using baby and adult 3D models (cell viability >80%) compared to Human Patch Test (predictive accuracy over 94%), eye irritancy assessment using BCOP (in vitro score mean of 3.2; classification between “No Category” / “No Prediction Category”) and acute oral toxicity using NRU (with LD50 extrapolated–1891mg/kg, category “Negligent in case of ingestion”). In addition, indirect skin penetration measurement, histology of epidermis cross section and screenings for skin sensitization are gathered together contributing in obtaining a decision regarding human safety of the product and confirm the need to combine different complementary methods in order to obtain a reliable animal-free risk assessment.

1878 A Framework for Assessing Chemical/Nonchemical Interactions: A Case Study of Lead and Psychosocial Stress


Chemical and nonchemical stressors are factors that may contribute to negative health consequences in certain individuals and groups. Nonchemical stressors include, but are not limited to, poverty, crowding, noise, and exposure to violence. Recent research has suggested that some nonchemical stressors may alter chemical toxicity. We propose a conceptual framework to explore the evidence for the interaction of chemical and nonchemical stressors. Specifically, the proposed framework is used to evaluate the potential interaction of lead exposure and psychosocial stress associated with low-socioeconomic status. We conducted a literature review and analyzed NHANES data to answer the following questions: 1) Does lead exposure occur disproportionately in low-SES groups that typically may also face higher
levels of psychosocial stress? 2) Do lead and stress result in similar neurodevelopmental outcomes via similar pathways, particularly, affecting the hypothalamic-pituitary axis (HPA)? 3) Do epidemiological and experimental studies demonstrate that stress alters the dose response for lead neurotoxicity? We found, that although overall blood lead levels continue to decline, lower-SES individuals are still disproportionately exposed to lead and that both lead exposure and stress result in cognitive impairments through their interaction with the HPA axis. We also note that many human and animal studies demonstrate that psychosocial stress increases the toxicity resulting from lead exposure. Currently, many data gaps exist regarding interactions of other chemical and nonchemical stressors. This conceptual framework may be useful in assessing possible interactions of nonchemical stressors with chemical agents.

Disclaimer: The views expressed in this paper are those of the authors and do not necessarily represent the views or policies of the U.S. EPA or the State of Connecticut.

1879 Assignment of Hazard-Specific Notations for Occupational Health Purposes in the Pharmaceutical Industry

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Hazard notations are assigned as key supplement information to occupational hazard categorization in the process of risk assessment as communication tool for pharmaceutical manufacturing employees. The link between the hazard and the risk is captured in the occupational exposure limit (OEL) which indicates the level at which those hazards appear. Novartis decided to assign the hazard notations for occupational health purposes based on intrinsic hazard of drug substances (DS) and pharmaceutical intermediates (IM). In this poster we would like to present Novartis strategy for assignment of these notations and to provide the scientific rationale for determination each notation through several well-known marketed Novartis drugs. These hazard notations are based on the following intrinsic toxicological hazards and their subcategories: carcinogenicity (immunosuppressive, possible carcinogen, carcinogen), genotoxicity (genotoxic), reproductive toxicity (fertility effects, embryo-fetal developmental effects, peri-postnatal developmental effects), corrosion (corrosive), irritation (irritant), and sensitization (skin sensitizer, respiratory sensitizer). The purpose of this poster is to present that for communication purposes, the scientific based assessed hazard has to be conveyed through few and clear hazard notations and OEL calculation.

1880 The Use of Glial Changes in Neurotoxicity Risk Assessments


Central nervous system (CNS) glia (i.e., astrocytes, microglia, and oligodendrocytes) are essential for normal brain function, and they orchestrate the CNS response to injury. While effects on glia are important to consider when evaluating neurotoxicity risk from exposure to xenobiotics, interpreting these changes is not always straightforward. Glial responses have the potential to be mischaracterized due to a difficulty in discerning homeostatic changes from adaptive changes initiated in response to sub-acute insults, or from pro-neurotoxic phenotypes. To better understand how glia have been characterized and used in neurotoxicity assessments of environmental chemicals, a survey of human health assessments in the U.S. EPA Integrated Risk Information System (IRIS) database was performed. The results indicate that IRIS assessments finalized in recent years more frequently include characterizations of CNS effects in general, and effects on glia in particular. However, even in recent assessments, descriptions of glial changes are brief and non-specific (primarily documenting gliosis following overt neuronal injury), and they do not appear to influence the identification or mechanistic understanding of CNS injury. The data are discussed in the context of the future use of glia in human health assessments, including examples of difficulties in interpretation from the viewpoint of risk assessors, as well as research needs that could improve the usability of these data in neurotoxicity risk assessments. As our understanding of glia is refined, glial changes highlighting subtle disruptions in neural function may eventually be used as markers early in the progression of an insult towards frank neurotoxicity, or as indicators useful for identifying potential susceptible populations. Overall, the appropriate inclusion and interpretation of glial responses to environmental agents is expected to improve the quality of CNS hazard characterization descriptions, and perhaps even dose-response analyses, in human health assessments. Disclaimer: The views expressed are those of the author and they do not represent U.S. EPA policy or guidance.

1881 Evaluating the Evidence for Developing Categorical Occupational Exposure Limits for Nanomaterials

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Nanomaterials toxicity studies are providing increasing data for hazard and risk assessment, yet few occupational exposure limits (OELs) have been developed to date. These OEL values (as 8-hr time-weighted average airborne concentration) vary by up to an order-of-magnitude or more for the same nanomaterials, largely due to differences in the methods and assumptions used in the risk assessments (e.g., to account for variability or uncertainty in interspecies deposited lung dose, long-term clearance and retention; and sensitivity to adverse response). In the absence of individual OELs for most nanomaterials, categorical OELs have been proposed in the following categories: soluble, poorly-soluble low toxicity, poorly-soluble high toxicity, and fibrous particles. Proposed adjustment factors of the current OELs for “bulk” (micro-diameter) particles to nanoparticles of similar chemical composition range from approximately 0.05 to 1. One approach to evaluating such OEL categories is to develop a set of risk estimates (i.e., the probability of adverse health effects associated with exposure) for a set of benchmark particles, which are well-studied and with sufficient dose-response data for risk estimation. Those benchmark particles would be included as controls in a standard set of experimental assays of nanomaterials to facilitate quantitative comparison and hazard or risk ranking. For example, risk estimates for poorly-soluble respirable particles based on subchronic and chronic inhalation studies in rats show that chemical composition and particle size both influence the risk estimates for adverse lung effects, and the OELs developed from those data. (The findings and conclusions in this abstract have not been formally disseminated by the National Institute for Occupational Safety and Health and should not be construed to represent any agency determination or policy.)

1882 Characterizing Factors That Modify Chemical Exposure or Response: Application of a Susceptibility Framework to Inorganic Arsenic Hazard Identification


Factors that may modify exposure or response to environmental chemicals may increase the potential risk of an individual developing health effects associated with the chemicals. Risk assessors have historically accounted for susceptibility factors in human health risk assessments by incorporating uncertainty factors in dose-response calculations. Yet, risk assessors and others recognize the potential value of developing new methods to more explicitly incorporate susceptibility factors, and their potential interactions, into the hazard identification portion of assessments. We started with a strength of evidence framework for susceptibility previously used for evaluating criteria air pollutants to facilitate explicit evaluations of the potential impact of life stage, human variability, and environmental factors. The framework supports evaluations of the coherence of effects, and biological plausibility of susceptibility factors from evidence across disciplines. We also developed a decision tree for making a susceptibility determination using the framework in order to clearly communicate how decisions are made. In this presentation, we demonstrate the utility of this approach with inorganic arsenic (iAs). We first conducted a focused literature search for susceptibility factors associated with iAs. Using the framework and decision tree, we characterized potential impacts of individual-level factors and life stage on the determination of susceptibility to effects associated with iAs exposure. Preliminary results show that life stage and smoking may be particularly important to include in the hazard identification process for iAs-induced health effects. Our results demonstrate the potential benefit of using a strength of evidence framework in tandem with a decision tree to explicitly characterize susceptibility factors and to identify potential groups at risk. Disclaimer: The views expressed are those of the authors and do not necessarily represent the views or policies of the US EPA.

1883 Nanoparticles from Municipal Waste Incinerators: Real Risk or a Sheep in Wolf’s Clothing?

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Municipal waste incinerators (MWIs) are viable alternatives to waste management because it extends the life of existing landfills while providing energy back to the community. An issue raised by concerned citizens is whether incidental nanoparticles (INPs) created by the incineration process, will cause adverse health effects. This study compares the size and composition of INPs generated from MWIs with
those generated by other common exposure sources, as well as the potential for exposure and risk associated with the exposure. Data from peer-reviewed literature were used to assess nanoparticle (NP) size, composition, particle numbers per volume, and emission control technologies. The exposure and risk were then assessed for NP exposure from MWI and other common NP generators. NP generation by MWIs is dependent on the starting material’s physicochemical properties and the MWI’s emissions control technologies. INPs often consist of complex metal mixtures that agglomerate during condensation, resulting in larger-sized particles. High incineration temperatures often decompose carbon-based contaminants (polyyclic aromatic hydrocarbons (PAHs)), making flue gas cleaner. Simple filter bags are capable of removing >98% of INPs generated by MWIs, while additional technologies further reduce INP emissions. In contrast, other sources often produce larger amounts of INPs. Transportation contributes >55% of fine particulate matter in the ambient atmosphere. These INPs are often incompletely combusted, resulting in high levels of PAHs and organic vapors on exhaust soot. Indoor conditions with natural gas stoves often generate higher concentrations of INPs than MWIs. Current epidemiological data do not take into consideration the intricacies of micromolecules, making current NP risk assessments difficult to interpret. Advances in characterization and analytical methodology, as well as NPs fate and effect in biological systems, will greatly enhance our ability to more accurately differentiate risk due to MWIs versus other sources.*

1884 Increased Susceptibility to Chemical Toxicity Associated with Pre-Existing Diseases: Framework and Case Studies
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Numerous host and environmental factors may modulate vulnerability and risk. An area of increasing interest to risk assessors is the potential for chemicals to interact with pre-existing diseases and aging that may yield cumulative damage, altered chemical response, and increased disease susceptibility. In the presentation are those of the authors and do not necessarily reflect the views or policies of the US EPA.

1885 An Approach to Standardize the Concepts of “Low-Dose” and Nonmonotonic Dose Response in Toxicological Research and Regulatory Science
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In order to facilitate impartial interpretation of research / discussions concerning the emerging concerns of non-monotonic ‘low-dose’ response; the need to establish a mutual understanding of its fundamental terminology is essential. Although ‘non-monotonic’ ‘low-dose’ responses are most commonly interpreted as non-linear response at doses below previously tested levels, currently the literature is littered with variants of these terms in a variety of contexts each with varying degrees of precision and accuracy that confound debate of more important issues relevant to the ‘low-dose hypothesis’. In the wake of emerging concerns of non-linear ‘low-dose’ response beyond endocrinology, such as nutrition (e.g. vitaminosis) and nanotechnology this work sought to assess key literature and 1) Identify the vast variation of interpretation of core concepts such as ‘low-dose’, ‘response,’ and non-monotonic dose response, often limited to qualitative, circumstantial and subjective criteria; 2) Present unbiased approaches to standardize interpretation of these terms based on a quantitative framework; 3) Identify critical flaws that are prevalent amongst the body of literature that comprises the ‘low-dose hypothesis’; and 4) Offer suggestions for future consideration. In doing so we have found, drastic inconsistencies in core concepts of the ‘low-dose hypothesis’ and offered a suggested framework by which stakeholders can consider moving forward. In addition we have highlighted pervasive experimental inadequacies in key aspects of study design, statistics and inconsistent experimental replication, all of which collectively undermine the proposed findings and conclusions from exploratory ‘low-dose’ studies. Ultimately we culminate our work with suggestions to help further facilitate toxicological consideration of future ‘low-dose’ studies.

1886 A Rules Engine Approach to Supporting Adverse Outcome Pathway-Based Rapid Toxicological Assessment
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Despite gains in the efficiency of gathering data about chemical activity on biological assays, especially via High Throughput Screening (HTS) methods, there remains a large backlog of potentially harmful chemicals with little or no assessment of toxicity. Our project addresses that backlog in part by taking advantage of the recent development of Adverse Outcome Pathways (AOPs), and their collection in the AOP knowledge base. The part of the project described here is twofold: 1) scientists use AOP information to create a set of logical rules that define when knowledge of an upstream event(s) is sufficient to infer the existence of a downstream event(s). 2) A rules engine was built that can ingest data on a chemical derived from the logical rules and use the rules to infer biological activity that results from that chemical. While the rules engine approach is not sufficient at this time to directly infer toxicity without a scientist’s interpretation, this process will allow toxicologists to use HTS and other data to quickly screen chemicals and prioritize chemicals for further assessment, or to group chemicals together by disease endpoint. This process may also be effective in illuminating data gaps and identifying where new assays may need to be developed or new data to be gathered to further aid toxicological assessment. The views expressed in this abstract are those of the authors and do not necessarily represent the views or policies of the US Environmental Protection Agency.

1887 New In Vitro Gastrointestinal Model Accurately Predicts Arsenic Bioavailability in Soils
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Arsenic (As) is a naturally occurring metalloid commonly found in soil and a key chemical of concern at many brownfield sites. Risk assessment calculations typically utilize default oral toxicity values, which are based on ingestion of readily soluble forms of As dissolved in water. Arsenic in soils, however, is bound to various other minerals that result in decreased solubility/bioavailability of As. Historically, the use of the juvenile swine method was the only available method for determining the relative bioavailability (RBA) of As in soils. The EPA recently released guidance recommending a default bioavailability of 60% for arsenic in soils. It has been demonstrated, however, that the RBA of arsenic in soils can be as low as 1%.

In vitro methodologies have proven to be useful surrogates for in vivo feeding studies in predicting RBA for other metals but lack precision for arsenic. The purpose of this study is to develop a single extraction in vitro procedure that conservatively estimates in vivo RBA of As for every test soil. Study soils were collected from mining sites throughout California with As ranging from 200 to 12,000 mg/kg and RBAs ranging from 1-40%. A modification of a previously published method (OSL-IVC) conservatively predicted As RBA in all study soils (n=18) and for most soils (9/11) with <1,200 mg/kg As the method provides a good estimate (within 90% CI) of RBA. This result holds true when ten non-California soils with swine RBA are included. The combined dataset provides enough data points for a robust regression (RBA= Modified OSL-IVC(0.8)+4.39, r²=0.82). In summary, we have developed a new method for predicting bioavailability of As in soils. While the data is still preliminary in nature, this affordable bench-top method could be used in place of the more expensive juvenile swine in vivo studies to estimate RBA of As in soils. This data can then be used to adjust human health risk assessment equations and provide a more reasonable estimation of risk.
Uranium is a naturally occurring element that is used for industrial and military applications, and predominantly accumulates in the skeletal system of both animals and humans following exposure. Evidence from animal bioassays and cell culture studies demonstrates that exposure can alter bone function, resulting in delayed bone development and increased fragility. However, a comprehensive mode of action analysis has not been performed for the existing database on uranium-induced bone effects. Adverse outcome pathways (AOPs) have been proposed as a framework for integrating evidence from multiple models, including both in vivo and in-vitro biological systems, as well as identifying relevant knowledge gaps. As a template for evaluating the available toxicological and mechanistic evidence for uranium-induced bone toxicity, an AOP was assembled based upon the established cellular, molecular, and endocrine mechanisms resulting in bone diseases such as osteoporosis and Rickets. Uranium toxicity studies were organized by species and endpoint and evaluated for consistency and biological plausibility. Two important biological elements were identified in uranium-induced bone disease: 1) inhibition of osteoblast differentiation, and 2) disruption of renal metabolism and production of Vitamin D. Application of the AOP framework also identified critical informational gaps in the available uranium database, such as: a) evaluation of the effects of uranium exposure during pregnancy and later life stages, and b) identification of the molecular initiating events which lead to altered osteoblast development, and reduced renal production of Vitamin D. Considering the role of Vitamin D, and normal osteoblast functions in bone development and maintenance, the proposed AOP suggests that both young and elderly populations may be especially susceptible to uranium-induced osteotoxicity. The views expressed are those of the authors and do not necessarily represent the views or policies of the US EPA.

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous contaminants of concern in the environment, and are predominantly found in soil due to their chemical characteristics. As a result of this, people may be exposed to PAH impacted soil through incidental hand to mouth contact. PAH oral bioavailability (BA) has been demonstrated to differ in various exposure media, and PAH BA from soil is generally agreed to be less than from other media. However, PAH BA from soil can differ between different soils, and it is thought that either soil PAH concentrations or soil characteristics such as carbon content or metal concentrations may affect BA. This is important in risk assessment as predicting exposure to PAHs is difficult if BA can change between soils. BA may be affected in these instances by the soil characteristics affecting the toxicokinetics of PAHs in the organism. In this study, juvenile swine were exposed to PAHs in one of either four PAH spiked artificial soils, or 18 soils collected from PAH impacted sites in Canada, Britain, or Sweden. Plasma PAH time course data was collected from each swine and used to determine the area under the curve (AUC) calculations, as well as absorption and elimination rate constants for benzo[a]pyrene and anthracene in each soil. AUC and soil PAH concentration had a positive linear relationship in swine exposed to spiked artificial soil; however, no relationship was seen between AUC and soil concentration with the real-world soils. This demonstrates that concentration of PAHs in soil does not affect the toxicokinetics of PAHs, but rather that soil characteristics do. Absorption and elimination rate constants were also compared to soil concentration, as well as soil organic carbon content and soil copper concentration, and no relationships between kinetic parameters and soil characteristics were observed. This may indicate that only a portion of PAHs are released from the soil particles and are available for systemic uptake.

Aflatoxin B1 (AFB1), with ubiquitous exposure and a rich database, was selected for this case study. AFB1 has been determined to induce HCC via a DNA-reactive MOA in many species, including humans. The sequential KEs identified for AFB1 are as follows: pre-MIE: Hepatic metabolic activation; MIE: Formation of a pro-mutagenic DNA adduct (N7-AFB1-guanine or AFB1-FAPy); KE1#: Inadequate or mis-repair of the pro-mutagenic DNA adducts; KE2#: Induced mutation in genetic gene(s); KE3#: Cellular proliferation and clonal expansion of mutant cells (pre-neoplastic lesions); AO: HCC. These KEs and the various KERs—both direct and indirect—are mapped out with supporting data for each. Assessment of quantitative aspects of the dose-response relationships for the KEs and KERs will support its use in quantitative risk assessment.
Tetrabromobisphenol A (TBBPA), a nongenotoxic flame retardant, was shown to cause uterine tumors in female rats. A proposed mode of action (MOA) for these tumors is disruption of estrogen-signaling pathways. Thus, establishing an AOP for estrogen related uterine tumors would aid in the integration of data for this MOA. As a critical first step, this assessment involved the compilation and strategic review of information on TBBPA within an AOP framework with the objective of identifying the MIE. Two potential MIEs associated with alteration of estrogen signaling were investigated: interaction with estrogen receptors (ER) and inhibition of estradiol sulfotransferase (ES). Results from 27 assays were identified that characterized measures of estrogenicity through interaction with ER. A review of these data showed a lack of interaction between TBBPA and ERs (e.g., TBBPA was inactive in 18 of 19 assays in ToxCast), though in 1 assay (of 8) reported in the literature showed that TBBPA exhibited weak ER agonist/antagonist activity with very low potency compared to known ligands (EC50 TBBPA ~ 19 μM vs. BPA ~ 0.63 μM). Collectively, these data did not provide support for ER binding as the MIE. In contrast, an in silico analysis showed that TBBPA binds to ES, and several in vitro assays reported TBBPA inhibited ES with high potency (IC50 0.012 -0.033 μM), thus demonstrating that the ability of TBBPA to interfere with the metabolism of estradiol to its sulfated conjugate is a plausible MIE. Several data gaps were identified; notably, the quantitation of TBBPA inhibition of ES and its influence on circulating levels of estrogen would provide critical information on the subsequent key events leading to the development of an AOP for altered estrogen signaling and uterine tumors. Identification of the MIE is the first step in mapping this MOA. As a critical first step, this assessment involved the compilation and strategic review of information on TBBPA within an AOP framework with the objective of identifying the MIE. Two potential MIEs associated with alteration of estrogen signaling were investigated: interaction with estrogen receptors (ER) and inhibition of estradiol sulfotransferase (ES). Results from 27 assays were identified that characterized measures of estrogenicity through interaction with ER. A review of these data showed a lack of interaction between TBBPA and ERs (e.g., TBBPA was inactive in 18 of 19 assays in ToxCast), though in 1 assay (of 8) reported in the literature showed that TBBPA exhibited weak ER agonist/antagonist activity with very low potency compared to known ligands (EC50 TBBPA ~ 19 μM vs. BPA ~ 0.63 μM). Collectively, these data did not provide support for ER binding as the MIE. In contrast, an in silico analysis showed that TBBPA binds to ES, and several in vitro assays reported TBBPA inhibited ES with high potency (IC50 0.012 -0.033 μM), thus demonstrating that the ability of TBBPA to interfere with the metabolism of estradiol to its sulfated conjugate is a plausible MIE. Several data gaps were identified; notably, the quantitation of TBBPA inhibition of ES and its influence on circulating levels of estrogen would provide critical information on the subsequent key events leading to the development of an AOP for altered estrogen signaling and uterine tumors. Identification of the MIE is the first step in mapping this MOA. As a critical first step, this assessment involved the compilation and strategic review of information on TBBPA within an AOP framework with the objective of identifying the MIE. Two potential MIEs associated with alteration of estrogen signaling were investigated: interaction with estrogen receptors (ER) and inhibition of estradiol sulfotransferase (ES). Results from 27 assays were identified that characterized measures of estrogenicity through interaction with ER. A review of these data showed a lack of interaction between TBBPA and ERs (e.g., TBBPA was inactive in 18 of 19 assays in ToxCast), though in 1 assay (of 8) reported in the literature showed that TBBPA exhibited weak ER agonist/antagonist activity with very low potency compared to known ligands (EC50 TBBPA ~ 19 μM vs. BPA ~ 0.63 μM). Collectively, these data did not provide support for ER binding as the MIE. In contrast, an in silico analysis showed that TBBPA binds to ES, and several in vitro assays reported TBBPA inhibited ES with high potency (IC50 0.012 -0.033 μM), thus demonstrating that the ability of TBBPA to interfere with the metabolism of estradiol to its sulfated conjugate is a plausible MIE. Several data gaps were identified; notably, the quantitation of TBBPA inhibition of ES and its influence on circulating levels of estrogen would provide critical information on the subsequent key events leading to the development of an AOP for altered estrogen signaling and uterine tumors. Identification of the MIE is the first step in mapping this MOA.
in stainless steel cage pans previously cleaned with one of six different procedures. Water was mixed with one of the following in 50 mL conical tubes to allow complete sample saturation – NHP, BD or SD rat feces; commercially formulated diets for each species; or dietary enrichments (vegetables, fruits, and various treats). Ten samples were collected for each animal after 1 hour of exposure, tested with the RTSA, and graded according to manufacturer instructions. Results: Artificial positive blood reactions were associated with samples contaminated with NHP, BD, and SD rat feces, formulated diets, dietary enrichments, and 2 of 6 cleaning procedures. Contamination with feces from all three species was associated with the strongest positive blood reactions. Positive reactions for other RTSA, most notably the glucose and protein reactions, generally occurred in highest incidence with dietary enrichments and certified diets. Conclusions: We identified a high incidence of artificial positive RTSA reactions associated with common urine contaminants in NHP, BD, and SD rats used in preclinical toxicology studies. These findings add cautionary perspective as to the liability and limitations of urine reagent test strip assays as markers of renal toxicity in preclinical studies.

Evidence from different types of studies (in vitro assays, laboratory animal and occupational and epidemiological studies) and across multiple species point to fibrosis, pleural plaques, autoimmunity, and cardiovascular toxicity as possible non-cancer disease outcomes of interest after exposure to Libby Amphibole asbestos (LAA). We systematically reviewed and evaluated the published literature to identify potential mechanisms by which LAA may induce these effects, but a mode of action (MOA) specific to LAA cannot be established. Similar to carcinogenic endpoints following exposure to LAA, chronic inflammation, cytotoxicity and regenerative proliferation can be hypothesized as relevant to an MOA for LAA non-cancer effects, but the temporal relationships and relative contributions of each mechanism remain to be elucidated. The precise mechanisms causing toxic injury from inhalation exposure to LAA have not been established. However, nearly all durable mineral fibers with dimensional characteristics that allow penetration to the terminal bronchioles and alveoli of the lower respiratory tract have the capacity to induce pathologic response in the lung and pleural cavity. The physicochemical attributes of mineral fibers are important in determining the internal dose and type of toxicity observed. Fiber dimensions (width and length) and density determine initial deposition; chemical composition, surface area, solubility, and durability additionally influence clearance, the type of toxicity observed, and the biologically significant dose. Although the limited mechanistic data demonstrate biological effects similar to those of other mineral fibers following exposure to LAA, the existing literature is insufficient to establish a MOA for any of these disease outcomes. DISCLAIMER: The views expressed are those of the authors and do not necessarily represent the views and/or policies of the Environmental Protection Agency.

Pharmaceutical research organizations have been using phenotypic-based cell analysis to develop, screen, and validate drug candidates, and to study toxicity and/or mechanisms of action of candidate compounds. Traditionally, imaging platforms have broad application in pharmaceutical compound screening, while flow cytometry (FC) has had less impact on this field. FC is a leading technology for single cell analysis, providing information-rich data sets which can measure the phenotypic effects of external perturbants on multiple characteristics of cell populations. Herein we report a fully automated compound screening system using a Hypercet-Cyan HTS platform using phenotypic markers including Glutathione content, ROS, Mitochondrial Membrane Potential, multiple viability markers, and cell cycle analysis. The described framework compiles multiple cell-population derived response curves from these phenotypes into a multi-way tensor fully characterizing the toxicological profile of the tested compounds. The described hardware/software combination allows the analysis of multiple concentration series of compounds using standard 384-well plates, in less than 30 minutes per plate. This presentation will focus on important technical aspects of multiplexed phenotypic assays, instrumental pitfalls and solutions to common sample handling problems. We will also discuss data processing approaches, which must proceed from simple single-parameter toxicity descriptors, such as IC50 values. We demonstrate more informative multidimensional alternatives that provide researchers with insight into the toxicological profiles of tested compounds, and convey complexity of the measured phenotypic signatures.

Evaluation of clinical pathology data is an integral part of the assessment of toxicity. Cynomolgus monkeys are frequently used in toxicology studies, but reference intervals for cynomolgus monkeys of various sources (origins) have not been published. Clinical pathology data was evaluated from 120 male and 120 female experimentally naïve cynomolgus monkeys of Chinese, Mauritian, and Cambodian origin varying in age from 2.4 to 9.4 years. Hematology, clinical chemistry, and coagulation parameters were obtained using an Advia 120/2120®B, an Olympus AU640e® and a Stago STA Compact® instrument, respectively. Reference intervals are published for cynomolgus monkeys from the three sources. Clinical pathology parameters from monkeys of Mauritian and Cambodian origin were compared with monkeys of Chinese origin. Monkeys of Mauritian origin have smaller-sized erythrocytes affecting RBC indices, higher globulins, and lower cholesterol and total lymphocyte count. Numerous parameters in Mauritian and Cambodian monkeys were statistically different, but considered biologically unimportant. Based on these comparisons, monkeys of Chinese and Cambodian origin do not exhibit biologically important differences in clinical pathology parameters. Monkeys of Mauritian origin do exhibit biologically relevant differences to some clinical pathology parameters compared with monkeys of Chinese and Cambodian origin. The source of monkey should be standardized throughout toxicology programs in order to decrease variations in data interpretation between studies.

The European chemical control regulation (REACH) requires registration of chemical substances in commerce at volumes greater than one ton per year. The regulation requires data on physical/chemical, toxicological and environmental hazards be compiled and summarized. However, REACH goes further in requiring a formal assessment to ensure substance use conditions are safe. To accomplish this, reference values (Derived No Effect Levels, DNELs) are calculated from toxicity data and compared to estimated exposure levels. If the ratio of the predicted exposure level to the DNEL, i.e., the Risk Characterization Ratio (RCR), is less than 1,
the risk is considered acceptable; otherwise, additional Risk Management Measures (RMM) must be implemented. These requirements pose particular challenges for complex substances with unknown or variable compositions. In this paper we use a hydrocarbon solvent, white spirit, as an example to illustrate how complex substances and uses can be factored into the DNEL determination. A series of adjustment factors were applied in the calculation. Exposure assessments were made using a set of generic exposure scenarios (GES) which incorporated predicted exposure estimates reported by ECETOC. Targeted Risk Assessment. GESs were established following a control banding process in which DNELs and volatility were grouped as high, medium or low. Newly-developed computer-based tools helped automate RCR calculations and ensured appropriate RMMs were applied and uniform communications made to users via safety data sheets.

1902 What Is an Acceptable Risk of Cancer Due to Occupational Exposure to a Carcinogen?

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It is widely accepted that there is no completely “safe” dose of a genotoxic carcinogen, and that there is some excess risk of developing cancer from any non-zero exposure. The excess risk from low exposures may be vanishingly small, but it is generally presumed to be greater than zero. Since it is not feasible to totally eliminate all exposures to suspect carcinogens in the workplace, some target or acceptable risk level is needed for purposes of establishing occupational exposure limits for carcinogens. Target risk levels for carcinogen exposures in the general population are usually set quite low, in the range of 1 in 10,000 to 1 in 1,000,000 lifetime excess risk. In the occupational setting, the 1980 U.S. Supreme Court “benzene” decision implied that a 1 in 1000 lifetime excess risk is a significant risk, while a 1 in 1 billion risk is not. The Court noted that it is the responsibility of the relevant government agency to determine what it considers to be a “significant” risk. A review of international policies for developing occupational exposure limits for carcinogens reveals a range of acceptable lifetime excess risks of up to 4 in 1000 (Netherlands) to 1 in 100,000 (Sweden). By comparison, the lifetime fatality rates in occupations generally thought of as low risk, such as the wholesale and retail trade sector and the services sector, has been reported to be in the range of 1-2 per 1000 workers. Taken together, these data suggest that lifetime excess cancer risks in the range of 1 in 100,000 to perhaps 1 in 1000 may be acceptable in the occupational setting. The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the National Institute for Occupational Safety and Health.

1903 Comparison of Inhalation Occupational Exposure Limits (OELs) and European Worker’s Inhalation-Derived No-Effect Levels (DNELs) for Volatile Organic Compounds

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The European Chemical Agency (ECHA) previously released manufacturer/importer REACH (Registration, Evaluation, Authorization and Restriction of Chemicals) chemical registration files, making available the Derived No Effect Levels (DNELs) for hundreds of substances. The DNEL is defined as “the level of exposure above which humans should not be exposed” and needs to reflect the likely route(s), duration and frequency of exposure. Specifically, worker DNELs are levels intended to protect workers during manufacture. Typically, DNELs are developed by Industry using ECHA’s guidance; the registrant has the final decision on the selection of the key studies, endpoints of concern, and the assessment factors to account for sources of uncertainty. By comparison, currently accepted occupational exposure limits (OELs), such as permissible exposure limits (PELs) or threshold limit values (TLVs) developed by the Occupational Safety and Health Administration (OSHA) and the American Conference of Industrial Hygienists (ACGIH), respectively, may have been derived using different methods, some of which incorporate economic and technical feasibility concerns and/or health-based assessment. In addition, these other occupational values may have been based on different toxicological data and endpoints. Because the worker DNELs may potentially be used as guideline levels for worker exposures in Europe and have the potential to become de facto OELs in other regions due to the global extent of REACH, a comparison analysis between the long-term inhalation DNELs for the worker population and OELs developed within Europe and by OSHA and ACGIH was completed for several volatile organic compounds (VOCs) of toxicological concern. This analysis showed that in most cases the long-term inhalation worker DNELs for VOCs that were used for comparison were equivalent to or lower than the accepted OELs. This is consistent with our previous findings in which the same analysis was conducted on metal substances.

1904 Characterizing Risks from Exposure to Hazardous Air Pollutants: Consideration of Health Risks from Acute Exposure


Section 112 of the Clean Air Act establishes a two-stage regulatory process to address emissions of hazardous air pollutants (HAP) from stationary sources. In the first stage, the EPA is required to develop technology-based standards for categories of sources. In the second stage, EPA is required to assess the health and environmental risks that remain after implementation of the technology-based standards. If additional risk reductions are necessary EPA must develop standards to address these remaining risks under the Risk and Technology Review (RTR) Program. The potential risks due to acute inhalation exposure to HAP is one of the factors considered in RTR assessments. The acute risk assessment methodology is tiered and iterative in nature, and designed to identify and eliminate from further consideration those sources of emissions (i.e., industrial facilities) for which we have confidence that no acute health effects of concern will occur. Available health effect reference values from various sources are considered in the characterization of potential risks due to acute inhalation exposure to HAP. Consideration of a reference value in a regulatory decision requires a critical evaluation of the available acute health effect reference values in the context of the available toxicity database. This presentation includes specific HAP examples to illustrate the critical elements considered when selecting appropriate reference values to be used in risk assessments that support regulations (e.g., biological relevance to humans, methods used to derive a given reference value). 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.

1905 Reanalysis of Angina Study Cited by US Environmental Protection Agency As Primary Basis for National Ambient Air Quality Standards on Carbon Monoxide

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The Environmental Protection Agency has never changed the National Ambient Air Quality Standards (NAAQS) for carbon monoxide (CO) adopted in 1971 but it has changed their basis. In a 2011 review, EPA rejected all epidemiological evidence and gave “primary consideration” to one controlled exposure study of men with angina. EPA commissioned this in 1983 from the Health Effects Institute (HEI) to “replicate and extend” study done by Dr. Wilbur Aronow in the 1970s after an audit could not find his records. HEI’s Multicenter CO Study Team, Allred et al., evaluated the effect on men with angina of exercising in clean air after 1-hour CO exposures producing average carboxyhemoglobin (COHb) levels of 2.2% and 4.4%. Results were published thrice: in HEI Research Report 25 and The New England Journal of Medicine in 1989 and Environmental Health Perspectives in 1991. HEI, like Aronow, discarded its CO study archives but it published enough individual data to reanalyze all the primary results and reconstruct most of the published figures and summary tables in all 3 versions. Over 100 errors and inconsistencies were found in the methods, results and conclusions. Some defy laws of toxicology, cardiology and statistics, including flat and flip-flopping dose-response curves; misinterpreting venous COHb as a measure of cardiac exposure; finding adverse effects only as COHb fell during exercise in air but never as it rose during CO exposure; and deriving p-values from permutation tests of trimmed means that exactly match t-tests, which should only happen in the limit. Most critically, the study’s 3 centers could not replicate Aronow’s results or each other’s. Conclusions about significant risks posed to men with angina by 2-4% COHb are contradicted by results showing no correlation between CO exposures 1.5-10 times the 1-hour NAAQS of 35ppm and the onset of angina or ECG changes (Pearson r<0.3). Given that men with angina are demonstrably not at risk from CO, EPA should lower the NAAQS to protect fetuses who epidemiology studies show are most at risk from exposure to current ambient CO levels.
Assessing Acute Exposures to Hazardous Air Pollutants to Inform Regulatory Decisions under Section 112 of the Clean Air Act: Challenges and Potential Improvements


Section 112 of the Clean Air Act establishes a two-stage regulatory process to address emissions of hazardous air pollutants (HAP) from stationary sources. In the first stage, the EPA is required to develop technology-based standards for categories of sources. In the second stage, EPA is required to assess the health and environmental risks that remain after implementation of the technology-based standards. If additional risk reductions are necessary, EPA must develop standards to address these remaining risks under the Residual Risk Program, Risk and Technology Review (RTR). As part of this evaluation, EPA models the potential for cancer and chronic non-cancer health risks based on annual emissions and meteorological data. EPA screens for acute health risks by assuming that for a given year, the worst 1-hour of emissions occurs at the same time as the worst 1-hour of meteorology. Thus, there is more uncertainty associated with acute risk estimates when compared to those for chronic. These conservative acute screening assumptions are designed to eliminate from further consideration those sources of emissions for which we are confident that there are no acute health effects of concern. In this presentation, we discuss the various assumptions made in the acute risk screening scenario, including those used to estimate worst-case 1-hour peak emissions and meteorology. We also discuss challenges and uncertainties in interpreting the results of the acute screen when it suggests the potential for acute health risk. Further, we discuss additional information and refinements that could make the results of the acute screen more useful in the risk management process. These refinements could include an evaluation of frequency and duration of exposures. 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.

Adjustment of the US EPA Draft Oral Slope Factor for Hexavalent Chromium to Be More Predictive of Risk at Lower Exposure Levels Based upon Dose-Dependent Differences in the Fraction of Dose Absorbed into Target Tissues

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Animal studies are often conducted using doses orders of magnitude higher than environmental human doses. The mouse dose (0.38 mg/kg-day) at the lowest water concentration used in the National Toxicology Program hexavalent chromium (CrVI) drinking water study (NTP 2008) is about 74,500 times higher than the approximate human dose (5.1E-06 mg/kg-day) corresponding to the 35-city geometric mean (0.00018 mg/L) reported in the Environmental Working Group survey (EWG 2010), and over 1,000 times higher than the approximate human dose (5.7E-04 mg/kg-day) based on the city with the highest reported CrVI tap water concentration (0.0129 mg/L). With experimental and environmental doses differing to such a great degree, it is a regulatory challenge to extrapolate high-dose study results to environmental doses that are multiple orders of magnitude lower in a meaningful and toxicologically predictive manner. This seems particularly true for the low-dose extrapolation of results for CrVI-induced carcinogenicity due to oral exposure since gastrointestinal (GI) reduction to CrIII prior to CrVI absorption is a detoxification mechanism that may result in dose-dependent differences in the dose fraction absorbed into target tissues. Such differences are apparent in the target tissues of the mouse GI tract (duodenum, jejunum, ileum) based on data collected as part of the CrVI mode of action research project (e.g., Kirman et al. 2012). These data can be used for a straightforward adjustment of the draft oral slope factor (SFO) to be more predictive of risk at doses lower and more environmentally-relevant than those used in NTP (2008). More specifically, the evaluation of observed and modeled differences in the fraction of dose absorbed by target tissues at the point-of-departure used for the draft SFO calculation versus lower doses suggests that the draft SFO at least be divided by an order of magnitude to be less over-predictive of risk at more environmentally-relevant doses.
awareness of NTP's products. Intermediate outcomes of NTP's work to inform science were many. NTP's research on Cr6 was cited in scientific publications to justify further studies, inform study designs, or interpret new data. Stakeholders used NTP's work to identify Cr6 as a hazard in legal and policy documents, science reports, and congressional testimonies or pre-emption regulations, and non-regulatory actions. Pinpointing distal outcomes showing NTP's products led to a positive change for public health was challenging because NTP has no regulatory authority, the time lag to impact varies, and external factors may affect use or progress of intended actions. Although proposed federal laws or state regulations cited NTP's products, often they were not enacted or were delayed by external factors. Notably, NTP's research was key to the nation's first-ever drinking water standard for Cr6 adopted by California in 2014. This case study demonstrated NTP's science on Cr6 had impact in many areas including public health. Furthermore, it identified broad and objective approaches for assessing NTP's effectiveness.

**1191 Using Acute Oral Toxicity Data to Estimate Acute Dermal Hazard Classification and Labeling of Pesticides**

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The U.S. Environmental Protection Agency (EPA) requires acute dermal systemic toxicity testing for hazard classification and labeling of pesticides to protect human health and the environment during the handling and use of chemicals. This study considered whether acute oral LD50 data could be used to determine EPA acute dermal hazard classifications. Oral and dermal LD50 data were collected for 225 pesticide active ingredients. Two approaches were used to predict dermal hazard classifications. First, oral hazard categories based on oral LD50 were compared to dermal hazard categories based on dermal LD50. Concordance with the reference dermal hazard categories was 65% (146/225), overclassification was 31% (70/225), and underclassification was 4% (9/225). In the second approach, the oral LD50 was used directly to assign the dermal hazard category. Concordance with the reference dermal hazard categories was 43% (96/225), overclassification was 56% (126/225), and underclassification was 1% (3/225). For substances in EPA Category IV the predictivity was 100% (22/22) with either approach. These data suggest that if only acute oral toxicity data are used for predicting both oral and dermal hazards, the dermal acute toxicity of many pesticide actives and formulations could be overstated. This project was funded in whole or in part with Federal funds from the NIH, NIH under Contract No. HHSN27320140003C.

**1192 A Global Initiative to Refine Acute Inhalation Studies through the Use of “Evident Toxicity” As an Endpoint: Toward Adoption of the Fixed Concentration Procedure**

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Acute inhalation studies are conducted in animals for hazard identification and risk characterisation of chemicals for a number of regulatory schemes. Current accepted methods use death as an endpoint (OECD TG403 and TG436). The Fixed Concentration Procedure (FCP) (draft OECD TG433) uses fewer animals and replaces lethality as an endpoint with ‘evident toxicity.’ Evident toxicity is defined as clear signs of toxicity that predict exposure to the next highest concentration will cause severe toxicity or death in most animals. The FCP was dropped from the OECD work plan in 2007 because of a lack of evidence for comparable performance with TG403 and TG436, suspected sex differences in toxicities (FCP originally only used females) and the ill-defined and subjective nature of evident toxicity. The first two issues have been resolved (1.2). A global initiative including 19 organisations, led by the NC3Rs, has addressed the last concern with the aim of making evident toxicity more objective and transferable between laboratories. The group has shared data on the clinical signs recorded during acute inhalation studies for 188 substances. Clinical signs such as tremors, hypoxia, body weight loss (>10%), and irregular respiration, recorded at least once in at least one animal in a group of five, are highly predictive of severe toxicity or death at the next highest dose (positive predictive value (PPV) >80%). Preliminary results suggest that signs such as ano-genital staining, gasping, hunched posture and noisy respiration are also highly predictive (PPV at least 77%). The working group has used this data to develop a revised draft TG that incorporates a clearly defined use of evident toxicity clinical signs. 1. Price et al., 2011. *Human & experimental toxicology*, 30:217-238. 2. Stallard et al., 2011. *Human & experimental toxicology*, 30:239-249.

**1193 Read-Across of Existing Hazard Data Fulfills HPV Chemical Program Requirements and Avoids Unnecessary Chemical Testing**

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The American Cleaning Institute® (ACI) is a leading manager of chemical consortia fulfilling commitments to the voluntary global International Council of Chemical Associations (ICCA) and U.S. Environmental Protection Agency (EPA) high production volume (HPV) chemical programs. ACI’s commitment is to compile and make publicly available a baseline set of health and environmental effects data, representing the OECD screening information data set (SIDS), for approximately 300 chemicals sponsored by 62 companies within ten chemical consortia. Sponsored chemicals include surfactants and other substances relevant to the cleaning products industry. The chemical categories represented by these consortia include: aliphatic acids; long chain alcohols (C6-22 primary aliphatic alcohols); aldehydes; alkyl sulfates, alkanol sulfonates and α olefins; amine oxides; glycerides; hydroxethanes; LAS/ABS; and methyl esters. Due to the structural similarity of the chemicals within a category, their environmental fate, physicochemical and toxicological properties are likely to be similar, which can be confirmed by examining available data from across the range of substances in a category. This has allowed the utilization of the read-across technique where the data available for some substances satisfies the data needs for other chemicals within the same category that lack data. ACI has determined the extent of read-across utilized within these chemical data sets. Furthermore it has determined the number of vertebrate animal tests avoided and the testing costs saved related to the read-across utilized. ACI has found that using read-across has greatly avoided unnecessary chemical testing while allowing ACI consortia to fulfill data requirements. The ACI-managed chemical consortia have realized significant benefits by using read-across. By applying this process the use of thousands of test animals has been avoided, and millions of dollars in testing and administrative expenses have been saved.

**1194 Toxicology Databases: Aggregating Human Food Safety Toxicology Information for Veterinary Drugs in Food-Producing Animals**

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Preclinical toxicology studies are usually recommended by FDA/CVM as part of the safety information for the evaluation of animal drugs used in food-producing animals. Recommended studies include two subchronic studies, a chronic study, one/two developmental studies, a reproductive study, and a standard battery of genotoxicity studies; these studies should be performed according to the Veterinary International Conference on Harmonization (VICH) Guidelines. Other studies to assess special endpoints of concern may also be recommended when appropriate. Based on the evaluation of the results from these studies, FDA establishes an acceptable daily intake (ADI) for chronic exposure of human consumers to the animal drug residues. The purpose of this work was to create a user-friendly toxicity database containing the parameters related to ADIs, and to analyze the database. Information from a total of 91 animal drugs that have been approved for use in food-producing animals was gathered from 21 CFR 556, Freedom of Information Summaries, and CVM files. Toxicology information was obtained for 64 out of the 91 drugs. The information collected includes class of drugs, type of study, animal species, NOEL, safety factor, and ADIs. It was noted that the NOEL for the ADI was most commonly based on a chronic study (dogs or rats), with the NOELs ranging from 0.83 μg/kg bw/day to 500 mg/kg bw/day. The most commonly used safety factors ranged from 100 to 1000, and the ADIs ranged from 0.7 to 1000 μg/kg bw/day. The database can be expanded as more drug information becomes available. The collated information can be used by FDA to respond to internal and external inquiries. However, this database needs to go through a quality assurance process before it can be considered fully reliable.

**1195 Effectiveness of Toxicology-Based Webinars in Promoting Nonstandard Methods Using REACH As a Case Study**

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Acting within the spirit of the European Directive 2010/63/EU on the protection of animals used for scientific purposes, the European Union chemicals testing programme, Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), contains a number of specific measures and general provisions designed
to establish and ensure the principle that animal testing should only be performed as a last resort. For example, non-testing approaches such as chemical categories, quantitative Structure Activity Relationships as well as non-animal testing methods must be used wherever possible. Integrating and interpreting nonstandard information for key events within a rational framework can be used to provide an assessment of a toxicity endpoint that is more predictive of human health effects than testing on animals, can minimise the use of animals and promote the efficient use of resources. However, reports show that the uptake of non-testing methods has been low and a number of animal tests have been conducted where an alternative is available. As a new initiative to enhance awareness of specific methods and strategies which may be used to minimise the use of animals for the Annex VII and Annex VIII endpoints, a series of interactive webinars have been designed for registrants preparing for the REACH 2018 deadline. Being easy to access, available to an international audience and on a platform that allows for open discussion, webinars offer an effective learning tool for professionals and are being utilised with increasing frequency. Here we report on the value of such learning tools in educating toxicologists and increasing the use of novel test methods and strategies.

Additionally, detailed information is provided on how Annex VII and Annex VIII REACH dossiers can be successfully completed using nonstandard information using a case study as an example.

1916 The Role of the Toxicologist in Required Food Safety Plans: A Case Study of Ochratoxin A (OTA) in Coffee
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The Food Safety Modernization Act (FSMA), signed into law in 2011, is the most significant food safety regulation adopted in the U.S. since 1958. An important contribution of FSMA is the requirement that the food industry help the U.S. FDA increase the agency’s capacity to prevent food safety risks. This public health benefit will be partially achieved through food safety plans, which include a hazard analysis and risk-based preventive controls. This presentation will use ochratoxin A (OTA) in coffee as an example of the important role toxicologists should play in determining suitable hazard control steps. OTA is a mycotoxin produced by several fungal species that can sometimes be found in various agricultural commodities, including green coffee beans. The FDA has not identified a regulatory limit for OTA in green coffee beans; however, some authoritative agencies (JECFA, EFSA) have established tolerable weekly intakes of 100-120 ng OTA/kg-BW based on renal toxicity observed in pigs. In preparation for characterizing significant chemical hazards under FSMA, the coffee industry assembled a group of coffee scientists, as well as several toxicologists, to review all available information on the potential hazard of OTA in green coffee and its hazard potential in coffee following roasting. The key assessment steps included: determining the hazard potential of OTA, identifying the factors associated with OTA development, evaluating potential control measures and identifying the appropriate level of documentation. Through this consultation, it was determined that existing published data substantiate that the concentration of OTA in green coffee, if present, is typically low (<10 µg/kg) and levels are reduced substantially (60-100%) following roasting. The application of risk-based decision making led to the conclusion that good agricultural control (to reduce OTA in green coffee beans) and typical roasting will be sufficient to effectively minimize the hazard without implementation of critical control parameters for OTA.

1917 EU Cosmetics Regulation Driving Acceptance of In Vitro Alternatives

Globally there is a drive to reduce the number of animals used in experimentation for safety assessment. This drive is particularly evident in the cosmetics industry within the European Union. The EU Cosmetics Directive (76/768/EEC), including the 7th Amendment of the Cosmetics Directive (2003/15/EC), imposed animal testing bans on cosmetic products and ingredients which took effect in 2004 and 2009, respectively. To ensure the need for public safety, the EU extended the deadline until March 2013 for the most complex human health effects (endpoints): repeated dose systemic toxicity, skin sensitisation, carcinogenicity, reproductive toxicity and toxicokinetics. From this date, the ban has covered all animal testing, regardless of how such tests are performed and irrespective of the availability of alternative non-animal tests. The same provisions were included in the Cosmetics Regulation (1223/2009/EC) which replaced the Cosmetics Directive from July 2013. In conflict with the cosmetics legislation, other legislation in the EU (e.g. for plant protection products, Regulation 1107/2009) and for chemicals, REACH Regulation 1907/2006), although promoting the 3Rs and mandating the use of alternatives where validated in vitro tests exist, REACH maintains the need for animal testing at higher tonnage levels to ensure the protection of human health and the environment. The decision to implement the blanket ban for cosmetics appears to be a sector-specific political choice made within the EU at odds with the conservative approach taken in other regulations. The value of the cosmetics industry sales in the EU was EUR 72.3 billion in 2012. The industry needs to maintain its sales and develop new products so is a driving force in the development of alternative methodologies. In this poster we examine the steps that have been taken over the last 40 years and are now being taken within the EU to develop alternatives to animal testing and how these have already benefited safety assessment in general. These latest efforts are being focused on the SEURAT-1 “Safety Evaluation Ultimately Replacing Animal Testing”, programme supported by the EU Commission.

1918 Cosmetic Safety Testing Roadmap to Regulatory Approval in North America, Europe, and Japan
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The cosmetics industry is a robust business, achieving greater than $250 billion in worldwide sales in 2013. Although major recognizable brands comprise the largest fraction of this total, there are thousands of small companies that operate in the same markets. While the industry association International Cooperation on Cosmetic Regulation (ICCR) seeks long-term harmonization of testing requirements, currently unaligned governmental agencies in different countries make navigation of safety requirements daunting for major manufacturers and Kalkaesque for smaller firms. While also laudable, the development, validation, approval and implementation of new non-animal safety assays often make the path to cosmetic approval seem more formidable than ever. To aid in navigation of the kaleidoscopic variables of cosmetic ingredient and final cosmetic formulation safety testing, the authors have developed a roadmap to safety assessment, focusing on requirements of the United States, the European Union, Japan and Canada.

1919 Interaction between GHS and Chemical Registrations in the EU
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The Globally Harmonized System (GHS) for classification and labelling of chemical hazards is now widely adopted globally, and becomes a requirement in the USA in 2015. Any given chemical is now likely to be similarly classified in whichever country it is considered; for instance, chemicals classified for physical and health hazards in the United States will likely have the same classification in the European Union. However, in Europe there are “downstream consequences” to GHS classification that are written into legislation, and which may be crucial for the use of that chemical. For example, -For agrochemicals and biocides, GHS classifications as category 1 carcinogen or mutagen or toxic to reproduction, excludes the chemical from registration as an agrochemical or biocide (except under derogation). -GHS classification as a reproductive toxicant plus a carcinogen fulfills temporary criteria for an endocrine disruptor, and similarly excludes the chemical from registration as an agrochemical or biocide. -Classification as a carcinogen, mutagen or reproductive toxicant may require testing of environmental metabolites for these hazards. The application of the weight of evidence approach and defining adverse effects is another important aspect of GHS. With particular respect to classification as a reproductive toxicant, under GHS the presence of maternal toxicity is no longer a reason to not classify; maternal toxicity needs to be shown to be causative of the developmental effect if classification is not to be applied. Classifications conducted under GHS in the USA or other authorities may therefore have significant consequences for registration in Europe, and this poster illustrates some recent pitfalls.
The Globally Harmonized System of Classification and Labelling of Chemicals (GHS) was developed to harmonize classifying and labeling of chemicals internationally. GHS was developed through the cooperation of the International Labour Organization, the Organization for Economic Cooperation and Development, and the United Nations Sub-Committee of Experts on the Transport of Dangerous Goods. The intent was a harmonization of the categorization process, not necessarily a harmonization of the categorization result, leaving the door open for organizations to interpret and apply the data differently. This study compared the carcinogen categorizations by four organizations that have compiled GHS categorizations (the European Union (CLP Annex VI), Safe Work Australia, Japan METI, and German IFA (GESTIS)) for 100 chemical substances. These GHS categories were compared to NTP, EPA, and IARC carcinogen classifications. Overall there was broad agreement on carcinogenicity, but out of 100 substances, there were disagreements among the four GHS categorization compilations for 41 substances. Of the 28 substances that at least one of the carcinogen classification agencies (NTP, EPA and IARC) ranked as known human carcinogens (or equivalent), 14 were categorized by the GHS-ranking agencies as a GHS carcinogen category 1B or 2 (presumed or suspected carcinogenic). Of the 69 substances that at least one of the NTP, EPA or IARC classified as possible or probable carcinogen (or equivalent), 14 of the substances were classified by at least one of the GHS-ranking agencies as not classifiable or insufficient data. This analysis demonstrates that the harmonization of process does not always lead to harmonization of decision. Understanding the advantages and pitfalls of carcinogen classification is important for interpretation of hazards. The findings and conclusions in this report are those of the author and do not necessarily represent the views of the National Institute for Occupational Safety and Health.
A Systematic Review of the Toxicological Hazards of the Waterpipe

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Waterpipe use has increased greatly worldwide in recent years. Vapor generated from heating flavored tobacco and smoke from the heating source, usually charcoal, is passed through a water basin before inhalation. A water filtration step leads to a common perception among users that water removes potentially harmful toxicants and the further perception that the waterpipe is a safer alternative to cigarettes. Although the toxicological profile of the waterpipe is poorly defined, an emerging evidence base demonstrates common themes regarding the hazards incurred from use of these tobacco products. To elucidate the toxicological profile of waterpipe tobacco smoke, and assess toxicological hazard relative to the use of conventional tobacco products, a systematic literature search of reference databases was conducted using general terms related to waterpipe concatenated with terms specific to tobacco smoke, and assess toxicological hazard relative to the use of conventional tobacco products, a systematic literature search of reference databases was conducted using general terms related to waterpipe concatenated with terms specific to tobacco products. A pilot study was conducted to assess five influential hazard levels for waterpipe use. Data analysis of individual NPs and cell types revealed distinct NP agglomeration determined by DLS. Statistical analysis determined that TiO₂ and CeO₂ NPs over a dose range of 3.2-200 μg/ml. NP cellular uptake was greater for TiO₂ and CeO₂ NPs than for other NPs. This highlights the need for continued discussion to distinguish these concepts for consistent application in regulatory decisions. To facilitate this dialogue, we support the consideration of a BRP continuum, where qualitative AOPs are used to organize data based on hypothetical or inferred linkages with limited utility in regulatory decisions and qualitative AOPs and MOAs can be used in regulatory decisions due to the increased confidence afforded them based on established causality.

Manufactured Metal Oxide Nanoparticles In Vitro Vascular Toxicity: Role of Size Profile and Cellular Specificity on Delivered Dose and Cytotoxicity

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Metal oxide nanoparticles (NPs) are used in a range of products and applications due to their unique physicochemical properties. In vitro studies have demonstrated the ability of NPs to translocate to the distal organs, including the cardiovascular system, following various routes of exposure. Therefore, it is essential that vascular toxicity be incorporated into characterizing NP systemic health effects. Our studies were undertaken to assess the vascular toxicity of 15 different metal oxides (8 TiO₂ and 7 CeO₂) NPs using human primary endothelial cells derived from aorta, coronary artery, lung microvasculature and umbilical cord. Endothelial cell viability and proliferation was assessed by WST-1 and cell counts following 24 h exposure to TiO₂ and CeO₂ NPs over a dose range of 3.2-200 μg/ml. NP cellular uptake was determined optically and NP agglomeration determined by DLS. Statistical analysis of all data from all endothelial cell subtypes and particles revealed that TiO₂ NPs exhibited greater cytotoxicity than CeO₂ NPs and correlated with cellular uptake. However, data analysis of individual NPs and cell types revealed distinct NP cytotoxicity profiles for each type of endothelial cell and regulated by NP agglomeration. For example, dispersed 10 nm TiO₂ NPs were more cytotoxic to coronary endothelial cells at lower delivered dose when compared to aggregates of this same NP independent of other confounding factors. Our results demonstrate that NP biological delivered dose, degree of NP agglomeration, and endothelial cell type must be accounted for when assessing NP vascular toxicity in vitro.

This abstract does not necessarily reflect those of the USEPA.
1929 Potential Toxicity of Titanium Dioxide Nanoparticles in Cultured Rat Pleural Mesothelial Cells

Y. Chen and J. M. Cerreta. PH.S, St. John’s University, Queens, NY.

Titanium dioxide nanoparticles (TiO2 NPs) are used in many applications, i.e., sunscreen, cosmetics and pigments. Possible routes of exposure to TiO2 NPs include ingestion, skin contact and inhalation. In addition to wide use and high possibility of exposure, the lack of safety standards demands more consideration regarding toxicity of TiO2 NPs. To investigate the toxicity of TiO2 NPs, rat pleural mesothelial cells (RPMCs) were cultured at 37°C in Ham’s F12 medium supplemented with FBS, glutamate and penicillin/streptomycin with 5% CO2. Initially, a series of concentrations of TiO2 NPs (50, 100, 200, 400 and 500μg/ml) were examined using the MTT cytotoxicity assay to determine the working concentration (400μg/ml) for further experiments. After 24hrs of treatment, cell viability via MTT assay was found to be 94.6±6.9%, 71.7±5.0%, 59.5±8.1%, 55.5±6.6% and 56.5±2.3% respectively to the above concentrations, whereas after 48 hrs, viability was 69.3±3.3%, 47.3±7.7%, 40.4±5.1%, 37±2.5% and 36.9±4.5% respectively. Following treatment, lactate dehydrogenase (LDH) increased by 4.3±3.6%, 13.2±16%, 23.6±25.8%, 35.1±25.5% and 46.2±16.1% respectively for the above concentrations, with respect to control cultures. Using the working concentration, cultures were assessed following 24 hrs of treatment for caspase 3, 8 and 9 activity, cytochrome C levels (cytosolic and mitochondrial) and by TUNEL assay and SEM. Caspase 3, 8 and 9 activity in treated cultures were increased to 152.4±26.3% (p<0.05), 235.3±20.9% (p<0.001) and 177.9±48.7% (p<0.05) respectively when compared to controls. Cytochrome C levels were increased in both cytosolic (1.6ng/ml) and mitochondrial fractions (1.7ng/ml) in treated groups when compared to control cultures (0.8ng/ml respectively). In the TUNEL assay, the average number of apoptotic bodies per field of treated cultures was significantly higher (42±5.5, p<0.01) when compared to controls (3.3±0.7). SEM indicates that TiO2 NPs were concentrated over the nucleus. These results suggest that TiO2 NPs can trigger cell apoptosis in RPMC cultures and such is occurring via the intrinsic pathway.

1930 Tungsten (IV) Oxide Nanoparticles Induce Apoptosis in Rat Pleural Mesothelial Cells

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Tungsten (IV) oxide nanoparticles (WO3 NPs) are extensively being used in the manufacture of conducting, semi-conducting materials and has mechano-chemical properties. Tungsten (IV) oxide nanoparticles (WO3 NPs) are extensively being used in the manufacture of conducting, semi-conducting materials and has mechano-chemical properties. Tungsten (IV) oxide nanoparticles (WO3 NPs) are extensively being used in the manufacture of conducting, semi-conducting materials and has mechano-chemical properties. Tungsten (IV) oxide nanoparticles (WO3 NPs) are extensively being used in the manufacture of conducting, semi-conducting materials and has mechano-chemical properties. Tungsten (IV) oxide nanoparticles (WO3 NPs) are extensively being used in the manufacture of conducting, semi-conducting materials and has mechano-chemical properties.

The side chains of ligand gold nanoparticles (AuNPs) potentially affect the toxicity and uptake of nanoparticles, possibly due to the interactions with the cell membrane. In this study, the effect of the presence of ligands of six different 14 nm AuNPs was investigated. The 14 nm AuNPs studied included citrate-stabilised AuNP, and AuNPs conjugated to functional groups via polyethylene glycol (PEG), namely hydroxyl-PEG (POH), carboxyl-PEG (PCOOH), biotin-PEG (PBTn), nitrotriacetic acid-PEG (PNTA), and azide-PEG (PZ). Cell impedance toxicity studies on BEAS-2B cells showed no toxicity of any of the AuNPs to the cells. Cells were treated with AuNPs for either 2 or 24 hours and their uptake was visualised by dark field microscopy. The citrate stabilised and PEGylated AuNPs entered the cells after 2 hours, whereas the other AuNPs did not. In conclusion, surface chemistry and modifications can influence the uptake of AuNPs, however this uptake did not result in cytotoxicity. A complete characterisation of the AuNPs to establish any differences in charge, size and aggregation state in culture media may provide an explanation for the observed differences in uptake.

1933 In Vitro Assessment of Vascular Nanoparticle-Induced Toxicity: Implications for a Susceptible Human Subpopulation

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Nanoparticles (NPs) associate macromolecules forming a biocorona (BC) when introduced into a physiological environment, altering their clearance, distribution and toxicity. Association of the BC is dependent on the NP physicochemical properties and the physiological environment. Individuals suffering from obesity and cardiovascular disease exist with altered physiological environments, which may influence NP toxicity. We hypothesize that a BC formed on NPs following incubation in hyperlipidemic serum will result in altered NP-BC protein content, cellular uptake, and toxicity compared to normal serum conditions. We utilized rat aortic endothelial cells (RAEC) and Fe3O4 NPs that are being developed as MRI contrast and tumor targeting agents. Further we used a dynamic flow in vitro exposure system to depict the in vivo environment. A BC was formed on 20nm PVP-suspended Fe3O4 NPs following incubation in water (control), 10% normal serum, or 10% hyperlipidemic serum. Addition of both BCs resulted in increased hydrodynamic size and a decreased surface charge. Fe3O4 NPs associated more cholesterol after incubation in hyperlipidemic serum compared to normal serum. We identified differences in BC protein components via liquid chromatography mass spectrometry. To assess BC-induced differences in uptake, RAEC were exposed under flow conditions for 2h to 10μg/ml of Fe3O4 NPs, Fe3O4 NPs with normal or hyperlipidemic BCs and analyzed by ICP-MS and dark field microscopy. Fe3O4 NPs without a BC were readily internalized by RAEC however addition of both BCs reduced uptake. To evaluate BC-induced differences in Fe3O4 NP cell activation we utilized an endothelial cell specific PCR array. For a number of genes including IL-6, TNF-α, Cxcl-2, VCAM-1, ICAM-1, and Selectin-E addition of the BCs was found to exacerbate RAEC responses to Fe3O4 NPs (Fe3O4-Hyperlipidemic > Fe3O4-Normal > Fe3O4). These findings demonstrate the possible influence of disease-induced variations in physiological environments and their impact on NP toxicity. Supported by Colgate-Palmolive.
Small Airway Epithelial Cells Exposure to Printer-Emitted Engineered Nanoparticles Induces Cellular Effects on Human Microvascular Endothelial Cells in an Alveolar-Capillary Coculture Model

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The printer is the one of the most common office equipment. Recently, it was reported that toner formulations for printing equipment constitute nanoenabled products (NEPs) and contain engineered nanomaterials (ENMs) that become airborne during printing. To date, insufficient research has been performed to understand the potential toxicological properties of printer-emitted particles (PEPs) with several studies using bulk toner particles as test particles. These studies demonstrated the ability of toner particles to cause chronic inflammation and fibrosis in animal models. However, the toxicological implications of inhalation exposures to ENMs emitted from laser printing equipment remain largely unknown. The present study investigates the toxicological effects of PEPs using an in vitro alveolar-capillary co-culture model with Human Small Airway Epithelial Cells (SAEC) and Human Microvascular Endothelial Cells (HMVEC). Our data demonstrate that direct exposure of SAEC to low concentrations of PEPs (0.5 and 1.0 μg/mL) caused morphological changes of actin remodeling and gap formations within the endothelial monolayer. Furthermore, increased production of reactive oxygen species (ROS) and angiogenesis were observed in the HMVEC. Analysis of cytokine and chemokine levels demonstrates that interleukin (IL)-6 and MCP-1 may play a role in the cellular communication observed between SAEC and HMVEC and the resultant responses in HMVEC. These data indicate PEPs at low, non-cytotoxic exposure levels are bioactive and affect cellular responses in an alveolar-capillary co-culture model, which raises concerns for potential adverse health effects.

Nanoparticle Ingestion Alters Nutrient Absorption in a Physiologically Based In Vitro Model of the Gastrointestinal Tract

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The gastrointestinal (GI) tract serves as an interface between the internal circulation and external environment by absorbing nutrients and defending from outside threats. Nanoparticle (NP) ingestion from food and food packaging is nearly unavoidable, and the role of NP exposure on GI health and function is not well understood. We are testing the overall hypothesis that ingestion of some types of nanoparticles can alter mineral and nutrient absorption, change cellular gene and protein expression, and ultimately result in physiological consequences. Our in vitro model is composed of Caco-2 and HT29-MTX cells co-cultured on a semi-permeable membrane, and contains a mucus layer and simulated digestion. This in vitro model was exposed to physiologically relevant doses of 30 nm TiO₂, or SiO₂, NPs for a total of 6 hours (or) chronic (5 days) time periods. Following NP exposure, transport of stable isotopes (⁵⁸Fe and ⁶⁷Zn) across the cell monolayer was used to model iron or zinc transport into the bloodstream. The degree of nanoparticles in the blood was measured at different concentrations. The results suggest that nanoparticles significantly elevated the amount of iron and zinc transport, while decreasing the amount of iron and zinc nutrition functionality, as transcellular epithelial resistance (TER) and claudin protein expression remained statistically the same between controls and NP-exposed cultures. Gene expression analysis showed that acute TiO₂ NP exposure decreased DMT1, which codes for the Fe²⁺ import protein, to 50% of control levels. ZIP1, which codes for a zinc import protein, was increased two-fold compared to untreated controls following acute SiO₂ NP exposure. Overall, these results suggest that intestinal epithelial cells are affected at a functional level by physiologically relevant exposure to NPs, and that the cells are working to regulate the iron and zinc transport mechanisms disturbed by NP ingestion.

Cytotoxicity of Titanium and Cerium Dioxide Nanoparticles in HaCaT Cells

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Metal oxide nanoparticles have the potential to contact skin due to their use in commercial products and potential release into the environment. The objective of this study was to assess the cytotoxicity of six titanium dioxide and three cerium dioxide nanoparticles using the human-derived keratinocyte cell line, HaCaT cells. Titanium dioxide particle sizes were 22, 25, 31, 59, 142 and 214 nm. Cerium dioxide particle sizes were 8, 40 and 58 nm. Approximately 1 x 10⁴ cells were plated in 96 well plates and placed in an incubator (37°C, 5% CO₂, 95% relative humidity). Twenty-four h later, the cells were exposed to the particles in media (Dulbecco’s minimum essential medium with 9% fetal bovine serum) at doses ranging from 1 to 500 μg/mL. The particles were probe sonicated before exposing them to the cells. The exposed cells were incubated for 24 h and then assessed for cytotoxicity using the lactate dehydrogenase assay. Additional experiments were conducted to assess formation of reactive oxygen species by quantitating the formation of 2',7'-dichlorofluorescein. All of the titanium dioxide particles showed significant (p < 0.0001) dose-response relationships with respect to cytotoxicity. The cytotoxicity of the titanium dioxide particles at 500 μg/mL ranged from 22.2% to 58.2%, with the
31 nm particle having the highest potency. Only one of the three cerium dioxide nanoparticles, 8 nm, showed a significant dose-response relationship (p<0.001) with respect to cytotoxicity. The cytotoxicity for this nanoparticle at 500 μg/mL was 40.7%. For the titanium (31 nm) and cerium dioxide (8 nm) nanoparticles with the highest cytotoxicity, a concentration of 500 μg/mL, the relative intensity of oxidant formation was increased 151- and 47-fold, respectively, relative to control. Reactive oxygen species appear to be formed in response to titanium and cerium dioxide nanoparticles and contribute to cytotoxicity in HaCaT cells. (This abstract does not necessarily represent U.S. EPA policy.)

1939 In Vivo Cardiotoxicity Screening of Silver and Metal Oxide Nanoparticles Using Human-Induced Pluripotent Stem Cell-Derived Cardiomyocytes


Exposure risk to silver and metal oxide nanoparticles (NPs) continues to increase due to their widespread use in products and applications. In vivo studies have shown Ag, TiO2, and CeO2 NPs translocate to the heart following various routes of exposure. In this study, we examined cytotoxicity and immune responses of metal oxide nanoparticles in human hematopoietic cell line THP-1. [Methods] Al2O3, CeO2, TiO2, SiO2, CuO, NiO and ZnO were examined in this study. The physicochemical properties, such as the size distribution and the zeta potential were measured by dynamic light scattering. After the nanomaterials were exposed to THP-1 cells, the cytotoxicity was assessed by using intracellular ATP method and the immune response was evaluated by measuring cytokines in the culture medium. [Results and Discussion] The hydrodynamic diameter of nanomaterials observed in suspension and culture medium were 41 to 224 nm and 153 to 407 nm, respectively. Although the zeta potentials of nanomaterials measured in suspension were positive (+45 to +62 mV), except SiO2 and ZnO(Afa Aesar) (+54 to 7.5 mV), all of the zeta potentials measured in culture medium were negative (-22 to -8.2 mV). Cytotoxicity in THP-1 cells was observed following treatment with CuO, ZnO and NiO. As the results of cytokine measurement in the cell culture supernatant, IL-8 content was increased following treatment with CuO, ZnO and NiO in a dose-dependent manner. TNF-c content was slightly increased by ZnO and NiO treatment. On the other hand, CuO, ZnO and NiO did not affect IL-6 and IL-1β productions. In conclusion, THP-1 cell showed both cytotoxicity and cytokine release following treatment with CuO, ZnO and NiO, but the effect on these cellular responses was different in each nanomaterial. A further molecular-level analysis would help the better understanding of the relation between biological effects and the physicochemical properties of nanomaterials.

1940 Tracking Translocation of Industrially Relevant Engineered Nanomaterials across Alveolar Epithelial Monolayers

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Relatively little is known about the fate of industrially relevant engineered nanomaterials (ENMs) in the lungs regarding translocation across the epithelial lining layer. Such processes may lead to subsequent effects on particle clearance, toxic effects or both. To allow precise quantification of translocation across lung epithelial cells, we developed a method for tracking metal oxide ENMs in vitro using neutron activation. The versatility and sensitivity of the proposed method for tracking industrially relevant ENMs while accounting for dosimetry can be used for tracking metal oxide ENMs in the lungs regarding translocation across the epithelial lining layer for large panels of materials. The reported INVET system for tracking industrially relevant ENMs while accounting for dosimetry can be used for tracking metal oxide ENMs in the lungs regarding translocation across the epithelial lining layer. 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intracellular oxidative stress was evaluated by DCFH-DA assay and pro-inflammatory cytokine release (IL-8) was measured by ELISA. 2D-DIGE/MALDI-ToF/ToF approach was used in order to identify possible mechanisms of toxicity. The uptake and localisation of the particles and ions was assessed by Secondary Ion Mass Spectrometry (NanoSIMS50). Contribution of Ag ions to toxicity was also evaluated (ultratransliteration and ICP-MS). Results and discussion: AgNO3 induced a reduction in metabolic activity in a dose dependent manner whereas no reduction was observed in the case of Ag 20nm and 200nm. The presence of mucus showed a protective effect against oxidative stress upon exposure to H2O2. Ag 20nm led to an increase in IL-8 release. Ag was found to be distributed homogenously in the cell with aggregates observed in specific locations in the case of Ag 20nm. Proteomic data revealed that AgNO3 and Ag particles induced an up-regulation of oxidative stress pathways, modulation of cytoskeleton machinery and apoptosis-related proteins. Ag 20nm and 200nm were found to behave in different manner compared to in solution ions. A size dependent effect was observed: 20nm particles seemed to be more toxic than Ag 200nm, that were found to be close to the negative control. Calculations: We described a co-culture model for intestine that is more physiological and relevant for toxicological studies compared to Caco-2 cells alone. Observed differences in effects cannot be attributed solely to ions while the effects were also particle size dependent.

1944 Silica Nanoparticle-Induction of CXCL8 and IL-6 in BEAS-2B Cells via Activation of NF-kB and a p38/TACE/TGF-α/EGFR Pathway: Role of ROS


Silica nanoparticles (SiNPs), used in a range of applications, are known to trigger inflammatory responses. We have previously shown that SiNPs of 50 nm size (Si50) induced interleukin (IL)-6 and CXCL8 responses in BEAS-2B cells through combined activation of several pathways, including NF-kB and p38/TACE/TGF-α/EGFR signaling. In the present study, we investigate the role of Si50-induced ROS and scavenger receptors in CXCL8- and IL-6 formation via Western blotting; release of TGF-α, CXCL8 and IL-6 by ELISA; and heme oxygenase expression by Real Time PCR. ROS was measured by spectrofluorometer after staining by DCFDA. Si50 exposure induced formation of ROS, and a time-dependent up-regulation of the mRNA expression of heme oxygenase. The anti-oxidant NAC partially reduced the release of IL-6 and CXCL8, suggesting a role for ROS. Furthermore, NAC-partially reduced phosphorylations of p38 and p65, and the release of TGF-α. Transfection with siRNA against Ddx2-1 exerted similar effects, but did not inhibit the release of TGF-α. Data suggest that the role of scavenger –receptors in these Si50-induced responses will also be presented. In conclusion, ROS formation seems to participate in the Si50-induced cytokine (IL-6 and CXCL8) release via MAPKInase p38, NF-kB and a cleavage of pro-TGF-α, via NADPH oxidase (Duox-1)-dependent and –independent mechanisms, respectively.

1945 Preliminary Validation Study of a 3D In Vitro Inhalation Model, Using Cytokine and Gene Expression Responses of Copper Oxide Nanoparticles

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Human 3D airway models are fully differentiated and functional models of the respiratory epithelium. They are cultured at an air-liquid interface (ALI), allowing relevant exposure via air. It is anticipated that these models may predict a more realistic bioavailability of inhaled compounds. To investigate the effects of donor, exposure unit, exposure session and insert, we performed air exposures of copper oxide nanoparticles using the MucilAirTM human 3D bronchial model. MucilAirTM (Epithelix SARL) were exposed at ALI conditions in Vitrocell exposure modules to aerosolized CuO (0, 50, 224, 1000 mg/m3) for 1 hour. Donor and exposure module unit were rotated among the four different exposure sessions using a statistical experimental design. Deposition of CuO nanoparticles was 4%. After a 24 hours post-incubation period, exposure to CuO showed a slight but significant LDH response for the highest dose. For inflammation markers MCP-1, IL-8 and IL-6 a dose-response was observed, where this was significant for IL-6. The influence of the parameters ‘concentration’ is the largest, followed by ‘donor’, ‘unit’ and ‘session’ which are in the same order of magnitude, which is then followed by the parameter ‘insert’. Gene expression analyses (using Illumina beadchip (humanHT-12v4)) showed a significant increase in regulated genes (adjusted p-values <0.05) in a concentration dependent way. For the highest dose up to 5852 genes were up regulated and down regulated, PCA showed clearly distinct groups for ‘concentration’, as well for ‘donor’. Statistical analyses showed that differences in ‘concentration’ were larger than those among ‘donors’, while donor differences were more substantial than differences between sessions. We conclude that the MucilAir model can be used to assess the effects of nanoparticles, as long as donor-, session- and chip differences are taken into account of the experimental design and subsequent statistical analyses.

1946 The Role of Valence State in Cerium Oxide Nanoparticle Toxicity

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Nanoparticles are part of an emerging field of technology that offers unique manufacturing and engineering properties not easily attainable with micron or larger particles of similar chemical composition. Cerium oxide (CeO2) nanoparticles are one such material used in a variety of products, including solar cells and gas sensors. Increased industrial production will subsequently lead to additional occupational exposures, making toxicology screenings crucial. Previous toxicology studies have presented conflicting results on the extent of CeO2 toxicity, which is thought to be due to the ability of Ce to couple in both a +3 and +4 valence state. Thus, to study whether valence state is important in CeO2 toxicity, CeO2 nanoparticles were doped with gadolinium (Gd) to adjust Ce toward a +3 state. We hypothesized that doping would increase toxicity and decrease antioxidant abilities as a result of increased oxygen vacancies and inhibition of +3 to +4 transition. RLE-6TN rat alveolar epithelial and NR8383 rat alveolar macrophage cells were treated with a range of CeO2 doses to assess toxicity using an annexin V/propidium iodide stain, and neither doped nor pure CeO2 induced toxicity by 24 hrs. Darkfield microscopy was employed to observe nanoparticle-cellular interactions, and within 5 min both pure and doped CeO2 began to associate with cells. Electron spin resonance, used to assess the effects of CeO2 on free radical production, showed that with doping, antioxidant potential decreased. Further, Nrf2 activity and downstream antioxidant proteins, such as heme oxygenase-1, were quantified via western blot and immunocytochemistry to elucidate important antioxidant pathways. The results suggest that valence state plays a role in antioxidant potential but a minimal role in cytotoxicity; however, further studies are needed to understand the mechanisms by which CeO2 valence state affects toxicity.

1947 Proinflammatory Potential of Silica- and Silver-Nanoparticles in Different Epithelial Lung Cell Cultures


Amorphous silica nanoparticles (SiNPs) are used in a wide range of applications, including food, cosmetics and medicine. Silver nanoparticles (AgNP) are used in various types of products. Different particle sizes of the same material will inherit different properties and toxicity. The aims of this study were to identify and characterize the acute pro-inflammation of responses of AgNP and different sizes of silica nanoparticles (SiNPs) in two different epithelial lung cell lines, and to determine the role of signaling proteins. The human bronchial epithelial cell lines, BEAS-2B and HBEC, were used. The cell lines were grown in LHC-0-medium and substituted with DMEM/F12-medium 1 day prior to particle exposure. The cells were exposed with SiNPs of two sizes: 10 nm (Si10) and 50 nm (Si50), and AgNP of 20 nm (Ag20). The expression and release of the cytokines interleukin (IL)-6, CXCL8 and RANTES (CCL5) were analyzed by real time PCR and ELISA. LDH release, used as a cytotoxic marker, was measured by a colorimetric assay. Involvement of signaling proteins were studied by using chemical inhibitors, gene silencing (siRNA) together with activation/phosphorylation of the signaling proteins (MAPKs and NF-kB) by Western blotting. The pro-inflammatory responses varied between the different NPs. The expression and release of IL-6, CXCL8 and CCL5 were much higher for Si10 than for Si50. Although the Ag20 had a size between the two SiNPs, the cytokine responses were even lower than for Si50. The cytokine responses were similar in the two lung cell cultures. For both SiNPs the responses seemed to be mediated partly through epidermal growth factor receptor (EGFR) and the MAPKs p38 and JNK. Thus, Si10 and Si50 are suggested to mediate cytokine responses by the same
signaling proteins, but to different extent. In conclusion, particle sizes together with other physical/chemical characteristics seem important for the pro-inflammatory responses in bronchial epithelial cell lines.

**1948 Physicochemical Properties of Gold Nanomaterial Affected Content Binding and Adherence Dynamics of their Serum Plasma Protein Corona**


Gold (Au) NMs are advancing techniques for biosystem probing and selective targeting due to their lower toxicity, acceptable surface chemistry, and tunable optical surface plasma resonance properties. Toxicity and the overall cellular/tissue interaction of NMs are influenced by the composition and dynamics of the surface protein corona formed during NMs exposure to complex media and biofluids. NM corona studies have been conducted on silica and polystyrene. This study investigated the protein content of two Au (10 and 50nm citrate capped) spheres (NS) after exposure to human plasma for two early time periods (5 min and 1h), which are consistent with early and late protein binding prior to tissue or cell uptake. Initial fluid size characterization (DLS) revealed that both Au NS agglomerated slightly in water with sizes of 29 ± 7 nm (10nm) and 61 ± 5.3 (50nm), and their surface charge (zeta potential) was similar at -47.5 ± 45.0 ± 1.7 mV, respectively. SDS PAGE separation/mass spectrometry band analysis demonstrated both protein profiles had similarities and differences based on size and incubation time. Some key toxicological proteins were found in Au NS profiles for 10 and 50 nm at both time points, including peroxiredoxin-6, involved in cell redox regulation, and carbonic anhydrase-1, involved with tissue CO2 binding/transport. However, differences were also observed in protein profiles based the same conditions. Triosephosphatase isomerase, essential for energy production, was bound to the 50nm NS after 5 mins, but was not detected on the 10nm NS or on the 50nm NS after 1hr. These results taken together revealed that NM of the same composition can differ beyond size and surface dose/mg, and supports the finding that cells and tissues are exposed to complex, dynamic NM surface conditions based on medium protein content and affinity surface dynamics.

**1949 Impact of Silver Nanoparticle—Ovalbumin Protein Corona on Antigen Presentation**

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Recent research has focused on the impact of protein coating on the surface of engineered nanomaterials (ENM) when they enter a biological medium (such as blood, plasma interstitial fluid, or cell culture media), which is known as the ENM-protein corona (ENM-PC). It is being increasingly recognized that the ENM-PC influences biodistribution, cellular uptake and toxicity. However, little is known about the protein structural changes that are dependent upon the physicochemical properties of nanoparticle surface and the possibility of immune responses to the altered protein. We hypothesized that the protein structural changes will result in protein structural changes that are dependent upon the physicochemical properties of nanoparticle surface and the possibility of immune responses to the altered protein.

**1950 Extended and Repeat-Dose Percutaneous Penetration of Dendrimers into Pig and Human Skin**

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Dendrimers are highly branched stable polymeric nanoparticles synthesized into specific nano-sizes, compositions, and surface chemistries. We previously saw differences in skin penetration of generation 3 (G3) amine terminated (NH2) and generation 4 (G4) glycyl (Gly) polyamidoamine (PAMAM) dendrimers in 24 h. We conducted extended and repeat dose studies for 48 and 72 h to determine if the dendrimers can penetrate deeper into the epidermis potentially becoming more systemically bioavailable. Two dendrimers, (neutral charge) dendrimers were conjugated with Alexa Fluor 568 (fluorophore), G3-NH2 and G4-OH were formulated in an aqueous solution or cosmetic emulsion formulation (0.2% concentration) and applied and re-applied (repeat dose) to viable pig and human cadaver skin assembled into diffusion cells for up to 672 h. Extent of skin penetration was determined by fluorescence in the skin layers using laser scanning confocal microscopy. In pig skin, G3-NH2 penetration from solution increased slightly after 48 h, and became more visible after 72 h and more prevalent with repeat dosing, G3-NH2 dosed in emulsion penetrated after 72 h. In human skin, G3-NH2 penetration into the epidermis occurred more after 72 h and 72 h repeat dosing, including penetration from emulsion. For G4-OH, pig skin penetration was prevalent when dosed in solution and emulsion after 48 h and became more apparent at 72 h (solution only). In human skin, G4-OH penetration from solution remained marginal and did not increase after 72 h. Some G4-OH penetration occurred with the emulsion application after 48 h, but did not increase during extended or repeat dose studies. Human skin remains less permeable to G3-NH2 and G4-OH dendrimers, but penetration from the cosmetic vehicle may occur after 48 and 72 h.

**1951 Cellular Uptake and Clearance of TiO2 Nanoparticles**

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Differential rates of cellular uptake and clearance of engineered nanomaterials may influence the propensity for tissue accumulation under chronic exposure conditions. A retinal pigment epithelial cell line (ARPE-19) was used to investigate 1) if TiO2 (Degussa, P25) nanoparticles affect cell proliferation, 2) if cellular uptake differs when exposed to a single dose or the same total dose level divided into fractional daily doses, and 3) clearance of particles from cells relative to cellular proliferation. ARPE-19 cells were plated at 2 densities (confluent and non-confluent), stained with CellTrace Far Red, an agent used for cell proliferation assays, and treated with TiO2 nanoparticle suspensions (3 or 10 μg/ml). For 24 or 48 hours, the cells were examined by flow cytometry. Changes in the fluorescent profile (indicative of cellular proliferation) were compared to light scattering data (indicative of internalized nanoparticles). Cells treated with nanoparticle suspensions showed a decreased rate of cell proliferation. Two assays supported reduced cell cycling (Side scatter signal was not changed in confluent cells over time, suggesting that the particles were not cleared. We then examined cellular response using various in vitro treatment regimens. Cells were exposed to an equivalent dose of TiO2 nanoparticles delivered either in one day (5 μg/ml) or over a course of three days (1 μg/ml/d). Nanoparticle internalization was again evaluated with flow cytometry light scattering. Delivered dose was also evaluated based on phototoxicity in which cell viability was measured by a live/dead assay (calcium-AM/propidium iodide) after exposure to UV light. Results suggest that cellular uptake was equivalent in the single and split-dose conditions, the presence of internalized nanoparticles did not inhibit subsequent uptake of nanoparticles, and that low level, long term exposure has a potential for cumulative cellular uptake. This abstract does not reflect EPA policy.
The aim of this study was to determine the immunological effects of iron oxide nanoparticles and to link the effects to the nanoparticles’ physicochemical properties. Iron oxide nanoparticles (21, 43 and 75 nm) were characterized as to their hydodynamic diameter in different biological media, zeta potential, chemical composition of the iron core and surface area. Human blood monocytes were isolated from buffy coat and treated with particles alone or particles in combination with lipopolysaccharide (LPS). Pro-inflammatory cytokines such as TNFα, IL-β and IL-6 were measured using ELISA. Furthermore, a leuprind-based human whole blood model was used to determine complement activation (soluble TCC) and monocyte and granulocyte stimulation (CD11b up-regulation). All the nanoparticles had a negative zeta potential in cell culture medium (-11 to -25 mV) and a larger hydodynamic diameter when suspended in cell culture medium compared to water. Only the 43 nm nanoparticles induced cytokine production by monocytes which was attributed to Toll-like receptor 2 (TLR2) and TLR4 activity as shown by a reporter assay. Interestingly, co-stimulation of the 21 nm nanoparticles and LPS led to a reduction in LPS-induced cytokine production with increasing particle concentration (up to 100 μg/mL). In the whole blood model, the iron oxide nanoparticles induced elevated levels of soluble TCC and up-regulated CD11b expression on monocytes and granulocytes in a concentration-dependent, but size-independent manner. These data suggest that iron oxide nanoparticles activate the complement system in a particle concentration-dependent, but size-independent manner, whereas neutralization of LPS is likely to be particle size-dependent.

### 1953 The “Spot Test”: A Novel Assay for the Analysis of Biocidal Properties of Nanomaterials


Toxicity testing of nanomaterials (NMs) is experimentally challenging because NMs may interfere with assay components and the turbidity of the suspensions may cause false results in the case of optical endpoints. In this work we propose a novel and reliable method – a ‘spot test’ to evaluate inherent biocidal potency of NMs for unicellular microorganisms. The novelty of this method lies in using deionized water (DI) as the test environment: cells are incubated in DI suspensions of NMs for up to 24 h and then pipetted as a ‘spot’ on agarized growth medium. Comparatively, a standard microdilution assay in ‘rich’ growth medium was applied. Ten chemicals were selected: two different Ag NPs, CuO and TiO2 NPs, multi-wall C-nanotubes (MWCNTs), AgNO3, CuSO4 and antimicrobials 3,5-dichlorophenol, triclosan and H2O2. Altogether 6 bacterial strains, yeast and a microalga were used as test species. The least toxic were MWCNTs (minimum biocidal concentration, MBC > 250 mg/L) and TiO2 NPs (MBC ≥ 1000 mg/L) and by far the most toxic substances were Ag and CuO NPs and the soluble salts of these metals (MBC ≤ 10 mg/L) in DI. Higher toxicity of Ag-ions and AgNPs and Cu-ions and CuO NPs (10-1000 times) to almost all of the test organisms in DI compared to the ‘rich’ medium shows that Ag and Cu-ions and NPs are promising biocides towards bacteria and fungi in a low-complexing environment.

The proposed test format - exposure of living cells to chemicals in DI allowed comparing the inherent toxic properties of chemicals, especially that of heavy metals or metal-based NMs, which bioavailability strongly depends on presence of complexing ligands in the test media. Based on the similar patterns of acute toxic effects of ten chemicals on eight unicellular organisms in DI we conclude that toxicity mechanisms of chemicals designated for general biocidal action (organic chemicals, metals) seems to be similar, whatever the organism (bacteria, yeast, alga). This work was supported by IUT 23-5 and ETF9001.

### 1954 Synthesis, Characterization, and Antimicrobial Activity of Drug-Loaded Calcium Alginate Nanocapsules: Perspectives on Their Potential Biomedical Use

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In this project, we performed the synthesis and characterization of polymer-based calcium alginate nanocapsules containing commercial antibiotics (Ciprofloxacin and Amoxicillin) to evaluate their antibacterial activity. Calcium alginate nanocapsules were prepared in our laboratory to obtain innovative drug carriers with selective and efficient antibacterial activity. The synthesis was performed by crosslinking emulsion technique in presence of surfactant agents and employing mild conditions during the reaction process. The characterization of nanocapsules was realized by Dynamic Light Scattering (DLS) and Low Voltage Electron Microscopy (LTEM). The antimicrobial activity of these materials was evaluated by Kirby-Bauer method (disc diffusion method) employing Staphylococcus aureus and Escherichia coli as models. DLS demonstrated that calcium alginate nanocapsules (NCA) were obtained in the range of 14 – 18 nm, and hydrodynamic radius decreased slightly as Ciprofloxacin and Amoxicillin were encapsulated by calcium alginate in the conditions described above. It was demonstrated that antimicrobial activity of NCA containing both drugs upon Escherichia coli and Staphylococcus aureus was remarkable compared with the commercial drugs by themselves, due to that NCA were able to inhibit bacterial growth in both cases. Considering that the concentration of drugs inside the nanocapsules was lower than commercial antibiotics applied to sensidiscs (3 μg per disc), NCA showed antimicrobial activity potentially stronger. Characterization considering LTEM is being carried out.

### 1955 Effects of Dose, Dissolution, and Ion Aging on Silver Nanotoxicity In Vitro


Nanomaterials composed of soluble metals such as silver undergo dissolution when suspended in cell culture media, producing ions, and altering particle properties (e.g. mass, morphometry, etc.), which can alter particle diffusion, sedimentation, and ultimately cellular dose. Cultured cells are exposed not just to particles, but to a complex, dynamic mixture of particles, free ions, and ion-ligand complexes. Without quantitative data on the role of each of these elements, interpretation of conventional in vitro experiments for soluble particles is challenging. In this project, effects of Ag nanoparticle and Ag ion cellular dose, dissolution rate, and Ag ion aging (complexing) were quantified using both experimental and computational approaches. RAW 264.7 macrophages were exposed to particle-coated Ag NPs (20 and 110 nm), freshly mixed Ag acetate ions, Ag ions aged in cell culture media, and ions formed from nanoparticle dissolution. Cytotoxicity and total cell associated Ag were measured. The In Vitro Sedimentation, Diffusion, and Dosimetry Model (ISD) was used to predict nanoparticle deposition, dissolution, and ion partitioning to cells (verified experimentally). Intracellular nanoparticle surface area was most highly correlated to cytotoxicity (r = 0.96), indicating that Ag activity from or on internalized nanoparticle surfaces is important. Freshly mixed ions (LD50 1.64 μg/mL) were more toxic than aged ions (LD50 >2.8 μg/mL) or ions formed from nanoparticle dissolution (LD50 >2.8 μg/mL). These results suggest that Ag ions are more toxic than Ag-ligand complexes, and ions from extracellular nanoparticle dissolution may rapidly bind to ligands in cell culture media. Overall these results implicate intracellular dissolution or nanoparticle surface area interactions as important determinates of silver nanoparticle toxicity, as ions formed extracellularly may be rapidly detoxified by complexing with media ligands. Supported by National Institute of Environmental Health Sciences (NIEHS) U19 ES019544.

### 1956 Distinct Patterns of Apoptosis and Necrosis in A549 Cells by Fourth-Period Transition Metal Oxide Nanoparticles


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Nanoparticles are being used in biomedical, industrial and consumer products and applications with increasing frequency. Nevertheless, little is known about adverse effects of these particles. In previous studies, we demonstrated a trend of cytotoxicity for seven transition metal oxide NPs in human lung cancer cells (A549) and immortalized human lung cells (BEAS-2B). The particles could be divided into three groups on the basis of cytotoxicity level: low (ZnO, Fe2O3 and TiO2), moderate (Mn2O3 and NiO) and high (CuO and ZnO). In the present study, we analyzed NP toxicity in A549 cells by apoptosis analysis using flow cytometry and immunofluorescence microscopy. The dyes were used for both studies were Annexin-V conjugated fluorescein isothiocyanate (FITC), an apoptotic dye, and 7-Aminoactinomycin D (7-AAD), a dye that stains the nuclear membrane and reveals late apoptotic, necrotic or dead cells. Flow cytometry analysis confirmed our tiers of toxicity. The percentage of total apoptotic (early + late apoptosis) cells at the highest concentration (100 μg/mL) of NPs for the low toxicity group were 5.8% ± 0.7% for CuO, 5.8% ± 2.56% for Fe2O3, and 12.5% ± 5.5% for TiO2. For the moderately-toxic group, the corresponding apoptotic percentages were 30.56% ± 15.9% for Mn2O3 and 14.0% ± 3.0% for NiO. The total apoptotic percentages for the high toxicity group were 74.8% ± 12.4% for ZnO and 88.2% ± 6.5% for CuO. In the high toxicity group, the highest concentration of NP used was 20 μg/mL. 

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Nanotechnology has grown rapidly over the past decade, promising benefits in diverse areas of society. However, the rate of toxicological analysis of nanoparticles (NPs) has not kept pace with the rate of development, leading to concerns over the potential biological toxicity and environmental contamination of NPs. Here, we report the application of a high-throughput screening suite of sub-lethal assays as well as a growth inhibition assay to a series of Cu particles, including nano Cu, nano CuO, nano Cu(OH)2, micro Cu and micro CuO as well as ionic Cu (CuCl2 and CuSO4) in bacteria (Escherichia coli and Lactobacillus brevis). Fluorescent assays such as PI/SYTO, XTT, DtBAC and H2DCFDA were used to measure membrane damage, respiration rate, membrane potential, and Reactive Oxygen Species (ROS) production, respectively. IC50 values were calculated from growth inhibition curves, revealing that Cu and CuO NPs are more toxic than their micron-sized counterparts, with toxicities approaching that of ionic Cu. Strikingly, the NPs showed distinct differences in their mode of toxicity when compared to the Cu ions, highlighting the unique toxicity properties of materials at the nanoscale. In vitro DNA damage assays revealed that all of the particles caused levels of DNA lesions. However, only two of the most potential oxidizing agent, nano Cu and micro Cu, were capable of gradients of plasmid DNA suggesting that nanoparticles lead to genomic instability by DNA oxidation. Sucrose gradient centrifugation coupling with ICP-MS revealed that the NPs, but not the micro-sized particles, were internalized by cells. 3D tomography images were constructed from electron microscopy of cells exposed to the NPs, confirming the presence of intact NPs inside the cells. Consistent with this, confocal images using fluorescent-tagged NPs revealed the co-localization of CuO NPs and the bacterial cells.


Crystalline silica nanoparticles (NPs) have been shown to be involved in lung diseases after inhalation. In comparison, the toxicity of manufactured fumed silica NPs remains poorly investigated. In addition to NP characterization, we used an integrative toxicological approach combining transcriptomics and proteomics to assess the cellular and molecular effects of fumed silica (AEROSIL® 200 from Evonik, Germany) in A549 human alveolar epithelial cells. The shape of NPs was visualized by atomic force microscopy and size was investigated in culture media using direct live cell counts and viability assays. Our results showed that the NPs are visible throughout the layers of the skin. This study indicated that positively charged AgNPs can diffuse into human skin, with higher concentrations resulting in increased silver content. More positively charged AgNPs penetrated into skin when applied in aqueous solution compared to cosmetic emulsion.

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The rapidly increasing manufacture and utilization of nanomaterials fuels the demand for fast and reliable toxicity screening assays. The ToxTracker assay is a recently developed mouse embryonic stem (mES) cell-based reporter assay that uses GFP-tagged biomarkers for detection of DNA replication impairment, oxidative and general cellular stress upon exposure to chemicals [Hendricks et al. Toxicol Sci. 2012]. The aim of the current study was to evaluate the applicability of the ToxTracker assay to identify the (geno)toxic properties of a panel of metal oxide- and silver nanoparticles (NPs) as well as additional non-metallic materials (diesel, carbon nanotubes and quartz). The NPs were characterized in terms of agglomeration and ion release in cell culture media (PCCS, ICP-OES); acellular ROS production (DCFH-DA assay); cellular uptake (TEM); GFP reporter induction and cytotoxicity (flow cytometry); genotoxicity (alkaline and FPG- comet assay, yH2AX and RAD51 foci formation). The results show that the mES cells were able to take up the NPs and that exposure to CuO, ZnO, and NiO NPs as well as to quartz resulted in activation of the oxidative stress reporter. NiO NPs also activated a p53-associated cellular stress response, suggesting additional reactivity. The conventional comet assay confirmed the ToxTracker response. The findings also suggested that for CuO NPs the reporter induction was likely the consequence of released Cu ions whereas the effect of NO was related to the particles per se. Furthermore, the genotoxicity of metal oxide NPs seems to occur mainly via oxidative stress. We conclude that the ToxTracker reporter system can be used as a rapid mechanism-based tool for the identification of hazardous properties of metal oxide NPs.

PS 1958 Toxicogenomics Evaluation of Fumed Silica Nanoparticles in Human Lung Cells Reveals a Hierarchical Stress Response

PS 1959 In Vitro Penetration of Branched Polyethyleneimine (bPEI)-Coated Silver Nanoparticles into Human Skin

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Silver nanoparticles (AgNPs) are used as an antimicrobial agent in cosmetics. Safety concerns associated with human skin exposure to AgNPs exist. Previously, we observed silver penetration in human skin after 24 h treatment with citrate (negative charge) and polyethylene glycol (PEG; neutral charge) coated 20 nm AgNPs in aqueous solutions. In this study, we investigated the in vitro penetration of branched polyethyleneimine (bPEI; positive charge) coated 20 nm AgNPs in human skin. AgNPs were characterized to confirm size, shape, and agglomeration state. bPEI coated AgNPs were prepared in aqueous solution and cosmetic emulsion formulations (0.01% and 0.001% concentrations) and applied to human cadaver skin in flow-through diffusion cells. After 24 h, skin was washed, removed from diffusion cells, and tape-stripped twice to remove unabsorbed AgNPs. Samples were analyzed by ICP-MS and TEM to assess AgNP skin penetration. ICP-MS revealed an increase in total silver content (ng Ag/g skin) from solution and emulsion treatments at both concentrations compared to controls (treated skin with no AgNPs). Silver uptake was greater using the higher concentration (0.01%) solution and emulsion compared to the lower concentration (0.001%) solution and emulsion, with a 20-fold and a 7-fold increase in silver uptake, respectively. Silver uptake was greater using the solution vehicle, with a 6-fold increase in silver content in skin treated with 0.01% solution compared to the emulsion, and a 2-fold increase in silver content in skin treated with 0.001% solution compared to the emulsion. TEM images showed that ICP-MS findings were confirmed; 20 nm AgNPs are visible throughout the layers of the skin. This study indicated that positively charged AgNPs can diffuse into human skin, with higher concentrations resulting in increased silver content. More positively charged AgNPs penetrated into skin when applied in aqueous solution compared to cosmetic emulsion.

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Nanoclays on Human Lung Cells

1960 The ToxTracker Reporter System Enables Rapid Screening with Mechanistic Insight into the Genotoxicity of Metal Oxide Nanoparticles


Crystalline silica nanoparticles (NPs) have been shown to be involved in lung diseases after inhalation. In comparison, the toxicity of manufactured fumed silica NPs remains poorly investigated. In addition to NP characterization, we used an integrative toxicological approach combining transcriptomics and proteomics to assess the cellular and molecular effects of fumed silica (AEROSIL® 200 from Evonik, Germany) in A549 human alveolar epithelial cells. The shape of NPs was visualized by atomic force microscopy and size was investigated in culture media using direct live cell counts and viability assays. Our results showed that the NPs are visible throughout the layers of the skin. This study indicated that positively charged AgNPs can diffuse into human skin, with higher concentrations resulting in increased silver content. More positively charged AgNPs penetrated into skin when applied in aqueous solution compared to cosmetic emulsion.

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The rapidly increasing manufacture and utilization of nanomaterials fuels the demand for fast and reliable toxicity screening assays. The ToxTracker assay is a recently developed mouse embryonic stem (mES) cell-based reporter assay that uses GFP-tagged biomarkers for detection of DNA replication impairment, oxidative and general cellular stress upon exposure to chemicals [Hendricks et al. Toxicol Sci. 2012]. The aim of the current study was to evaluate the applicability of the ToxTracker assay to identify the (geno)toxic properties of a panel of metal oxide- and silver nanoparticles (NPs) as well as additional non-metallic materials (diesel, carbon nanotubes and quartz). The NPs were characterized in terms of agglomeration and ion release in cell culture media (PCCS, ICP-OES); acellular ROS production (DCFH-DA assay); cellular uptake (TEM); GFP reporter induction and cytotoxicity (flow cytometry); genotoxicity (alkaline and FPG- comet assay, yH2AX and RAD51 foci formation). The results show that the mES cells were able to take up the NPs and that exposure to CuO, ZnO, and NiO NPs as well as to quartz resulted in activation of the oxidative stress reporter. NiO NPs also activated a p53-associated cellular stress response, suggesting additional reactivity. The conventional comet assay confirmed the ToxTracker response. The findings also suggested that for CuO NPs the reporter induction was likely the consequence of released Cu ions whereas the effect of NO was related to the particles per se. Furthermore, the genotoxicity of metal oxide NPs seems to occur mainly via oxidative stress. We conclude that the ToxTracker reporter system can be used as a rapid mechanism-based tool for the identification of hazardous properties of metal oxide NPs.
on BEAS-2B cells, with the toxicity of the thermally degraded nanoclays varying with respect to both the time and the type of nanoclay being assessed. In particular, our results revealed that as-received Cloisite 30B showed a time-dependent decrease in live cell count and cellular viability, while the thermally degraded Cloisite 30B and Cloisite Na+ respectively showed potential increases in cellular proliferation, all relative to their respective control counterparts. Preliminary analysis of the toxicity mechanisms revealed possible damages to the mitochondria, membrane, and genetic material of the cell. While further investigation needs to be performed to determine mechanisms and conclude on the toxicological profiles of the nanoclays, our results show that care needs to be taken when handling and implementing these nanoclays in consumer products as well as during their disposal.

**1962 Surface Manipulation of Gold Nanorods: Toxicity Analysis of Chemical Modifications to Provide High Biocompatibility, Enhanced Uptake, and Unique Intracellular Distribution Pattern**

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Gold nanorods (GNRs) possess a number of shape-specific biosystem use advantages, including enhanced surface area, unique spectral signature and tunable optical properties to target imaging and/or deploy substances. GNR toxicity remains a use obstacle where surface chemistry accounts for a dominant portion, but where surface functionalized GNRs often results in lower cell uptake and optical property losses. Two surface GNR chemical modifications that hold significant promise to ameliorate toxicities while providing enhanced uptake are MTAB (16-mercaptopentadecyl trimethylammonium Br) a thiol analogue of CTAB and tannic acid (TA). MTAB binds to the GNR replacing CYAB and provides GNR good biocompatibility while maintaining a high level of GNR cellular uptake. TA combines with CTAB or MTAB and provides a GNRs surface amenable to endosomal uptake while maintaining optical properties. Here we report that MTAB GNRs of varying aspect ratio (1–4) showed no significant decrease in cell viability or stress activation in the alveolar epithelial A549 cell line. In addition, MTAB and MTAB-TA GNRs demonstrated a substantial level of cellular uptake with MTAB-TA GNRs displaying a unique intracellular appearance. In contrast, CTAB, CTAB-TA and polyethylene glycol (PEG) GNRs induced a significant degree of cytotoxicity and/or significantly lower internalization rates. In conclusion, these results demonstrate the biocompatible nature and high rate of internalization of MTAB and MTAB-TA GNRs, identifying them as prime chemical modifications for use in nano-based bioimaging and therapeutic applications.

**1963 Identifying Novel Gene Expression Targets following Metallic Nanomaterial Exposures Using Next-Generation Sequencing: A Comparison of Mouse and Human In Vitro Neuronal Models**

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For this study, we wanted to explore changes in microRNA (miRNA) expression since miRNAs are known to regulate a wide range of mRNA targets and limited studies have been performed evaluating the impact of nanomaterials (NMs) on their expression. Using a mouse neuronal co-culture model consisting of the C6/C13 neuronal cell line and the C8-B4 microglial cell line along with the human neuroblastoma cell line, SH-SY5Y, we evaluated changes in miRNA gene expression following metallic NM exposure. Manganese (Mn), a known neurotoxicant, was chosen along with gold (Au) as a reference NM. Both Mn and Au had a primary diameter of 80 nm. The Mn displayed aggregation in cell culture media while the Au remained relatively monodispersed. In both cell models, Mn NMs demonstrated the greatest decrease in cell viability and the greatest increase in reactive oxygen species production, whereas, the Au was non-toxic. Our time course gene expression data revealed that changes for select mRNA and miRNAs peaked at approximately 8 h following Mn exposure with Au demonstrating no changes in gene expression. Therefore, next generation sequencing (NGS) of miRNAs was performed following an 8 h exposure to a low and high dose of Mn NMs. The results demonstrated a dose-dependent response in gene expression changes for both the mouse and human neuronal models. Furthermore, multiple novel targets were identified that showed similar expression in human and mouse cells, while others were species specific. Using these unique expression profiles, we can target the miRNAs thereby affecting downstream gene expression and potentially cellular responses to NM exposure.

**1964 Identification of Novel Gene Targets and Putative Regulators of Arsenic-Associated DNA Methylation in Human Urothelial Cells and Bladder Cancer**

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There is strong epidemiologic evidence linking chronic exposure to inorganic arsenic (iAs) to carcinogenesis. This study set out to identify promoter methylation patterns associated with iAs and its metabolites in exfoliated urothelial cells (EUCs) that originate mainly from the urinary bladder, one of the targets of arsenic (As)-induced carcinogenesis. Genome-wide, gene-specific promoter DNA methylation levels were assessed in EUCs from 46 residents of Chihuahua, Mexico, and the relationship was examined between promoter methylation profiles and the intracellular concentrations of total As (tAs) and As species. A set of 49 genes was identified with increased promoter methylation associated with EUC tAs, iAs, and/or monomethylated As (MMAs) enriched for their roles in metabolic disease and cancer. Notably, no genes had differential methylation associated with EUC dimethylated As (DMAs). Further analysis showed that two of the 49 As-associated genes, specifically the genes COL12A1 and PSMB2, are among 1082 bladder cancer associated genes identified using The Cancer Genome Atlas repository. In addition, both the As- and cancer-associated genes are enriched for the binding sites of common transcription factors that are known to play roles in carcinogenesis, demonstrating a novel potential mechanistic link between iAs exposure and bladder cancer.

**1965 Modulation of AhR-Regulated Genes by Trimethyl Arsin Oxide in the Lung, Kidney, and Heart of C57BL/6 Mice**

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Rationale. Arsenic is a human carcinogen that has been extensively studied over decades; however, no definitive understanding for underlying mechanisms has been established. Arsenic is capable of differentially modulating the expression of phase I and Phase II AhR-regulated genes in extrahapatic tissues. However, whether organic arsenicals have similar effects or not need to be investigated. Experiments. C57BL/6 mice were received trimethyl arsine oxide (TMAO; 13 mg/kg ip) with or without the prototypical AhR ligand, TCDD (15 μg/kg). Thereafter, extrahapatic tissues were harvested at 6 h for gene expression or 24 h for protein and catalytic activities determination. Results. TMAO increased Cyp1a1 mRNA, protein and activity in lung; and increased Cyp1b1 mRNA and protein in lung and kidney. Upon coexposure, TMAO potentiated the TCDD-mediated induction of Cyp1a1 at mRNA, protein as well as activity levels in the lung, and kidney; and Cyp1a1 at mRNA in the heart. TMAO potentiated the TCDD-mediated induction of Cyp1a2 at mRNA, protein and activity in the lung. As for Cyp1b1, TMAO potentiated the TCDD-mediated induction of Cyp1b1 mRNA and protein in the kidney and Cyp1b1 mRNA in the heart. TMAO induced Nqo1 mRNA in the lung, kidney and heart, with subsequent increase in Nqo1 protein and activity in the lung. TMAO increased Gsta1 mRNA in the heart; and increased Gsta1/2 protein and Gst activity in the lung and kidney. Upon coexposure, TMAO increased Nqo1 mRNA compared to TCDD in the kidney and heart. TMAO potentiated TCDD-mediated induction of Gsta1/2 protein and Gst activity in the kidney. Conclusions. Our results demonstrate for the first time that, TMAO modulates constitutive and TCDD-induced AhR-regulated genes in a tissue-, and AhR-regulated genes-specific manner. In addition, induction of Cyp1 family in extrahapatic tissues could be crucial for activation of arsenic toxicity and carcinogenicity. Acknowledgments. NSERC Discovery Grant RGPIN 250139 to A.O.S. O.H.E. is the recipient of ACF and ATIF Scholarships.
1966  Arsenite- and Cadmium-Transformed Human Bladder Cancer Cells and Tumor Transplants Contain Elevated Levels of Gap Junctional Protein Connexin 43
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This study uses the previously developed model of Cd2+ and As2+ induced human urothelial cancer via the malignant transformation of the UROtsa cell line to validate microarray results indicating an elevation of the gap junctional molecule connexin 43 (Cx43) in transformed cells. In this study, Cx43 was found to have elevated mRNA and protein levels in 5/6 of the As3+ transformed cell lines and in 2/7 Cd2+ transformed lines. Interestingly, tumor transplants generated with the UROtsa transformed cell lines showed that all 6 As3+ and 7 Cd2+ tumors had high levels of both Cx43 message and protein. Next, the effects of low dose, short term As3+ and Cd2+ exposure on Cx43 levels in the parental UROtsa cells was determined. Results showed that while the mRNA levels decreased over 48 hrs, Cx43 protein levels were initially increased at 12 and 24 hrs and returned to basal levels after 48 hrs of exposure to either heavy metal. Finally, the immunohistochemistry of Cx43 expression was determined in normal urothelium, tumor transplants, and in human urothelial cancers. Results showed that normal bladder urothelium contains Cx43 protein in the stroma but not in the epithelium. Conversely, Cx43 expression was detected in all 6 As3+ and 7 Cd2+ tumor transplants although the highest staining for Cx43 was found in well-differentiated tumors in the center of tumor nests. In human urothelial tumors, Cx43 expression was found to be negative in 6/7 invasive, high grade cancers but both non-invasive and invasive urothelial cancers that have squamous differentiation were positive for Cx43. Overall, the results of the study show that Cx43 is increased in several but not all transformed urothelial cell lines and in human bladder tumors that contain squamous differentiation.

1967  Gestational Arsenic Exposure Affects Gene Expression in the Kidney and Lung in the F1 and F2 Mice
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Numerous epidemiological studies have linked high levels of arsenic in drinking water to a number of diseases, including carcinogenesis, cardiovascular disease and metabolic disease. However, the transgenerational effect of arsenic exposure is not fully elucidated. Previous studies have shown that gestational arsenic exposure of C3H mice increases the incidence of adult-onset tumors in the F1 male offspring. Our recent study further showed that gestational arsenic exposure of C3H mice increases hepatic tumors in the male grandchildren (F2). In this study, we focused on the other target organs of arsenic such as lung, kidney, bladder and testis in this model. Gene expression analysis of lung and kidney using real time RT-PCR method showed differences between control and arsenic groups in the F1 and F2 males. Especially, the statistically significant changes were observed in the cholesterol transporter Abca1, which is a key regulator of HDL biogenesis. In the arsenic group, the expression of Abca1 was significantly suppressed in the lung of F1 and the kidney of F2 compared to the control group. These results suggest that gestational arsenic exposure of C3H mice disrupts the expression of cholesterol metabolism-related genes on the subsequent generations. The transgenerational Abca1-suppressive mechanism should be clarified by further studies. Although hepatic tumors, on the other hand, were increased in the arsenic group, the histopathological analysis could not detect any increase in the tumor incidence in the lung, kidney, bladder and testis in this model.

1968  Arsenic Methylation Is Required for Arsenic-Induced Atherosclerosis
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Arsenic exposure is linked to increased atherosclerosis, both in humans and in mouse models. Arsenical is metabolized through a series of methylation and oxidation/reduction reactions that are catalyzed by the arsenic (III) methyltransferase (As3MT) enzyme. However, the role of arsenic methylation via As3MT in the pro-atherogenic effects of arsenic has not been described. We hypothesized that arsenic methylation is important to promote atherosclerosis. We first exposed male ApoE-/- mice to methylated arsenicals at 200 ppb, and found they enhanced plaque formation and alter its constituents in a manner analogous to our observations with NaAsO2. Furthermore, we compared male ApoE-/- and ApoE-/- As3MT-/- (DKO) mice exposed to tap water or 200 ppb NaAsO2 for 13 weeks, and analyzed plaque size in the aortic arch and sinus. As expected, arsenic increased plaque size in ApoE-/- mice. DKO mice had similar plaque size to ApoE-/- mice. However, As3MT deletion prevented arsenic-enhanced plaque formation. Arsenic also did not alter the plaque constituents in the DKO mice, as we observed in the ApoE-/- mice. Macrophages are required for plaque formation, and although the plaques from DKO mice had similar number of macrophages (+/ NaAsO2, we hypothesized that they may contribute to arsenic methylation in situ. Bone marrow-derived macrophages (BMDM) express As3MT, assessed by qPCR and western blot, which increased after arsenic exposure. Methylated arsenicals were detected in BMDM exposed to NaAsO2, indicating the As3MT is functional. Moreover, we tested if As3MT expression altered BMDM lipid homostasis. DKO and wild type (WT) DKO BMDM failed to accumulate lipid in response to arsenic when compared to ApoE-/- mice. Together, our data suggest that arsenic methylation is required for arsenic-enhanced atherosclerosis and is the first report linking arsenic methylation to an increased pathological outcome.

1969  Activity of the Zinc Finger Transcription Factor HNF-4α Is Inhibited by Arsenic in the Livers of Mice with Diet-Induced Nonalcoholic Fatty Liver Disease
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Non-alcoholic fatty liver disease (NAFLD) starts with simple steatosis, but it can progress to steatohepatitis if the individual is exposed to another insult such as environmental arsenic. The purpose of the present study was to investigate the mechanisms by which arsenic promotes the progression of NAFLD. Proteomics was used to characterize the effects of arsenic in mice with steatosis, and that information was used to identify regulatory elements that contribute to progression to steatohepatitis. Mice were fed a high fat diet for 10 weeks to produce steatosis. Half of the mice were co-exposed to drinking water containing 5 ppm sodium arsenite. LC-MS-MS was used to identify hepatic proteins that were altered. Ingenuity IPA analysis was used to identify groups of proteins with regulatory elements in common. Real time PCR, western blotting and gel shift assays elucidate the effects of arsenic on the predominant regulatory element predicted by IPA. 1,800 unique proteins were identified in the livers of these mice, and 386 proteins had at least a 2-fold difference in abundance between the 2 groups. 34 of the 386 proteins were known or suspected to be regulated by the zinc finger transcription factor HNF-4α, and 28 of these (82%) were lower in the arsenic-exposed group. There was no change in HNF-4α mRNA or protein levels, but HNF-4α DNA binding activity was inhibited in mice exposed to arsenic. Therefore, in mice with diet-induced steatosis, arsenic inhibits HNF-4α mediated gene expression by inhibiting its DNA binding activity.

1970  Metabolomic Signature Associated with Knockout of Arsenic (+3 Oxidation State) Methyltransferase in C57B6 Mice
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Arsenic (+3 Oxidation State) methyltransferase (As3mt) is the key enzyme in the pathway for methylation of inorganic arsenic (iAs), a common drinking water contaminant and a potent human carcinogenic. The As3mt-mediated conversion of iAs to mono-, di- and trimethylated arsenic is the principal mechanism for detoxification of iAs in many mammalian species, including humans. The fact that As3mt was conserved during evolution from simple invertebrates to modern humans implies that it may be involved in essential functions independent of iAs metabolism. The present study used metabolomics to identify metabolites and metabolic pathways associated with As3mt in absence of iAs exposure. Adult wild-type (wt) and As3mt-KO C57B6 mice were fed a regular laboratory chow and drank deionized water. Plasma and urine metabolomes were analyzed using gas chromatography and liquid chromatography with time of flight-mass spectrometry (TOF-MS) detection. Metabolites were verified and annotated using an in-house library of >800 mammalian metabolites and the on-line databases. Comparison of metabolites associated with wt and As3mt-KO genotype showed significant shifts in iAs metabolites (ranged in trimethyl arsenicals, glutathione, arginine, ornithine, and carnosine) and with metabolism of glucophospholipids, specifically phosphatidylcholine. Chronic exposure to 1 ppm As in drinking water had little effect on metabolome of wt mice, but shifted a significant number of
metabolites in plasma and urine of As3mt-KO mice that are not able to effectively detoxify iAs. Future research focusing on the metabolites and pathways linked by the present study to As3mt expression should provide more detailed information about the physiological functions of this enzyme.

**1971 Metabolism and Disposition of Arsenic in Acute Promyelocytic Leukemia Patients Treated with Arsenic Trioxide (ATO)**

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Involving differential analysis of these metabolites are needed to better assess possible pharmacokinetics of As species among the APL patients, suggesting that individual ng/ml, respectively. iAs was the major As species in urine and EUC at day 1 and 32 ng/ml to 10 ng/ml within six hours, remaining practically unchanged for at least 15 days after ATO infusion. Inorganic As (iAs), methyl-As (MAs) and dimethyl-As (DMAs) were measured in plasma, urine and in exfoliated urothelial cells (EUC) using hydride generation-cryotrapping-atomic absorption spectrometry. Plasma iAs concentrations in APL patients averaged at 0.051 ng/ml before treatment. After ATO infusion, iAs was quickly metabolized from a peak plasma concentration of 32 ng/ml to 10 ng/ml within six hours, remaining practically unchanged for at least 24 hours. Plasma MAs and DMAs concentrations began to increase at 4 hours after infusion, and continue to rise by day 4 to an average concentration of 8.5 and 10.4 ng/ml, respectively. iAs was the major As species in urine and EUC at day 1 and 4 after infusion. There were substantial differences in plasma concentrations and pharmacokinetics of As species among the APL patients, suggesting that individual capacity to metabolize ATO may play an important role in the therapy outcomes. In summary, treatment with ATO leads to the formation of MAs and DMAs, whose effect in APL patients are poorly understood. Large scale investigations involving differential analysis of these metabolites are needed to better assess possible therapeutic effects or long term toxicity on non-targeted organ systems.

**1972 Maternal Genotype for Arsenic (+3 Oxidation State)-Methyltransferase Influences Inorganic Arsenic Metabolism and Newborn Birth Outcomes in a Pregnancy Cohort in Mexico**

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Prenatal exposure to arsenic has been associated with adverse birth outcomes such as increased risk of stillbirth, infant mortality, and low birth weight. The level of exposure and the rate or pattern of arsenic metabolism may be responsible for these outcomes. Genetic predisposition is assumed to be one of the factors influencing the individual arsenic metabolism and excretion ability. However, very little is known about the role of single nucleotide polymorphisms (SNPs) in the genes involved in arsenic methylation of pregnant mothers and birth outcomes. Thus, we have evaluated the relationship between arsenic (+3 oxidation state) methyltransferase (AS3MT) gene variants on the metabolism of arsenic in pregnant women of the Biomarkers of Exposure to ARsenic (BEAR) cohort in Gómez Palacio, Mexico, and its effects on newborn birth outcomes. Seven SNPs in AS3MT were analyzed with respect to the concentration of inorganic arsenic in drinking water and urinary maternal concentrations of inorganic arsenic and methylated arsenic metabolites in urine of 200 women and the newborn birth outcomes using multiple linear regression. Overall 6 out of 7 SNPs of AS3MT were identified to be associated with urinary maternal concentrations of arsenic metabolites and 4 out of 7 SNPs of AS3MT to be associated with newborn birth outcomes. When stratified by newborn sex, mothers who were pregnant with males showed a more significant correlation between maternal SNPs of AS3MT and both maternal arsenic metabolite concentrations and birth outcomes. This research highlights that specific maternal metabolic profiles of arsenic depend on AS3MT polymorphisms and are influenced by fetal sex. Further studies are needed to evaluate the potential long-lasting effects of prenatal iAs exposure on children’s health.

**1973 MicroRNA Profile Changes in Immortalized Human Keratinocytes after Low Arsenic Exposure**

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Arsenic exposure is the second most common cause of skin cancer. Drinking water is the most common route of exposure to arsenic worldwide. Oral exposure as well as inhalation of inorganic arsenic can cause skin lesions and tumors in the lung, urinary bladder, and other locations. However, only 10% of the exposed population develops skin abnormalities and cancers. Arsenic induced skin cancer differs in pathology and progression to malignancy from sunlight or UV induced skin cancer. UV exposure can cause squamous cell carcinoma (SCC), basal cell carcinoma (BCC) and malignant melanoma (MM), but only SCC and BCC can develop due to arsenic exposure. The mechanism of arsenic-induced skin cancer is not yet clear, but several studies indicate that mutation is not the driving force as it is for UV-induced skin cancers. Environmentally-driven epigenetic alterations influence disease development. MicroRNAs are an acknowledged component of the epigenetic gene regulation process. Therefore, the purpose of this work was to determine the impact of short-term arsenic exposure on microRNA expression profiles in immortalized human keratinocytes (HaCaT) in order to gain insight into the early mechanisms of arsenic transformation of keratinocytes. Cultures of HaCaT cells were maintained with 0 and 100 nM arsenite for 3 weeks. Total RNA was purified using miRNA kit and microRNAs assayed using a PCR array (panel A, Invitrogen). MicroRNAs induced by arsenite were miR-182, -200c, -193a and -758 and only miR-22c was suppressed (all significant at alpha <0.05). Mir-182 induction has been associated with endometrial, colorectal, cervical and ovarian cancers. Mir-200c has been associated with breast cancer, and is induced in vitro in human breast cancer cells by short-term arsenite exposures ≥500 nM. In summary, our data provide strong evidence of epigenetic changes in human keratinocytes related to carcinogenesis upon short-term exposure to arsenite.

**1974 Relationship between mRNA Expression Level and SNPs of As3MT in Residents from Arsenic-Contaminated Areas in Vietnam**


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To understand the association of mRNA expression level of arsenic (+3 oxidation state) methyltransferase (AS3MT) and its single nucleotide polymorphisms (SNPs), we investigated local residents from arsenic (As)-contaminated areas in the Red River Delta of Vietnam. Total arsenic concentrations in the groundwater were in the range of <0.1 - 502 μg/L. Concentrations of dimethylarsinic acid (DMA), monomethylarsinic acid (MMA), and inorganic arsenic (iAs) in human urine were positively correlated with total arsenic levels in the groundwater suggesting that people in these areas may be exposed to arsenic through the groundwater. There were no significant correlations between the concentrations or the individual As compounds in urine and AS3MT mRNA expression level in blood. Analysis of 18 SNPs of AS3MT revealed that blood AS3MT mRNA levels were significantly low in residents with AS3MT genotypes including 04760CC (rs12416607), 07359AA (rs12767543), 12590CC (rs3740392), 35803TT (rs1078672), 35991AA (rs10748835) and 37853AA (rs11191459).

**1975 Yes-Associated Protein Expression in Neoplastic and Non-Neoplastic Breast Tissue of Women Living in an Area with Hydroarsenicism**

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Yes-associated protein (YAP) is a major regulator of tissue homeostasis by controlling cell proliferation, cell division and apoptosis, and once dysregulated it may contribute to a malignant cellular phenotype. YAP is crucial for oxidative stress, as well as a central regulator of responses elicited by oxidants, including arsenic.
However the role of YAP in breast cancer has not been clarified, also has not been fully explained the role that have the protein in the detoxification of arsenic. To assess the expression of YAP in neoplastic and non-neoplastic breast tissue of women living in an area with hydroarsenicism. YAP expression was assessed by immunohistochemistry in 120 breast biopsies of women living in an area with hydroarsenicism (n=44). Arsenic concentration was quantified in urine and toenails by ICP-MS/HPLC. We found a higher percentage of negativity in YAP expression of cases compared to controls (p<0.02). There was a lower intensity in protein expression in the cytoplasm of cases compared to controls (p<0.03). YAP expression showed a protective factor for breast cancer (OR=0.28, 95%CI 0.09-0.88). Protective factor was obtained when the location of YAP was positive in the cytoplasm and nucleus (OR=0.31; 95%CI 0.01-0.96). The cytoplasm high intensity of YAP was a protective factor in breast cancer (p=0.27; 95% CI 0.09-0.81). No differences were found between cases and controls in terms of total urine arsenic, its species levels and in the methylation profile. YAP expression is lower in breast cancer cases than in controls. YAP intensity in cytoplasm is lower in cases. YAP expression suggests a protective role for breast cancer. There wasn’t relationship between arsenic concentrations and YAP expression.

1976 Remodeling of Mitochondrial Network Topology Defines Myogenesis Progression: Insights from Low-Dose Arsenic Exposure
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Chronic environmental exposure to arsenic is a major worldwide public health concern that promotes a number of diseases and morbidities associated with dysfunctional muscle metabolism. Mitochondrial function, long recognized as a target of arsenic toxicity, is essential for muscle maintenance and regeneration following injuries. We recently demonstrated that low-dose arsenite restructures mitochondrial morphology in both skeletal muscle and muscle progenitor cells in vivo with accumulation of dysfunctional elongated mitochondria, alteration of bioenergetics and consequent muscle dysfunction. Despite the importance of mitochondrial architecture in differentiation, its governance of myogenic cell fate and the molecular mechanisms involved remain poorly understood. Here, we show in C2C12 myoblasts that low-dose (20 nm) arsenite stimulation of dynamin-related GTPase optic atrophy 1 (Opa1) proteolytic cleavage promotes inner mitochondrial membrane fusion activity and results in inadequate myogenic differentiation. Changes in mitochondrial topology, polarization and cardioldin content are concomitant with the mitochondrial membrane accumulation of prohibitin 2 (PHB2) and the epimedium growth factor receptor (EGFR), which is known to be activated by arsinite in a number of pathogenic processes. By favoring fusion over fission, arsinite positions bioenergetic adaptation and cell fate determination during myogenesis as competing programs. Thus, we have identified an important segment of the myogenic molecular mechanism that coordinates external environmental cues with mitochondrial intrinsic mechanism that promotes the necessary energy and metabolites for differentiation. This mechanism may explain the pathogenic shift in muscle metabolism caused by arsenic exposure. Supported by NIH grants R01ES023696.

1977 Activating Transcription Factor 4 (ATF4) Regulates Arsenic Triage-Mediated Impairment of Macrophage Immune Functions

Chronic arsenic exposure to humans is thought to be immunosuppressive as it is associated with several infectious diseases. However, the exact molecular mechanisms involved in these disrupted immune responses remain unknown. We previously showed the involvement of unfolded protein response (UPR) signaling pathway in the impairment of innate macrophage functions. Here, employing genetic approaches, we provide evidence that arsenic-mediated disruption of macrophage functions is triggered by the enhancement of activating transcription factor 4 (ATF4), a major UPR regulatory transcription factor. Arsenic treatment to ATF4+/+ mice significantly impacted on CD11b expression and phagocytic functions of macrophages (peritoneal and lung) whereas, ATF4−/− were relatively resistant in this regard. Similar observations were also noted in our in vitro studies where arsenic-impairment mediated in murine macrophage functions was rescued by genetically knocking down the expression of ATF4. Sustained activation of ATF4 transcription by arsenic resulted in the induction of apoptosis in these macrophages. Employing ATF4+/+ and ATF4−/− MEFs, we demonstrated that ATO-induced ATF4-regulated signaling was crucial in orchestrating crosstalk between endoplasmic reticulum and mitochondria. In this regard, arsenic-mediated Ca++ release from ER by activated inositol 1,4,5-trisphosphate receptor (IP3R) directed disruption of mitochondrial membrane potential (MMP) and mitochondrial ROS (mtROS) production which was regulated via augmented expression of voltage-dependent anion channel (VDAC). Pharmacological blockade of IP3R or VDAC significantly restored both MMP and mtROS. These data indicate that pathogenesis of arsenic-mediated disruptions of innate immune functions of macrophage is regulated by the UPR transcription factor, ATF4. These data also reveal that ATF4 is important in regulating ER-mitochondrial crosstalk which is key to multiple toxicological manifestations and pathogenic responses induced by arsenic.

1978 Mechanism of Arsenic Synergistic Effect on Hexavalent Chromium-Induced Metaphase Damage in Human Lung Cells

Hexavalent chromium (Cr(VI)) and inorganic arsenic are well known environmental and occupational hazards and are often found as mixed contaminants and wastes. Both of them are known human lung carcinogens. Despite that people are exposed to these metals simultaneously under most conditions, little is known about the potential co-exposure impact. Arsenic is known to inhibit DNA repair and our previous studies show that Cr(VI) induces DNA double strand breaks and neoplastic transformation in human lung cells. Thus, it is likely that co-exposure to Cr(VI) and arsenic could cause greater toxic effect. The objective of this study is to determine the ability of arsenic to increase Cr(VI) genotoxicity and carcinogenicity in human lung cells. Our data show that 0.5 uM arsenic alone induces 23 percent of relative cell death. When cells are co-exposed to 0.1, 0.2 and 0.3 ug/cm2 lead chromate, the percent of relative cell death increased from 15, 46 and 67 to 35, 74 and 92, respectively. 0.5 uM arsenic alone causes 18 chromosome aberrations out of 100 metaphases. The chromosome aberration increases from 23, 50 and 64 to 89, 89 and 107 after cells were co-exposed to 0.1, 0.2 and 0.3 ug/cm2 lead chromate for 120 h, respectively. Moreover, we found that 0.5 uM arsenic alone induces 1 percent of metaphases with cohesion defect phenotype. When co-treated with 0.1, 0.2 and 0.3 ug/cm2 lead chromate, the percent of metaphase with this phenotype increases from 18, 24 and 40 to 37, 55 and 71, respectively. Arsenic has a similar effect on solubilized Cr(VI) compound, sodium chromate, toxicity. These data suggest that co-exposure to Cr(VI) and arsenic induces an additive effect on Cr(VI)-induced cell death and synergistic effect on metaphase and chromosome damage. Current works are focusing on the studies on underlying mechanisms of the synergistic effect. This work is supported by NIEHS grants R15ES021587 (H.X.) and E016693 (J.F.W.).

1979 Low-Dose Arsenite during Fetal Development Alters Energy Metabolism and Increases Susceptibility to Cardiovascular and Fatty Liver Disease
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The role of low-dose early-life As (III) exposure in cardiometabolic disease remains unclear. We aim to examine As (III) mediated disruptions of metabolism and the development of non-alcoholic fatty liver disease (NAFLD). Mice were treated with 100ppb NaAsO2 at embryonic day 5 (IU), after weaning (PN), or from embryonic day 5 onward (IU+). All mice were fed a western diet from day 21 to the end of the study. Liver to body weight ratio in the As (III) treatment groups was elevated. Increased triglycerides and HOMA-IR indicative of insulin resistance were detected in plasma from IU+ mice. Histology of liver sections revealed steatosis in all groups, but hepatocellular ballooning was most severe in the As (III) treatment groups. Picrosirius red staining found fibrosis in As (III) treated livers. Hepatic lipids showed increased triglycerides and HU-1V+ groups and increased free fatty acids and cholesterol detected only in the IU+ livers. Metabolomic analysis of plasma detected changes in As (III) exposed animals that support an effect on energy metabolism: with effects evident in glycolysis, the TCA cycle, and lipid metabolism. Hepatic expression of lipid uptake, metabolism, and packaging genes were found to be altered with As (III) treatment. Hepatic expression of isocitrate dehydrogenase was decreased in IU and IU+ groups. In related work, an Oil Red O stained lipid uptake detection system in HepG2s showed that As (III) as low as 50ppb significantly increased lipid content. These findings demonstrate increased cardiometabolic risk factors and development of NAFLD, suggesting that low-dose As (III) exposure during development, predisposes mice to more severe disease.
states. We propose that established As (III) dysregulation of TCA cycle dehydrogenases prevent Acetyl-CoA flux leading to an accumulation of Acetyl-CoA and inability of the liver to process excess dietary lipid resulting in NALFD.

1980 Identifying N-6 Adenine-Specific DNA Methyltransferase 1 (N6AMT1) Polymorphisms Associated with Arsenic Methylation in Two Population Studies


We previously demonstrated that N-6 adenosine-specific DNA methyltransferase 1 (N6AMT1) is involved in methylation of the toxic inorganic arsenic (iAs) metabolite, monomethylarsonous acid (MMA), to the less toxic dimethylarsenic acid (DMA) in human urothelial cells. Therefore, polymorphisms in the N6AMT1 gene may play a role in susceptibility to arsenic toxicity by altering methylation of MMA to DMA. A recent study by Harari et al. found associations between five tag single nucleotide polymorphisms (tag SNPs) located within the N6AMT1 gene and the percentage of MMA in the urine of arsenic-exposed and unexposed people. Building on these findings, we identified 14 tag SNPs that provide broader coverage of the N6AMT1 gene and designed a custom multiplexed, ligation-dependent probe amplification (MLPA) assay to genotype these tag SNPs. MLPA is an inexpensive and rapid method that highly correlates with TaqMan® results (r=0.993). Urinary metabolites (%iAs, %MMA, %DMA) and N6AMT1 tag SNP genotypes were measured in an arsenic-exposed population from Cordoba, Argentina (n=141). An initial analysis identified a statistically significant association, %DMAs in urine) and F- exposure were observed (p<0.001). In the multiple linear regression analysis, the urinary %DMAs was negatively associated with the urinary F level (β = -3.22, 95%CI -5.20 - -1.24, per one increment of 1 μg F/mL in urine the urinary %DMAs reduced 3.22), considering urinary tAs level, age and sex as covariates in the adjusted model. Thus, co-exposure to F in drinking water may result in a decreased capacity to convert iAs to DMA.

1981 Inorganic and Organic Arsenic Inhibit CFTR Cl Secretion by Human Bronchial Epithelial Cells

B. C. Goodale, R. Barnaby and B. Stanton.

Arsenic in drinking water increases morbidity and mortality from infectious lung disease at high exposure concentrations, and has been associated with adverse respiratory immune responses in US populations. Inorganic arsenic (iAs) is metabolized in vivo to monomethylarsonic acid (MMA), and dimethylarsinic acid (DMA). MMA and DMA are also present in the US food supply, notably in rice and fruit juices. However, the effects of organic arsenic at levels relevant to the US population on respiratory infections are unknown. One mechanism by which arsenic may increase respiratory infections is by inhibiting mucociliary clearance of respiratory pathogens. Mucociliary clearance requires adequate airway surface liquid volume, which is regulated by the cysic fibrosis transmembrane conductance regulator (CFTR), a cyclic AMP-regulated chloride (Cl) channel. In a previous study we demonstrated that iAs reduces CFTR Cl secretion in airway epithelial cells. In this study we hypothesized that chronic exposure to organic arsenic at levels relevant to the US population decreases CFTR Cl secretion by human bronchial epithelial cells. Here we exposed human bronchial epithelial cells expressing WT-CFTR to 5-10 ppb iAs, MMA, DMA, or a mixture, and measured cAMP-induced CFTR-mediated Cl secretion. All three of the individual arsenic species and the arsenic mixture significantly decreased Cl secretion in a concentration-dependent manner. The mixture of iAs, MMA and DMA representative of arsenic species in human serum decreased Cl secretion by 83% and 52% of control at 5 and 10 ppb, respectively. This level of inhibition will reduce mucociliary clearance of respiratory pathogens. Ongoing studies are investigating whether organic arsenicals act via similar or independent mechanisms to decrease CFTR Cl secretion. These data are important for determining safe levels of arsenic in foods, which can contain high levels of organic arsenic compounds.

1982 Coexposure to Fluoride and Inorganic Arsenic in Drinking Water May Decrease the Capacity to Methylate and Detoxify Arsenic


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The concomitant exposure to inorganic arsenic (iAs) and fluoride (F) is frequent in several parts of the world; however, very little is known about the effects of the combined exposures and the metabolic interactions between these two toxicants. In humans, iAs is enzymatically methylated to methyl-As (MAs) and subsequently to dimethyl-As (DMA) metabolites that are along with residual iAs excreted mainly in urine. Previous studies have suggested that altered profiles of arsenic species in urine reflect inter-individual differences in the efficiency of iAs metabolism and may determine individual disease susceptibility. The present cross-sectional study examined associations between F exposure and iAs metabolism capacity in a cohort of adult Chihuahua residents (n=679) who drank water containing 0.05 - 419.8 μg As/L and 0.22 to 9.8 mg F/L. The sum of As species (iAs) in urine ranged from 3.8 to 417.6 ng As/μL while the concentrations of F in urine ranged from 0.06 to 17.72 μg F/μL. A strong positive correlation was found between urinary iAs and urinary F concentrations (r=0.538, p<0.001). A significant negative correlation between the arsenic methylation capacity (increased %iAs and %MAs, and reduced %DMAs in urine) and F exposure were observed (p<0.001). In the multiple linear regression analysis, the urinary %DMAs was negatively associated with the urinary F level (β = -3.22, 95%CI -5.20 - -1.24, per one increment of 1 μg F/mL in urine the urinary %DMAs reduced 3.22), considering urinary tAs level, age and sex as covariates in the adjusted model. This level of inhibition will reduce mucociliary clearance of respiratory pathogens. Ongoing studies are investigating whether organic arsenicals act via similar or independent mechanisms to decrease CFTR Cl secretion. These data are important for determining safe levels of arsenic in foods, which can contain high levels of organic arsenic compounds.

1983 N-Cadherin Upregulation and EMT Progression in As3+ and Cd2+-Transformed Urothelial Cells

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Environmental agents are common causes of bladder cancer, and specifically, As3+ and Cd2+ are known carcinogens implicated in the development of bladder cancer. Previous studies from our laboratory have shown that As3+ and Cd2+ can cause malignant transformation of normal immortalized bladder urothelial cells, which can form tumors when injected subcutaneously or intraperitoneally into nude mice. Microarray analysis of repeated metal transformation in parallel revealed that N-cadherin was the most upregulated gene in As3+ transformants, and a top induced gene in Cd2+ transformed cells. The switch from E- to N-cadherin is a well-known indicator of the epithelial-to-mesenchymal transition occurring in bladder cancer. N-cadherin upregulation is correlated with tumor stage, increased recurrence, and decreased survival in patients. While the factors mediating the decrease in E-cadherin expression are well-established, little is known of the factors regulating the increase in N-cadherin expression. The goal of the present study was to determine how As3+ and Cd2+ regulate N-cadherin expression, whether this expression is maintained in heterotransplant models, and if N-cadherin is promoting the epithelial-to-mesenchymal transition in vitro. For this purpose real-time PCR was performed on DNA samples isolated from the transplanted cell lines and the tumor heterotransplants. The expression of N-cadherin was greatly increased in the As3+ and Cd2+ transformed cell isolates, with local expression present in intraperitoneal tumor heterotransplants. Twist and were also elevated, indicating epithelial-to-mesenchymal transition. Furthermore, exposure of nontransformed UROtsa to arsenic induced N-cadherin expression, suggesting that heavy metal exposure promotes the epithelial-to-mesenchymal phenotype and bladder cancer progression.

1984 Anterior Gradient 2 Expression in MCF-10A and UROtsa Cells Exposed to Arsenite and Cadmium


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The environmental carcinogens arsenic and cadmium have been implicated in various cancers. Our laboratory has shown that arsenite and cadmium can cause malignant transformation of a breast epithelial cell line, MCF-10A and a bladder epithelial cell line, UROtsa. Previous studies have shown that the proto-oncogene, Anterior Gradient 2 (AGR2) expression promotes breast tumorigenesis in mice.
This gene is known to play a role in promoting cellular transformation, tumor growth, and metastasis in various cancers. In this study, we were interested in determining the expression level of AGR2 in arsenite and cadmium transformed MCF-10A cells and the UROtsa cells. For this purpose, real-time PCR was performed on 28C A samples isolated from the arsenite and cadmium transformed MCF-10A cells and the UROtsa cells. Western analysis was performed on protein samples isolated from the cells. The data obtained indicated that the expression of AGR2 was significantly increased in the MCF-10A cells transformed with arsenite when compared to the cadmium transformed cells. Expression of AGR2 was also determined in 6 arsenite and 7 cadmium transformed UROtsa cells using PCR and Western analysis. The results indicated that AGR2 expression was increased in some of the arsenite and cadmium transformed cells. Exposure of the parent MCF-10A cells to 4, 8, and 16 μM arsenite or 2, 4, and 6 μM cadmium for 48 hours resulted in a significant increase in the expression of AGR2. These results suggest that arsenite has the potential to induce AGR2 in MCF-10A cells, the expression of which may increase the metastatic potential of the cancer cells. In addition, our data also shows that histone acetylation and DNA methylation may play a role in regulating the expression of AGR2 in MCF10A cells.

1985 Potential Involvement of Cadmium in the Induction of the Polyol Pathway of Hyperglycemic Glucose Metabolism in Human Proximal Tubule Cells
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Diabetic nephropathy (DN) is a major cause of end-stage renal disease (ESRD), where prolonged exposure to hyperglycemia induces damage to proximal tubule cells of the kidney. Since progression to ESRD relates to pathological changes in the tubular segments of the kidney, the effects of hyperglycemia in the proximal tubule portion of the nephron may be particularly relevant to the progression of DN. Development of this disease is also likely to occur in the context of exposure to other renal toxins, and the heavy metal cadmium may be the most relevant due to the accumulation of this metal in the major cell type involved in glucose reabsorption: proximal tubule cells. Preliminary microarray analysis has shown that human proximal tubule (HPT) cells exposed acutely and chronically to cadmium have an increased expression of an aldose reductase (AR) isoform, AKR1B10. This isoform along with AKR1B1 and sorbitol dehydrogenase (SORD) are involved in glucose metabolism under hyperglycemic conditions via the polyol pathway. The goal of this study was to verify and extend these observations in culture of HPT cells. For this purpose, HPT cells were exposed to one of the three following treatments: 5,5 (control), 7.5, 11, or 16 mM glucose concentrations for 8 days, 9, 27, 45 μM cadmium for 24 hours (acute), or 4.5, 9, 27 μM cadmium for 15 days (chronic). Real-time PCR was used to measure the expression level of these enzymes. Exposures to either hyperglycemia or cadmium stimulated a significant induction of AKR1B10 in HPT cells; however, exposure to these renal toxins had no effect on AKR1B1 or SORD expression. These results are suggestive of potential synergistic effects of cadmium and hyperglycemia in the toxic responses of the proximal tubule during the development of DN.

1986 Exposure of V79 Cells to Cadmium Chloride Results in the Production of Single-Strand Breaks, Double-Strand Breaks, and Cell Cycle Changes
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Cadmium is a toxic heavy metal with many industrial and commercial uses, resulting in widespread environmental pollution. Exposure to cadmium occurs through tainted food, tobacco, and living environment. Cadmium exposure causes neurological and respiratory problems, vitamin D deficiency, and cancer. This study was designed to examine the effects of cadmium exposure on cytotoxicity, genotoxicity, and cell cycle progression in V79 Chinese hamster cells. Cell cycle progression was examined by monitoring the expression of cyclin A, cyclin B, and CDK1/CDK2 through western blot analysis. Propidium iodide staining and cell cycle analysis were used to determine cadmium’s role in cell cycle arrest. Both time- and dose-dependent trials measuring expression of cyclin A, cyclin B, and CDK1/CDK2 through western blot analysis. Propidium iodide staining and cell cycle analysis further suggested this arrest. Genotoxicity was measured through double strand break, single strand break, and micronuclei formation. The formation of double strand breaks was dose and time-dependent. The presence of single strand breaks and micronuclei similarly increased with dose. Interestingly, inhibition of cell cycle progression using nocodazole significantly reduced the production of damage. An increase in mutations at the HPRT gene following exposure to cadmium was also observed. Collectively, this study suggests that cadmium alters cell cycle progression in the G2/M phase, and damages cell DNA by inducing single strand breaks.

1987 Changes of Gene Expression in Human Proximal Tubular Cells Treated with Cadmium
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Cadmium (Cd) is an environmental toxic heavy metal that causes severe clinical symptoms in various tissues including the kidney. Although many researchers have studied the molecular mechanism of Cd toxicity using various cultured cells, the underlying mechanism of Cd toxicity remains unclear. Recent studies have shown that changes in gene expression may be involved in Cd toxicity in several cell lines. In the present study, we examined the gene expression changes in HK-2 human renal proximal tubular cells following treatment with Cd using DNA microarray analysis to provide new insight into the mechanism of Cd renal toxicity. When HK-2 cells were treated with 40 μM Cd for 24 h, the viability was decreased by about 50%; however, 3 h treatment with 40 μM Cd did not show such toxic effects. Thus, to determine the gene expression pattern in Cd-exposed HK-2 cells before the development of cytotoxicity, we conducted DNA microarray analysis in HK-2 cells treated with or without 40 μM Cd for 3 h. In this condition, the expression levels of 30 genes were increased by two-fold or more, and those of 21 genes were decreased by half or less (1). Among the 30 up-regulated genes, it was shown that 7 genes (HSPA1, HSPA1B, HSPH1, HSP90AB1, HSP90AA1, HSPD1, and HSPA8) were involved in coding for chaperon proteins. Because Cd is known to cause ER stress in kidney cells, the increased expressions of these chaperon protein-related genes might be due to Cd-induced unfolded protein response. In addition, 4 transcription-related genes (AP2B1, HOXA7, HOXA9 and TCEB2) were down-regulated by Cd, suggesting that Cd may exert its toxic effect through transcript network disruption. Therefore, our present study provides new information that can be useful to identify the target genes involved in Cd renal toxicity in human. [This work was partly supported by the Study of the Health Effects of Heavy Metals, organized by the Ministry of the Environment, Japan]. (1) Lee et al., J. Toxicol. Sci. 38, 959-962 (2013)

1988 Involvement of FOXF1 Transcription Factor in Cadmium-Induced Suppression of UBE2D4 Gene Expression
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Cadmium (Cd) is a harmful metal, and its chronic exposure mainly induces a series of damage in renal proximal tubular cells. However, the molecular mechanism of Cd renal toxicity are still unknown. We previously have demonstrated that Cd induced apoptosis in proximal tubular cells via accumulation of p53 protein in vivo as well as in vitro. Interestingly, suppression of gene expression of UBE2D2 and UBE2D4, members of ubiquitin-conjugating enzyme E2 D (UBE2D) family, is directly involved in Cd-induced accumulation of p53 in HK-2 cells human proximal tubular cells. In this study, we examined which transcription factor might regulate the expressions of genes of UBE2D family in HK-2 cells. To identify the transcription factors whose transcriptional activity were changed by Cd, we employed protein/DNA binding assay with the nuclear extracts from HK-2 cells treated with Cd or not. Among 345 transcription factors, Cd increased the DNA binding activities of 20 transcription factors, and decreased those of 28 transcription factors. Interestingly, the DNA binding sequence of FOXF1, one of the transcription factors of which Cd decreased the DNA binding activity, exists in upstream of coding region of UBE2D4, a member of UBE2D family genes. In addition, we confirmed that Cd decreased DNA binding activity of FOXF1 using Cd Shift assay. UBE2D4 gene expression was significantly decreased by knockdown of FOXF1 using the siRNA transfection. Furthermore, western blot analysis indicated that Cd decreased cellular FOXF1 protein levels. These results suggest that Cd-induced suppression of UBE2D4 gene expression may be due to the inhibition of FOXF1 transcription activity. [This work was partly supported by the Study of the Health Effects of Heavy Metals, organized by the Ministry of the Environment, Japan]
1989 Pleiotropic Roles of Calmodulin-Dependent Pathways Regulating Cadmium Toxicity in Human Osteoblast-Like Cell Lines


Environmental cadmium pollution, predominantly through the improper disposal of electronic waste, is connected to impaired human bone health. Although bone is a known target site for cadmium, the mechanism by which cadmium exposure leads to osteotoxicity is under investigated. Our lab previously reported that cadmium induces apoptosis via extracellular signal-regulated kinase (ERK) activation in Saos-2 bone-forming osteoblasts. Cadmium, a divalent ion with similar ionic radii to calcium, may activate calmodulin dependent kinase (CAMK) pathways as a substitute for intracellular calcium. We hypothesize cadmium induces osteotoxicity via ERK activation, regulated through CAMK. MG-63 and Saos-2 osteoblast-like cells were treated with 5µM CdCl2 alone or in cotreatment with one of the following three inhibitors for 24 or 48 hrs: Cadmium-dependent Phosphodiesterase (PDE) inhibitor GGS-9343β; Cadmium-dependent Kinase Kinase (CaM KK) inhibitor STO-609; or Cadmium-dependent Kinase II (CaM KKII) inhibitor KN-93. After treatment, cell viability was evaluated using an MTT assay, ERK activation by Western blot, and apoptosis using the ApoPercentage assay. Treatment with the three inhibitors resulted in inhibitor-specific response to cadmium exposure. GGS-9343β protected against cadmium-induced toxicity, STO-609 enhanced toxicity, whereas KN-93 had no detectable effect on toxicity. Further investigation demonstrated that treatment with GGS-9343β prevented cadmium-induced ERK activation, whereas STO-609 further increased it. Additionally, cadmium-induced apoptosis was partially recovered with GGS-9343β co-treatment. These findings demonstrate the pleiotropic role of CAMK pathways in cadmium osteotoxicity. Our major finding shows that cadmium-induced ERK activation leads to apoptosis via PDE activation. In contrast CaM KK protects against cadmium-induced osteotoxicity. This result helps elucidate the mechanisms in which exposure to cadmium leads to the pathogenesis of bone diseases.

1990 Comprehensive Analysis of Transcription Factors Involved in Rat Proximal Tubular Cells Exposed to Cadmium

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Cadmium (Cd) is an environmental harmful metal and its chronic exposure causes kidney injury. Although recent studies have shown that changes in gene expression may be involved in Cd renal toxicity, the molecular mechanism of Cd toxicity is not fully defined. The gene expression is regulated by transcription factors. In this study, to clarify the cellular target transcription factors affected by Cd, we examined comprehensive analysis of transcription factors involved in Cd toxicity in rat proximal tubular cells (NRK-52E cells). After treatment of NRK-52E cells with 5 µM Cd for 3 h, nuclear extracts were used for the protein/DNA binding assay. Among 65 transcription factors, Cd increased the DNA binding activities of 6 transcription factors (MTF-1, CBF, C-Myc, Smad3/4, Myc/Max, CDP) by more than 2.0-fold. Especially, activity of MTF-1 was most strongly increased to 6.9 times of control by Cd treatment. MTF-1 is known to participate in induction of metallothionein and is activated by Cd. On the other hand, Cd decreased the DNA binding activities of 15 transcription factors (Y1, Sp-1, GATA, HNF-4, EGR, AP-2, etc.) by less than 0.5-fold. Activity of Y1 was most strongly decreased to 4% of control by Cd treatment. These comprehensive findings may provide new candidates of transcription factors associated with Cd renal toxic mechanism. [This work was partly supported by the Study of the Health Effects of Heavy Metals, organized by the Ministry of the Environment, Japan]

1991 Effect of Injection Timing of Cadmium on Severity of Testicular Toxicity

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Cadmium (Cd) is a common environmental pollutant and a major constituent of tobacco smoke. Our previous work indicated that Cd-induced toxicity (mortality and hepatotoxicity) was markedly different by injection timing. Since the Cd-induced testicular toxicity is known well, we examined whether injection timing would affect the severity of Cd-induced testicular toxicity. CdCl3 (4.5 mg/kg) was injected to male C57BL/6J mice (7 weeks of age) intraperitoneally at time 14:00 or 2:00, describing as zeitgeber time (ZT); ZT6 or ZT18. These injection time showed most difference in the severity of Cd-induced mortality and hepatotoxicity1. After one week of injection, mice were sacrificed at ZT6 or ZT18 followed by separating the testes, epididymides and cauda epididymides. The right cauda epididymis was minced with scissors to release sperm in 2 ml of medium 199 containing 0.5% bovine serum albumin at 37 °C. Several parameters of sperm motility were measured by CASA (HTM-IVOS, USA). Furthermore, sperm head numbers both in right testis and cauda epididymis were measured by CASA. The body weights and the testicular organ weights did not change in mice between injection timing. Significant decrease of main sperm motility parameters were observed in the group injected at ZT6 compared to control group. However, no such decrease was observed in the ZT18-injected group. The sperm numbers in the right testis and cauda epididymides were decreased significantly only in the group administrated at ZT6 compared to both control and injected at ZT18 groups. These results suggest that Cd-induced testicular toxicity was also affected by injection timing.1 Miura et al (2013), J Toxicol Sci, 38, 947

1992 Exposure to Cadmium Causes Changes in Gene Expression In Vivo and In Vitro Rat Liver and Kidney Models

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Cadmium is a metal present in trace amounts in the human diet, but in larger doses it can be acutely toxic or cause adverse health effects in chronic, long term exposures. Workers in the metal industry, and in certain regions of the world, are at a greater risk for exposure. While the toxic effects of cadmium have been widely studied, the exact mechanisms of toxicity remain unclear. In order to further elucidate these mechanisms and to identify novel biomarkers of exposure or effect, we exposed two rat derived cell lines and rats to several sub-lethal concentrations of cadmium chloride and examined changes in gene expression using microarrays. Since cadmium accumulates in the liver and kidney, we chose liver and kidney derived cell lines, and collected the liver and kidney from exposed rats. Enriched pathways, networks, and biological functions were determined using a variety of bioinformatic tools. We identified common and unique differentially expressed genes and biological processes among the model systems. Many of the observed changes may be due to an increase in oxidative stress and protein misfolding. Differentially expressed genes are involved in biological processes such as energy metabolism, response to oxidative stress and protein degradation pathways. We also observed an increase in metal accumulation in the liver and kidney, histopathological evidence of liver damage and changes in clinical chemistry measurements. This study provides further insight into the mechanism of cadmium toxicity and provides a basis for candidate biomarkers. Using multiple systems may also provide a basis for extrapolation to human populations. Research was conducted in compliance with the Animal Welfare Act, and all other Federal requirements. The views expressed are those of the authors and do not constitute endorsement by the U.S. Army. This project was supported in part by an appointment to the Research Participation Program for the USAMRMC administered by ORISE through an agreement between the US DOE and USAMRMC.

1993 Role of Metallothionein in Modifying the Toxicological Response to Cadmium in Humans

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Background: Metallothionein (MT) is important for transport of cadmium, Cd, (CAS No 7440-43-9) in the body and for protection against cellular toxicity, as demonstrated in animals and in vitro (1). Such roles may exist also in humans, but direct evidence has not previously been available. The present studies in humans examined the influence on Cd-induced kidney dysfunction by 1) MT gene expression and 2) MT mRNA levels, and biological processes among the model systems. Many of the observed changes may be due to an increase in oxidative stress and protein misfolding. Differentially expressed genes are involved in biological processes such as energy metabolism, response to oxidative stress and protein degradation pathways. We also observed an increase in metal accumulation in the liver and kidney, histopathological evidence of liver damage and changes in clinical chemistry measurements. This study provides further insight into the mechanism of cadmium toxicity and provides a basis for candidate biomarkers. Using multiple systems may also provide a basis for extrapolation to human populations. Research was conducted in compliance with the Animal Welfare Act, and all other Federal requirements. The views expressed are those of the authors and do not constitute endorsement by the U.S. Army. This project was supported in part by an appointment to the Research Participation Program for the USAMRMC administered by ORISE through an agreement between the US DOE and USAMRMC.
in humans. Also, increased levels of MTab increase the likelihood for Cd induced kidney dysfunction. Taken together these findings support the notion that MT serves important protective functions in humans. Reference. Nordberg G F et al: Handbook on the Toxicology of Metals 4th Ed, Academic Press, 2015.

1994 Cadmium Effect on Lipids Accumulation in Mouse Primary Culture

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Cadmium (Cd) is highly toxic metal. It is known that Cd favors the lipid deposit in hepatocytes. The hepatocyte is a not a lipid-storage cell. Nevertheless the mechanism underlying the hepatocyte lipidic accumulation remains elusive. Aim: to evaluate the lipogenesis role in hepatocyte primary culture that are expose to Cd and its relation with oxidative stress. Hepatocyte primary culture was perform by using the double perfusion protocol, C57Bl/6 mice were feed with a balanced diet, special for rodents for 48h; the hepatocytes obtained were treated or not with different concentrations of Cd for different times. Lipidic content was determined by using the Red Oil staining. Cellular viability was assay by using CCK8®, ROS were measured by spectrophotometry, and oxidized proteins were analyzed through Oxyblot®. Antioxidant and lipogenic enzymes were assessed by Western blot. Our data shows that Cd significantly decreases cellular viability dependently of concentration and/or time of exposure. We also found that ROS generation and protein oxidation increases in the hepatocytes after the Cd exposure. Coincidently antioxidant enzyme as the SOD-1, GPx, GCS and the HSP70 were increased. A time exposure dependent- lipid content increment was also reported, result that correlates with the overexpression of the SREBP1c and the lipogenic ACC. Finally our data shows that a pre-treatment with Trollox, an antioxidant, along with Cd exposure decreases the lipid accumulation. Together these data suggest that Cd promotes a cellular oxidant state through ROS overproduction and by increasing lipogenic-related enzyme expression. Hepatocyte lipid accumulation may be associated to oxidant stress being a key factor for the Cd-induced damage. Molecular studies of the pathways that are involved in cellular damage induced by Cd are primordial to identify molecular targets that could be used therapeutically against the toxicity generated by this metal. Conacyt 166042, INFR-2013-01-205941.

1995 Environmental Metabolomics of Low-Dose Cadmium (Cd) in Nonalcoholic Fatty Liver Disease

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Non-alcoholic fatty liver disease is common in the US and a recent epidemiologic study showed association of Cd with fatty liver disease. Our previous studies showed that environmental low-dose Cd oxidized protein redox states and stimulated inflammatory signaling and actin cytoskeleton disruption. However, little is known about the systemic effects of low-dose Cd exposure on liver function and associated metabolic pathways. In the current study, we investigated effects of low-level of Cd on mouse liver and plasma, analyzing markers associated with fatty liver and plasma metabolome, respectively. Livers and plasma were collected from mice exposed to Cd (10 mg/L) by drinking water or control for 16-weeks. Results show that Cd contents in livers were substantially higher (10-fold) in mice exposed to Cd than CR mice. Oil-Red-O staining of liver sections showed that increased Cd content in liver was associated with elevated amounts of lipids. Plasma levels of alanine transaminase and aspartate transaminase were elevated in mice exposed to Cd suggesting that low-dose Cd exposure resulted in liver damage. To evaluate the systemic effects of environmental levels of Cd exposure, high-performance metabolomics with liquid chromatography-high-resolution mass spectrometry was performed. From the total of 13,653 metabolites detected, 337 were affected by Cd (p < 0.05), including 29 at FDR < 0.2. Of these, 17 metabolites were decreased and 12 were increased. Cd-affected metabolic networks that are associated with these 29 metabolites include prostaglandin and phospholipid metabolism, and metabolism for lipid biosynthesis and inflammatory response. Together, the results show that this relatively low dose of Cd changes liver function consistent with Cd-dependent fatty liver and subsequent disease.

1996 The Human Exposome: Environmental Metabolomics of Low-Dose Cadmium (Cd)

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Federal guidelines and hygiene programs have largely eliminated disease burden from occupational exposure to Cd, with a decline in cigarette use further resulting in decreased Cd burden. However, surveillance data show low-dose environmental Cd exposure in humans continues from numerous sources. Recent epidemiologic studies using NHANES data found Cd to be associated with fatty liver disease and liver-related death after controlling for all known confounders. As part of the Emory-Georgia Tech HERCULES Center for exposome research, we investigated the in vivo toxidynamics of environmental Cd in humans, with urinary Cd/creatinine selected as an indicator of body burden. Plasma samples from 248 men (67 ± 7.6 y) were profiled using high performance metabolomics (liquid chromatography-high-resolution mass spectrometry), which detected 16,482 m/z features from chemicals in the plasma. A mean urinary Cd concentration of 0.66 ± 0.59 μg/g creatinine was observed, with four individuals having elevated Cd high enough to expect some degree of renal impairment. Each metabolite was tested for correlation with urinary Cd to produce a metabolome-wide association study (MWAS) of Cd. MWAS showed 901 features with raw p < 0.05; however, none were significant after false discovery rate (FDR) correction. Comparison of the top and bottom Cd quintiles revealed 39 metabolites, including lipids and mitochondria-related metabolites, associated with body burden at FDR<0.2. The results indicate that environmental Cd affects multiple biologic functions, many with small size of effect. These include intermediary metabolism, cell proliferation, mitochondrial function and cell death. Such diverse toxidynamic effects of low-dose Cd levels are consistent with the known pleiotropy of Cd toxicity, which affects liver, bone, lung, liver, kidney and other organ systems.

1997 Cadmium Accumulates within Pancreatic Islets at Levels Similar to the Renal Cortex in an Experimental Model of Long-Term Exposure

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Diabetes is a growing worldwide epidemic. Impaired insulin release is a hallmark of type I diabetes and is key in the progression of type II diabetes. Multiple epidemiological and experimental studies show that exposure to the metal cadmium (Cd), is associated with diabetes and reduced serum insulin. However, Cd is considered a classic nephrotoxicant that accumulates in the renal cortex at levels 8 to 20 times higher than any other tissue. The objective of the current study was to quantify and compare the amount of Cd in the renal cortex to that of pancreatic islets. To do this, male Sprague Dawley rats were injected subcutaneously with either saline (control) or Cd (0.6 mg Cd/kg/day, 5 days per week). After 6 or 12 weeks of Cd treatment, kidney cortex tissue and pancreatic tissue was removed that underwent an islet isolation process where islets were isolated and maintained in culture conditions overnight. 18 hours post isolation, islets were either exposed to low (0.5 mg/ml) or high (3.0 mg/ml) glucose for 4 hours. At the end of the incubation, islets were rinsed in saline and lysed with TPER reagent then stored at -80 for later protein, Cd and Zn determination. Kidney tissue from 12 week treated animals had the highest Cd content at 11,007 ± 777 (nmol/g or protein ± SE); in pancreatic islets incubated in 0.5 mg/ml glucose the average Cd concentration was 7,305.2 ± 403. Surprisingly, at the same 12 weeks of Cd treatment there was significantly less Cd content in islets incubated at 3.0 mg/ml glucose, 5,039 ± 515. Zn levels were approximately 4 fold higher in islets than in the renal cortex; similar to Cd, there was less Zn present in islets incubated in 3.0 mg/ml compared to 0.5 mg/ml glucose. These preliminary results show that Cd accumulates within pancreatic islets to levels approaching the renal cortex. Indicating that pancreatic islets are potential targets of Cd toxicity and are likely involved in etiology of Cd-induced hyperglycemia.
Cadmium (Cd) is an ubiquitous transition metal that enters the human body by ingestion and inhalation and is poorly eliminated with a decades-long half-life. Epidemiologic data link Cd to life-threatening diseases of multiple organ systems, yet the mechanism(s) of environmental Cd exposure in the respective etiologies remains poorly understood, in part because many toxicological studies of Cd use doses above the normal exposure range. In addition, potential roles of Cd in pulmonary toxicity are less well defined than in other organs. To identify pulmonary effects of typical Cd exposure, 6-week-old male C57Bl6 mice were placed on 10 mg/L (55 μM) CdCl2 in drinking water or vehicle (control, CR) for 16 weeks. Cd-exposed mice had increased lung Cd compared to CR and exhibited increased airway hyperresponsiveness to methacholine. Transcriptional analysis was performed on lung tissue mRNA (MoGene ST 2.0, Affymetrix). We identified 393 significant differential genes from Cd exposure at α=0.05. Of these, 56% represented a decrease in gene expression. Using Gene Set Enrichment Analysis (Broad Institute), we identified 100 pathways with family-wise error rate (FWER) < 0.05. Significant pathways inhibited by Cd exposure included mitochondrial energetics, muscle and cytoskeletal structure, ribonucleotide stability, membrane trafficking, cell cycle regulation and Wnt signaling. These data show environmentally relevant levels of Cd exposure could have wide-reaching effects on the development or exacerbation of pulmonary diseases.

Methylmercury is an environmental pollutant that is known as the causative agent of Minamata disease. However, the molecular mechanism involving methylmercury toxicity has not yet been elucidated. We have previously found that EGO complex, consisting of Gsα, Gt2α, and MeHg, is involved in the reduction of methylmercury toxicity in yeast. The EGO complex localizes to the vacuolar membrane. However, the role of the EGO complex in the reduction of methylmercury toxicity has not been studied. In this study, we have investigated the relationship between methylmercury toxicity and the EGO complex in yeast. We found that methylmercury reduced the level of EGO3 that binds to the MeH1, and the level of EGO3 that was dissociated from MeH1 was subsequently decreased. Moreover, methylmercury accelerates the turnover rate of Ego3 in the presence of protein synthesis inhibitor. These results suggest that methylmercury may promote the dissociation of Ego3 from MeH1 and then Ego3 is rapidly degraded. Since the EGO complex acts as an autophagy inhibitory factor on the vacuolar membrane, we next examined the effect of methylmercury on the vacuolar function. Methylmercury induced abnormal vacuolar morphology and the abnormalities were further increased by the loss of EGO complex. On the other hand, the addition of 3-methyladenine, which is a inhibitor of autophagy, decreased the frequency of abnormal vacuolar morphology induced by methylmercury and also reduced the methylmercury toxicity in yeast. Moreover, the high-sensitivity to methylmercury by the loss of EGO complex in yeast was hardly observed in the presence of 3-methyladenine. We conclude that methylmercury might exhibit cytotoxicity by causing the dysfunction of the vacuole through the induction of excessive autophagy with the inhibition of EGO complex formation.

Mercury was demonstrated histochemically in macrophages throughout the brain of Minamata disease patients. Brain inflammation from macrophages is a double-edged sword such as the control of regenerative response or cause of additional toxic effects on neuronal cells. However, the molecular mechanisms of their response to non-cytotoxic concentration of methylmercury (MeHg) exposure remain to be elucidated. We investigated how MeHg affects the expression of IL-6 and IL-8 in cultured human U937 macrophages. Compared with controls, IL-6 and IL-8 mRNA expression in MeHg-exposed U937 macrophages was maximal at 6 h and declined after 24 h. ELISA of the supernatants indicated enhanced secretion of IL-6 in MeHg-exposed U937 macrophages at 6 h. In contrast, IL-8 secretion was maximal at 24 h in control macrophages but was significantly reduced at 6 h in MeHg-exposed U937 macrophages. We examined whether the up-regulation of IL-6 and IL-8 in MeHg-exposed U937 macrophages was due to the antioxidant response element. The antioxidant response element binding protein 1 (Nrf2) expression was increased in MeHg-exposed U937 macrophages but was significantly reduced at 6 h in MeHg-exposed U937 macrophages. We concluded that ethanol alleviates MeHg-induced inflammation in vitro through up-regulation of Nrf2 expression.
functional activation of NF-κB after exposure to 10 μM MeHg for 6 h. These results indicate an early inflammatory response of macrophages to MeHg including the activation of IL-6 and IL-8 expression that may lead to macrophage recruitment in MeHg-exposed tissues.

2003 In Vitro Studies to Characterize the Bioaccessibility and Bioavailability of Methylmercury from Seafood

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Current risk assessment for mercury ingested during seafood consumption assumes that 100% of ingested methylmercury is absorbed into the body. However, this assumption may be overly conservative and may preclude some of the health benefits of fish. Emerging evidence suggests that the human gut bioaccessibility of methylmercury from fish is in some cases significantly less than 100%. Briefly, we digested samples of the 10 most commonly consumed seafood in North America in a static in vitro digestion model incorporating gastric, small intestinal, and colonic digestion phases. Bioaccessible mercury was measured in the soluble fraction resulting from these digestions. Soluble fraction was also added to a Caco-2 Transwell assay to assess bioavailability. Our data thus far from gastric and small intestinal digestions suggest that certain fish have mercury bioaccessibility lower than 100%; canned white tuna (75%), canned light tuna (65%), fresh tuna (97%), cod (99%), crab (77%), and halibut (89%). These results show that mercury bioaccessibility for seafood commonly consumed in North America is lower than 100%. Given that 100% of ingested methylmercury is assumed to be absorbed into the body, future risk assessments need to better consider that variations in bioaccessibility exist.

2004 Conversion Factors to Estimate Oral NOAEL Values from LD50 Values: Implications for Medical Device Toxicology

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The derivation of noncancer Tolerable Intake (TI) values for compounds released from polymeric materials typically involves the use of NOAEL or LOAEL values from long-term, repeat-dose toxicity studies. However, NOAEL or LOAEL values from well-conducted toxicity studies are not available for many compounds released from materials used to manufacture medical devices. In the absence of necessary NOAEL or LOAEL values, acute toxicity (LD50) values can be used to derive provisional TI values, providing that an appropriately conservative factor is used to convert the acute LD50 to a chronic NOAEL value. Earlier efforts to derive a factor to estimate a NOAEL from LD50 values were based on relatively small data sets. In this study, we derived conversion factors to conservatively estimate oral NOAEL values from LD50 values using a relatively large (n=498) data set as well as a reduced data set (n=263) that does not contain compounds used as pesticides or drugs. LD50 values were identified in the NLM ChemID Plus or HSDB databases for compounds with NOAEL values in the Munro et al. data set (Food Chem Toxicol 34: 829-867, 1996). Statistical distributions of LD50/NOAEL ratios were obtained and the 50th, 75th, 90th, and 95th percentile values of the distributions were identified for the full and reduced data sets. Conversion factors based on the 95th percentile of the distribution of LD50/NOAEL ratios were 533 for the reduced data set and 2048 for the full data set. Use of these upper-bound conversion factors was able to conservatively estimate the NOAEL values from their corresponding LD50 values for compounds in an independent validation data set (n=8). Utilization of this approach provides a method to conservatively estimate NOAEL values from corresponding LD50 values in the absence of data from long-term, repeat-dose toxicity studies and consequently provides a practical means to assess the potential systemic toxicity of many compounds that lack experimentally derived NOAEL or LOAEL values.

2005 Computational Toxicological Evaluation of DESMA: Challenges in Evaluating a Complex Reaction Product Mixture Used in Dental Devices

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Computational toxicology is an increasingly recommended tool in the biological evaluation of medical devices and their chemical components; however, detailed examples of its application are lacking, DESMA (CASRN 1101874-33-2) is a complex polyurethane methacrylate mixture used as a primary monomer in temporary dental crowns and bridges. DESMA is useful for evaluation of computational tools because ISO 10993-compliant toxicity test data exist for the key endpoints of sensitization and mutagenicity for comparison with computational predictions, and DESMA contains unique structures which may not be well represented in computational toxicology programs. This case study evaluated dermal sensitization and in vitro mutagenicity potential using three approaches: 1) Structural Alerts, 2) (Q)SAR predictions, and 3) Read-across, with a weight of evidence approach used to evaluate the predictions. The reaction intermediates, multiple reaction products, and predicted metabolites were evaluated. For both endpoints, identified structural alerts were not consistently supported by the (Q)SAR predictions, read-across or the available toxicity test data. The (Q)SAR programs did not provide predictions for all compounds due to the structures being outside of the applicability domain of the models, but generally the (Q)SAR predictions for mutagenicity were consistent with the available test data. Although the read-across approach struggled to find close structural analogs for many of the compounds, generally read-across did agree with the available toxicity data, and was more useful for mutagenicity than sensitization. The lack of consistency among tools emphasizes the need to incorporate multiple approaches and highlights the challenges with evaluating a chemically complex mixture like DESMA. Overall, this case study shows that the process could aid preliminary risk assessment of medical device ingredients when used and interpreted by experts with an understanding of the applicability and limitations of the approaches.

2006 Toxicology-Based Cancer Causation Analysis of CoCr-Containing Hip Implants: A Quantitative Assessment of Genotoxicity and Tumorigenicity Studies

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In this paper, quantitative methods were used to evaluate the weight of evidence regarding a causative relationship between cobalt-chromium (CoCr)-containing hip implants and increased cancer risk. We reviewed approximately 80 published papers and identified 629 adverse-effect level (NOAEL) and/or lowest-observed-adverse-effect level (LOAEL) values for specific endpoints of interest: genotoxic effects from in vitro studies with human cell lines as well as genotoxicity and tumor formation in animal biosays. Test articles included Co particles and ions, Cr particles and ions, and CoCr alloy particles and ions. The NOAEL/LOAEL values were compared with body burdens of CoCr particles and ions we calculated to exist in systemic tissues of hip implant patients under normal and excessive wear conditions. We found that approximately 40 tumor biosays have been conducted with CoCr alloy implants or CoCr particles and ions at levels hundreds to thousands of times higher than those present in hip implant patients, and none reported a statistically significant increased incidence of systemic tumors. Results from in vitro and in vivo genotoxicity assays, which are relatively less informative owing to false positives and other factors, also indicated that DNA effects would be highly unlikely to occur as a result of wear debris from a CoCr implant. Hence, the toxicological weight of evidence suggests that CoCr-containing hip implants are unlikely to be associated with an increased risk of systemic cancers, which is consistent with published and ongoing cancer epidemiology studies involving patients with CoCr hip implants.

2007 Risk Assessment of Cyclic Siloxanes (D3 to D19) As Extractables from Polymeric Components in Biopharmaceutical Manufacturing


Silicone tubing and polypropylene (PP) connectors are commonly used as polymeric components in the manufacturing of biopharmaceuticals. Because they come into direct or indirect contact with the drug product, it is important to assess the risk of the extractable and leachable (E&L) impurities to patient safety. This presentation will use cyclic siloxanes from two case studies to illustrate the risk assessment approach on structurally related compounds. Platinum-cured silicone tubing and PP connect coupling were certified to meet USP Class VI requirements were studied under experimental conditions using model solvents. The extracts were analyzed using GC/MS, LC/MS, HPLC/UV and ICP/MS. Low molecular weight cyclic siloxanes (D3 and D4) were detected in extracts from silicone tubing at levels < 3 μg/cm² (combined). In contrast, higher molecular weight cyclic siloxanes (D6 to D8) were detected in extracts from PP connectors at levels < 4 μg/mg (combined). In animal studies, D3 and D4 showed greater systemic toxicity relative to D6-D8, consistent with their different ADME profiles. Based on an exhaustive literature review, 2 categories were developed using OECD (2007, 2009), ECHA (2008) and ECETOC (2012) criteria. The first category included D3 and D4 and
of this case study support the use of the MCR approach for evaluation of potential mixture extracts and TTC evaluation of single extractable substances. The results for all experimental products fell into risk management Group II (low toxicological simplicity, extractable substances were assumed to have a similar mode of action; data for aqueous 5% ethanol extracts generated according to ISO 10993-12. For evaluation by repeated-dose animal testing and the threshold of Toxicological Concern (TTC) procedure used to assess thrombogenicity of direct blood contacting medical devices. The study purpose was to develop methods for an acute performance testing and subsequent thrombus assessment of a prototype peripheral device in the porcine model, in order to evaluate replacement initiatives for the standard thrombogenicity test method in a relevant model. One swine was enrolled in this study. Upon initiation of the performance study (Phase one), ACT was maintained between 200-350 using IV heparin. Following completion of the acute performance evaluation, IV heparin was discontinued. ACT was checked until it returned to normal levels to confirm heparin washout. In Phase 2 components of the test and control devices placed in the L and R Femoral veins and the L and R Jugular veins were anchored in situ and exposed to blood for 1 hour. ACT was checked at 0, 30 and 60 m during the test. The animal was euthanized 60 min after discontinuation of heparin, and veins with the devices in situ were removed. The venous wall of each sample was cut longitudinally, and the luminal surface of the vessel and device were scored for adherent thrombus similar to the scoring scheme used in the NAVI model (2013 FDA Guidance). Thrombogenicity scores were 0 for both components of the control article. The BSC prototype had scores of 0 for one component, and 1 for a second component. A film-like coating was observed over approximately 5% of the external surface of the coated component. Both the test and control devices were non-thrombogenic. The study method used an accepted model for peripheral device evaluation. It allowed initial device acute performance testing, with subsequent thrombus assessment after heparin washout, in the same animal. Future methods development will focus on device location, relevant blood exposure time, and monitoring blood components prior to and after heparin administration.

The 4hr canine non-anticoagulated venous implant (NAVI) model is a standard test procedure used to assess thrombogenicity of direct blood contacting medical devices. The study purpose was to develop methods for an acute performance testing and subsequent thrombus assessment of a prototype peripheral device in the porcine model, in order to evaluate replacement initiatives for the standard thrombogenicity test method in a relevant model. One swine was enrolled in this study. Upon initiation of the performance study (Phase one), ACT was maintained between 200-350 using IV heparin. Following completion of the acute performance evaluation, IV heparin was discontinued. ACT was checked until it returned to normal levels to confirm heparin washout. In Phase 2 components of the test and control devices placed in the L and R Femoral veins and the L and R Jugular veins were anchored in situ and exposed to blood for 1 hour. ACT was checked at 0, 30 and 60 m during the test. The animal was euthanized 60 min after discontinuation of heparin, and veins with the devices in situ were removed. The venous wall of each sample was cut longitudinally, and the luminal surface of the vessel and device were scored for adherent thrombus similar to the scoring scheme used in the NAVI model (2013 FDA Guidance). Thrombogenicity scores were 0 for both components of the control article. The BSC prototype had scores of 0 for one component, and 1 for a second component. A film-like coating was observed over approximately 5% of the external surface of the coated component. Both the test and control devices were non-thrombogenic. The study method used an accepted model for peripheral device evaluation. It allowed initial device acute performance testing, with subsequent thrombus assessment after heparin washout, in the same animal. Future methods development will focus on device location, relevant blood exposure time, and monitoring blood components prior to and after heparin administration.

Recent efforts to redesign an endoscopic guidewire revealed a cadmium-containing pigment that may pose a toxicological risk to patients. Cadmium zinc sulfide yellow, was formulated in a color paste which is added to a PTFE slurry. The mixture was then heat extruded to form a jacket that is used to encapsulate a metal alloy core wire. During clinical use, a guidewire is threaded through an endoscope and makes limited contact with mucosal membranes in the GI tract. Cadmium and cadmium compounds are known human carcinogens and can cause chronic renal tubular disease. An evaluation of the bioavailability of the cadmium in the guidewire was conducted by immersing finished guidewire samples in purified water under conditions of either 37°C for 1 hour (similar to clinical use) or 70°C for 24 hours (exaggerated extraction). The extracts were then analyzed for cadmium using ICP-MS. No cadmium concentrations above the quantitation limit (10 ng/mL or <0.1 µg/ device) could be detected from the extracts under the conditions of 37°C/1 h. Low levels of cadmium (88.6 ng/ml or 1.2 µg/device) were detected only in one sample extract under the exaggerated conditions of 70°C/24 h. Exposure estimate was calculated to be 0.01 µg/device based on known cadmium bioavailability data (0.0002%) from the pigment (ECHA). The exposure estimates are well below the average dietary intake (15-25 µg/day), drinking water acceptable level (10-80 µg/day)/EPA and oral chronic Minimal Risk Level (7 µg/day) (ATSDR). It was concluded that cadmium release would not be toxicologically significant and the presence of cadmium zinc sulfide yellow pigment would not pose any carcinogenic or non-carcinogenic health risks under the intended clinical use of the guidewire.

Curable dental materials, like other medical devices, have the potential to release low levels of multiple chemicals after placement in the body, resulting in patient exposure to a mixture of substances. Advances in risk assessment methodology for mixtures have generated new tools for evaluation of such exposures. Here we report the results of a case study to evaluate the application of the Maximum Cumulative Ratio (MCR) component of the Cefic Mixtures Ad hoc Team decision tree to the results of a case study to evaluate the application of the Maximum Cumulative Ratio (MCR) component of the Cefic Mixtures Ad hoc Team decision tree to the assessment of non-cancer health risk from co-exposure to multiple dental device extractable substances. The MCR is a tool for investigating the magnitude of the toxicity missed if a cumulative risk assessment is not performed and a single chemical approach is used. It can be calculated based on the hazard quotients of the individual substances and the cumulative hazard index, as described in a recent publication by Price et al. (DOI: 10.1080/19440049.2013.865145). Co-exposures are assigned to different risk management groups (I, II, IIIa, and IIIB) based on the calculated values for the hazard index and the MCR. For this work, the MCR approach was applied to extracts of nine experimental dental products previously evaluated by repeated-dose animal testing and the threshold of Toxicological Concern (TTC) approach. Exposure to extractable substances was estimated using analytical data for aqueous 5% ethanol extracts generated according to ISO 10993-12. For simplicity, extractable substances were assumed to have a similar mode of action; thus, the calculations represent a worst-case scenario. The predicted co-exposures for all experimental products fell into risk management Group II (low toxicological concern). This outcome is consistent with the results of other approaches for evaluating the extractable substances, including animal testing of product whole mixture extracts and TTC evaluation of single extractable substances. The results of this study support the use of the MCR approach for evaluation of potential interactions among substances released from curable dental materials.

The non-anticoagulated venous implant (NAVI) model for ISO 10993-4 in vivo thrombogenicity testing is often used to meet regulatory requirements for new, blood-contacting medical devices. The NAVI model is designed to evaluate the thrombogenic potential of a medical device in comparison to an approved, commercialized predicate device. The intent of the assay is to predict the potential for blood contacting devices to elicit a thrombogenic response upon implantation or deployment in the circulatory system. As medical devices continue to become more complex in both function and composition, the clinical relevance and predictability of this standard assay has come into question. Bind deployment in canine veins can result in unexpected “false-positive” adverse outcomes. Relative vessel size, venous vs. arterial deployment, blood flow and vessel wall contact vs. deployed device geometry should be considered prior to initiating in-vivo thrombogenicity assessments. The aim of this study was to identify specific interventional and surgical techniques that may be used to evaluate the in situ characteristics of a device and thereby assist in generating a more clinically relevant assessment of its true thrombogenic potential. In our studies, control devices (PICC or ablation catheters) were used to demonstrate critical deficiencies in techniques typically used in standard NAVI studies. Using fluoroscopy with contrast administration allows deployment site previewing and deployment monitoring, observation of device contact with the vessel walls and venous valves and a general view of overall blood flow. In addition, expanding species selection from dog to pig or sheep to better accommodate larger devices or allow surgical deployments to larger vascular structures (atria) may also overcome substantial obstacles for some device designs. Understanding the impact of these otherwise poorly controlled variables on the outcome of the acute assessments is key to interpreting unexpected results from the standard models.
ISO 10993-4 thrombogenicity testing is widely used for meeting regulatory re-
requirements for approval of new blood-contacting medical devices. Although in vivo
models such as the non-anticoagulated venous implant (NAVI) model are often
part of testing and submission packages, there is a need for faster and less costly
assays including in vitro screening models. This study describes an in vitro assay
which integrates freshly harvested ovine blood, minimal heparinization with shear
stress conditions induced by blood flow. Fresh ovine blood was collected from a
healthy donor and heparin added to 1 U per ml of blood. Blood was introduced
into the loop (total volume ~125 ml) no more than 25 minutes after the comple-
tion of the draw. Activated clotting time (ACT) of the blood was measured and
monitored throughout the procedure. Air was removed from the closed, sealed loop
and the blood was circulated at 37°C using a peristaltic pump. After equilibration
with flow, up to 2 positive or negative controls (polyurethane medical grade tubing, 10-20 cm in length) simulating tubular devices were inserted into access sites in the
loop. Up to four loops were prepared simultaneously for testing with positive or
negative control material. After 4 hours, the loop sections containing the tubing
segments were opened, photographed in situ and evaluated for the presence of
thrombus in accordance with standard in vivo thrombogenicity scoring for acutely
implanted devices. Scoring was based on a scale of 0-5, where: 0=no thrombus
visible and 5=severe, with thrombus covering >75% of material area. Typical scores
using standardized positive and negative control materials were reproducible over
several months of testing ranging from 0-2 for the negative control and 3-5 for the
positive control. This in vitro blood-loop model using characterized positive and
negative controls allows prediction of a materials’ in vivo thrombogenicity, can substantially de-risk the materials or coating selection process and can lead to successful in vivo testing outcomes.

### 2012 Development of a Minimally Heparinized Circulating Ovine Blood-Loop Model for In Vitro Thrombogenicity Testing of Medical Devices

M. E. Smith, S. Deline, S. Howard and K. Grove. American Preclinical Services, Minneapolis, MN.

The purpose of this test method is to determine if medical materials exposed to
human whole blood will reduce the platelet and leukocyte counts. Surface-induced
platelet and leukocyte activation, adherence and subsequent depletion from blood
are events predictive of potential thrombus formation on or associated with a sur-
face. A significant decrease of either of these cell types from whole blood when
compared to a control non-thrombogenic material implies a potential for an in vitro
thrombogenic response. Human blood was qualified prior to use according to
ASTM F2888-13 procedures, to ensure that the platelet and leukocyte counts fell
within a normal range. Whole blood was added to the test or control articles at a
ratio of 1 mL of blood/12 cm2 material. The samples were incubated at 37°C for
1 hour with continuous agitation. The blood samples were then introduced into a
Hematology analyzer and platelet and leukocyte counts obtained. Negative control
materials consist of coupons of medical-grade high-density polyethylene (Hatazo
or USP HDPE). Several versions of positive control material were evaluated for
reproducibility including black rubber and latex. The optimum positive control
identification was composed of glass microscope coverslip material coated with dilute
solutions of bovine thrombin and then air dried. Typical results yield a 0-10 %
reduction in platelet and leukocyte counts in negative controls while the positive
control material yielded a 70-85% reduction. Using a range of donors, platelet and
leukocyte count reduction was observed over 3 months with a mean of 84% and
range of from 91.3% to 74.4% for platelets and a mean of 70.2% with a range of
73.2% to 67.7% for leukocytes. This methodology will contribute to a more repro-
ducible assay for the standard method. The introduction of a dependable source
of a positive control material will enhance the value of the assay by improving the
uniformity of response across blood preparations and donors.

### 2013 Minimizing Systemic Toxicity via Catheter-Directed
Thrombolytic Drug Delivery in a Porcine Model of Deep
Vein Thrombosis


Purpose: Deep vein thrombosis (DVT) has been known to cause significant mor-
Bidity and potentially fatal conditions in the general population. Due to a vast
number of predisposing factors that can lead to DVT, extensive research is war-
anted to determine the optimal therapeutic regimen to prevent post thrombotic
syndrome (PTS). The continual development of therapeutics underscores the need
for a clinically robust animal model of DVT, where local delivery of thrombolytic
drugs can be evaluated. It is anticipated undesirable systemic toxicity can be avoided
while maintaining therapeutic tissue concentrations using this approach. Methods:
In this study, a reproducible method of inducing deep vein thrombosis was estab-
lished by intravascular balloon inflation in the porcine iliac vein. Confirmation of
DVT was determined by visually detecting flow stasis and thrombi formation via
angiography at 4 hours, 24 hours, and 7 days. During patency of thrombi, PTS was
objectively measured using the Villalta scoring system to assess severity of clinical
features. At the aforementioned time points, catheter-directed thrombolysis (CDT)
using a tissue plasminogen activator (Alteplase) was delivered to the thrombus and
evaluated for thrombolytic efficacy. Upon confirmation of thrombus dissolution
via CDT, the animals were survived for an additional one week with diagnostics
(clinical/neurologic exams, angiography, and necropsy) performed to detect any
migratory emboli that may have impacted overall health. Vessels and vital organs
were collected for histopathology analysis. Results: CDT as well as thrombolytic
activity was detected in all groups with restoration of venous flow confirmed.
Conclusions: As our study indicated, the formation of a thrombus in the iliac vein
has proven to be effective as a preclinical model for evaluating CDT as a treatment
option for DVT. Further evidence is required to establish long-term benefits of
CDT in minimizing systemic and local toxicity as well as determining the optimal
frequency and dosage of thrombolytic drugs in preventing PTS.

### 2014 Development of a Standard Test Method and Positive
Control Materials for the Platelet Leukocyte Count: An
In Vitro Assay for Hemocompatibility Assessment of Blood
Contacting Medical Devices

J. Pomson, M. E. Smith, S. Deline, K. Grove and S. Howard. American Preclinical Services, Minneapolis, MN.

In accordance with ISO 10993-10, the guinea pig maximization test (GPMT) or
murine local lymph node assay (LLNA) are used to assess dermal sensitization for
medical device biocompatibility, however, these methods are time-consuming and
efficient. The goal of our study was to determine if the in vitro SenCecTox® assay,
using the EpiDerm™ reconstructed human skin model (MatTek Corp.), could be
an acceptable alternative for assessing the sensitization potential of medical device
extracts. The SenCecTox® assay monitors specific genes controlled by Nfz2/ARE
which are known to be crucial in dermal sensitization. To that end, ten compounds
with known sensitization potencies were assessed. Six of the compounds were in-
corporated into samples of medical device silicone (10% final concentration) and
extracted in saline and sesame oil in accordance with ISO 10993-12. The resulting
extracts were further diluted in their respective vehicles prior to tissue exposures.
The remaining four compounds and assay controls were prepared directly in saline
and sesame oil at low concentrations immediately prior to exposure. This resulted
in 20 test solutions (10 compounds in both saline and sesame oil). Tissues were
exposed for 24 hours and tissue viability was assessed by LDH release. Expression
of multiple genes controlled by Nfz2/ARE was assessed by qRT-PCR. The test
solutions were also assessed for reactivity with glutathione (GSH). Sixteen of the
20 test samples (or 80%) were correctly identified as negative (non- or weak sensi-
tizers) or positive (moderate/strong/extreme sensitizers). In addition about half of
the potencies were correctly identified when compared to known in vivo potencies.
Our results indicate that the EpiDerm™ model can detect the presence of sensiti-
ers at low concentrations in medical device extract mixtures. Therefore, this model
can be a suitable replacement for the GPMT and LLNA to evaluate medical device
biocompatibility.

### 2015 Evaluation of an In Vitro Human Dermal Sensitization Test
For Use with Medical Device Extracts


Purpose: Bioadherence of medical device materials is often assessed in vitro
using standardized positive and negative control materials were reproducible over
similar test conditions induced by blood flow. Fresh ovine blood was collected from a
porcine iliac vein. Confirmation of thrombus dissolution via CDT, the animals were survived for an additional one week with diagnostics
(clinical/neurologic exams, angiography, and necropsy) performed to detect any
migratory emboli that may have impacted overall health. Vessels and vital organs
were collected for histopathology analysis. Results: DVT as well as thrombolytic
activity was detected in all groups with restoration of venous flow confirmed.
Conclusions: As our study indicated, the formation of a thrombus in the iliac vein
has proven to be effective as a preclinical model for evaluating CDT as a treatment
option for DVT. Further evidence is required to establish long-term benefits of
CDT in minimizing systemic and local toxicity as well as determining the optimal
frequency and dosage of thrombolytic drugs in preventing PTS.

In vitro...
Skin irritation is a condition caused by acute damage to keratinocytes following exposure to a test chemical. Testing for skin irritation potential of medical devices has typically involved the use of laboratory animals. In an effort to reduce the need for in vivo testing, alternative in vitro skin irritation test methods have been developed. While these methods have been shown to provide results consistent with in vivo data, they have involved the use of chemical solutions or spiked extracts as positive controls. In order to provide a method more applicable to medical device testing, attempts have been made to find an extractable material that will induce a positive irritation response in both polar and non-polar extraction vehicles. Here we report the findings of in vitro skin irritation testing performed with heat-pressed polyvinyl chloride (PVC) sheets spiked with Genapal X-080 at various ratios (Y-1, Y-2, Y-3 and Y-4). All materials were extracted for 72 hours in both saline and sesame oil at both 37°C and 50°C. Reconstructed human epidermis (RHE) tissues were exposed to test extracts. Tissue viability was determined by MTT reduction and interleukin-1α (IL-1α) values were measured by ELISA. Tissue viability of greater than 50% and IL-1α values of > 100 pg/mL were indicative of skin irritation. Significant activity for irritation response was not detected in Y-1-Y-3, while Y-4 consistently resulted in a positive irritation response under all extraction conditions. These results closely correlate with those of in vitro intracutaneous reactivity testing with rabbits. We submit that Y-4 should therefore be considered for use as an extractable positive control material for future in vitro skin irritation testing of medical device extracts.

In vitro skin sensitivity and irritation tests have been developed as alternative methods for animal tests. However, these tests have not been validated for medical device extracts. In the current study, the correlation of the in vitro and in vivo tests was evaluated for polar and non-polar extracts from 10 medical device materials (e.g., polysisoprene, polyetherimide, acrylonitrile butadiene styrene, and stainless steel, etc.). The test materials were extracted in normal saline or sesame oil according to ISO 10993-12. The test extracts were tested in the guinea pig maximization sensitivity, in vitro sensitization (IVSA - human reconstructed 3D skin model, the MatTek EpiDermTM), rabbit intracutaneous reactivity, and in vitro irritation (IVIA - MatTek EpiDermTM model) tests according to ISO 10993-10. All of the 10 materials were considered non-sensitizers and non-irritants in the in vivo tests for both polar and non-polar extracts. Consistently, the saline and sesame oil extracts of all 10 materials were negative (non-irritant) in the IVIA model. However, in the IVSA model, 4 of the 10 saline extracts were considered weak sensitizers (toxicity index: 1.5 - 3.5); five of the ten sesame oil extracts were considered sensitizers (three weak and two moderate/strong sensitizers, toxicity index: 2 - 5.5). Considering both polar and non-polar extracts, the in vivo and in vitro sensitization tests showed 30% agreement. The results from this comparison suggest that for medical device extracts the positive response from the IVIA does not necessarily predict the in vivo response, while the responses from the IVIA and in vivo skin irritation tests are likely to be more consistent. In order to apply these in vitro assays for the in vivo prediction of irritation and sensitization potential of medical devices, further evaluation and validation are essential.

Assessment of dermal irritation is an essential component of the safety evaluation of medical devices. Reconstructed human epidermis (RHE) models have replaced rabbit skin irritation testing for neat chemicals (OECD TG 439). However, medical device extracts are dilute solutions with low irritation potential, therefore the validated RHE-methods needed to be modified to reflect needs of ISO 10993. A protocol employing RhE EpiDerm was optimized in 2013 using known irritants and sensitizers (three weak and two moderate/strong sensitizers, toxicity index: 2 - 5.5). Considering both polar and non-polar extracts, the in vitro and in vivo irritation tests showed 50% and IL-1α values of > 100 pg/mL were indicative of skin irritation. Significant activity for irritation response was not detected in Y-1-Y-3, while Y-4 consistently resulted in a positive irritation response under all extraction conditions. These results closely correlate with those of in vitro intracutaneous reactivity testing with rabbits. We submit that Y-4 should therefore be considered for use as an extractable positive control material for future in vitro skin irritation testing of medical device extracts.

The ISO 10993-5 standard for in vitro cytotoxicity testing of medical devices suggests use of culture medium with serum as the extraction vehicle due to its ability to support cell growth and to extract both polar and non-polar entities; however, the standard also acknowledges that proteins in serum may bind to some compounds released from device materials, inhibiting toxicity of these compounds. Limited data are available on the effects of serum concentration on cytotoxicity of leachables from medical devices. The goal of this study was to determine whether serum concentration in cell culture extract media affects cytotoxicity of polymeric materials. Cytotoxicity testing was conducted per ISO 10993-5 using L-929 fibroblasts. UV-sterilized discs of various polymeric materials (latex, nitrile, EPDM, BUNA rubber, and polystyrene) were extracted per ISO 10993-12 guidelines (57°C x 24 hours in cell culture media; SAV ratio of 3 cm²/ml) with fetal bovine serum concentrations of 0.1%, 1%, 5%, and 10%. Cells were grown in media containing 10% serum for 24 hrs. Media was then replaced with polymeric extracts with various serum concentrations and cells were grown an additional 24 hrs. Cytotoxicity was assessed by MTT assay and flow cytometry (7-AAD). Results suggest that serum concentration in extract media strongly impacts cytotoxicity of some polymeric materials, but not others. Specifically, full strength extracts of latex, nitrile, and BUNA rubbers were cytotoxic regardless of serum concentration. No cytotoxicity was produced by extracts of the negative control material (polystyrene) at any serum concentrations. In contrast, cytotoxicity of EPDM extracts was dependent on extract serum concentration with 0% EPDM extract was toxic (7% cell viability); whereas EPDM extract containing 10% serum resulted in 87% cell viability. Similar results were obtained with both MTT assay and flow cytometry evaluation. Results of the study highlight the need to consider serum concentration in extract media when conducting cytotoxicity testing of medical devices.

Two cytotoxicity tests (the MEM elution (ME) and Colony Formation (CF)) are commonly used in the biological evaluation of medical devices. Previously, results from 43 samples used in medical devices were evaluated for both tests (2014 SOT poster 199). In this study, an additional 30 samples (e.g. titanium alloy, polyure-
thiane, polysisoprene, polysterathermide, and stainless steel, etc.) were evaluated to determine the correlation between the ME and CF tests and the consistency of data between the current and previous studies. Furthermore, the results from the two in vitro cytotoxicity tests were evaluated for their ability to predict in vivo test results for 21 samples. The test samples were extracted in cell medium or polar and non-polar vehicles according to ISO 10993-12. The ME including a serial dilution of the extract, CF, guinea pig maximum sensitization, rabbit intracutaneous reactivity, and mice acute systemic toxicity were conducted according to the appropriate ISO 10993 standards. One test sample was non-cytotoxic in ME but showed low cytotoxicity in the CF test (3% disagreement for 50 samples). When all 30 samples were evaluated, the concentration of the extract that showed no cytotoxicity from ME was correlated to the concentration of the extract that inhibited colony formation to 50% (IC50) from CF (R2 =0.88). Toxicity was not observed in any of the in vitro tests even though 4 of 21 samples showed cytotoxicity in at least one in vitro test. The results from this comparison suggest that ME and CF tests provide comparable results, and the positive response from the in vitro cytotoxicity assays does not necessarily predict the in vivo toxicity. The results from the current study were consistent with the previous one.

### 2021 EVA Foamed Excipient: Novel Way to Deliver Stem Cells, Growth Factors, and Biologics for Controlled-Release Applications

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VitalDose® EVA (ethylene vinyl acetate, pharma grade) is considered a neutral, biocompatible material suitable for use in medical/combination device applications. Our work is the first in the medical and pharma industries to address biocompatibility requirements (USP Class VI and ISO 10993). Available toxicological data include cytotoxicity/genotoxicity, sensitization, skin irritation, systemic toxicity, and implantation. Controlled release properties of foamed EVA were demonstrated for therapeutic agents highlighting capability for creating a microenvironment to prevent recurrence of brain tumors following resection. Methods: EVA microcellular foam (density 0.27 g/cm3) was used as a delivery vehicle. To simulate therapeutic agents, sodium-p-styrenesulfonate (PSS) was used at 70kD and 200kD. PSS seeding was done in vitro with EVA. The release profile of PSS was measured in HBSS solution, monitored by UV/Vis spectroscopy. Results: PSS release was monitored for a period > 200 hr. Five to ten weight percent of the loaded PSS was immediately released when the seeded EVA was initially placed in solution, simulating a therapeutic burst effect. We found that the release of the PSS depended on the PSS molecular weight (MW): between 25% and 50% of the total quantity of loaded PSS was released in the first 24 hr, with an extra 20% release occurring over the next 75 hr. Conclusion: Each high MW agent demonstrated controlled release over a 200 hr period. Because of the hydrophobic nature of EVA, controlled release delivery of agents in solution is possible without EVA disintegration. A stable cellular foam microstructure is ensured and maintained for long term controlled release vehicles whose diffusion pathways are invariant. In addition, a modified EVA delivery vehicle seeded with therapeutic agents could potentially provide a modified microenvironment suitable for mitigating brain tumor recurrence in patients with glioblastoma multiforme. The 24 to 100 hr controlled release timescale may also be targeted for biologic oral dosage applications.

### 2022 NTP Studies of N,N-Dimethyl-p-toluidine (DMPT), a Component in Medical Devices and Gestational Material

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N,N-Dimethyl-p-toluidine (DMPT) (CAS No. 99-97-8) is used as an accelerator for methyl methacrylate monomers in medical devices and in dental materials. In NTP 2-year studies, DMPT caused liver tumors in male and female F344/N rats and B6C3F1/N mice, nasal cavity transitional cell epithelium tumors in F344/N rats, and lung and forestomach tumors in female B6C3F1/N mice. We have examined the nasal cavity transitional epithelium of DMPT-treated F344/N rats to identify early toxicity changes. After a 5-day DMPT exposure (0, 1, 6, 20, 60, 120 mg/kg), hyperplasia of the transitional epithelium was seen at 60 and 120 mg/kg. Nasal cavity transitional epithelium was laser capture microdissected from controls and DMPT exposed rats, and global transcript patterns were obtained using the DMiss Rat Genome 230 2.0 Array platform. At a 5% false discovery rate threshold, there were more than 1,700 differentially expressed gene transcripts in the transitional cell epithelium due to DMPT treatment (120 mg/kg). These changes were associated with anti-oxidative defense response, cell proliferation and decreases in apoptotic function. The DMPT nasal cavity transcript changes overlapped those seen in the nasal cavity of rats exposed to formaldehyde. These findings support the hypothesis that DMPT nasal cavity transitional cell epithelium toxicity is due to oxidative damage.

### 2023 Should Respiratory Sensitizers Be Listed As Substances of Very High Concern (SVHC) under REACH?

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There is increasing regulatory pressure in Europe to list respiratory sensitizers as substances of very high concern (SVHC) based on “equivalent level of concern” criteria set out in REACH Article 57(f). This approach assumes that in certain cases, the negative impacts caused by sensitizers on the health and quality of life of affected individuals and on society as a whole are comparable to those elicited by carcinogens, mutagens, and reproductive toxicants (CMRs). Potential factors for comparison include seriousness of the effect, delayed onset and/or irreversibility of effects, potency, mode of action, impairment of life quality, or uncertainty about the dose-response relationships. As there are currently no applicable guidelines or generally accepted assays that can accurately identify respiratory sensitizers nor distinguish between respiratory and dermal sensitizers, all materials with sensitizing potential may be considered for inclusion as SVHC. While all respiratory sensitizers may test positive in animal-based dermal sensitization assays, skin sensitizing agents do not elicit respiratory effects under normal circumstances and respiratory sensitizers are generally considered to pose a greater health concern. Current guidance recommends a weight-of-evidence approach based on human and animal data to identify a potential respiratory sensitizer; however, some regulatory authorities may accept any positive indication of sensitizing potential as evidence for inclusion as a SVHC. A SVHC is subject to authorization within the EU and may not be used unless an authorization is granted for their specific use. These proposed regulatory actions will likely have a profound impact on the sale and use of materials due to regulatory authorization and/or de-selection of products containing a SVHC. The goal of this roundtable is to promote a free and open discussion of the rationale and scientific basis for handling chemical sensitizers as SVHC under REACH.

### 2024 Will Generally Recognized As Safe (GRAS) Become an Endangered Species?

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The current legislative and regulatory framework provides for self-determination of Generally Recognized As Safe (GRAS) ingredients in food products, with voluntary notification of the GRAS determination to the US FDA. Recent reports by the federal General Accountability Office, the Pew Institute, and the Natural Resources Defense Council have been critical of the Generally Recognized As Safe (GRAS) process for use of food ingredients. Specifically, GRAS has come under fire as not having adequate safeguards in place to protect the public from inadequate safety behind a decision of GRAS, specifically as being susceptible to conflicts of interest and generally, too far outside of regulatory oversight, which might otherwise provide a higher degree of assurance of public safety. More recently, the GRAS status of partially hydrogenated oils (aka trans fats) has been revoked. Does this mark the start of a new era where the US FDA will have more oversight of the GRAS process? Will GRAS notification be required? Would this requirement increase the safety of food ingredients? This roundtable will provide an in-depth look at the history and future of GRAS determinations, where the GRAS process has worked, why the GRAS status of a few ingredients has been revoked, and how the process can be improved. Principles followed in the self-determination and notification processes of GRAS ingredients, safeguards that are in place to ensure the safety of GRAS ingredients, and proposed steps to increase transparency and rigor of the assessments will be discussed.
2025 Risk Communication and Management in the Era of Social Media and the Internet: Serving Society’s Needs with Accurate Information

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Ample evidence exists that the source of chemical safety information for people today has shifted, at least in part, from traditional sources of textbooks, academia, and government authorities to bloggers, websites, and email. This has multiple implications for society as well as our science. Adapting to this new world of communication is critical. Unfortunately, communicating and engaging with the lay public is not addressed as part of graduate training, and, therefore, even accomplished toxicologists who are effective scientific communicators find themselves unprepared. The good news is that tools and guidance to help communicate in this new world are rapidly evolving. Expanding the use of these tools and developing new methods requires effort by the risk assessment and risk communication communities. This begins by understanding the tools, learning the methods, and, occasionally, taking a risk by trying out these new communication techniques. Sharing experiences cross functionally will enable communication across the risk management community. Four individuals with varying risk management roles across society come together in this informational session to share their experience and insight into the new world of risk communication. A discussion panel will follow the completion of the formal presentations.

2026 What Toxicologist Do You Wanna Be? The Role of Toxicologists across Diverse Organizations

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Participants across SOT, namely students and postdocs, who are geared up to transition into their full-time career paths of choice as toxicologists do not have a good understanding of what toxicologists do on a day-to-day basis while working in diverse industries/organizations. Although academic toxicology training programs across the globe are training the students well in the principles and concepts of toxicology, they come short in educating the students/postdocs on the role they play as toxicologists in real-world job scenarios across diverse industries/organizations. Based on these needs and to better equip our young toxicologists, an informational session/education career development session that highlights or summarizes the different roles toxicologists play in the real-world job settings would be of immense value to students/postdocs in evaluating whether their training/personality suits them better in a specific industry/organization over the others. Although it is impossible to cover all the organizations where toxicologists play an important role in one CRAD/informational session, we attempted to represent the major organizations where toxicologists are hired predominantly in the recent years. Each of the five speakers covered the following general topics as presented in the 8-minute presentations: (1) How and why the speaker ended up with their respective current affiliated organizations; (2) How they went about securing their first job; (3) The kinds of training/soft skills/interpersonal skills needed to find a job in their respective organizations; (4) The kind of career-growth opportunities an entry-level toxicologist will have with the organization or respective industry; (5) The ONE thing the speaker most likes about their job; (6) The ONE thing the speaker most hates about their job.

2027 Liver-Specific miRNAs in Human Plasma As Potential Clinical Biomarkers for Liver Injury

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Liver specific miRNAs in plasma have been demonstrated to be promising biomarkers of hepatotoxicity in rats. In this study, we investigated the potential application of four liver-specific miRNAs, albumin (ALB), apolipoprotein h (APOH), group specific component (GC) and α-1 microglobulin/bikunin precursor (AMBP) miRNAs, as biomarkers for liver injury in humans. We firstly confirmed the liver-specific expression of our target miRNAs by relative expression analyses in major human organs and tissues. Real-time quantitative reverse transcription-polymerase chain reaction was performed to determine the plasma levels of the four target miRNAs in healthy donors and hepatitis patients, including patients taking transcatheter arterial chemoembolization (TACE) which results in artificial but specific injury to the liver. As compared to the healthy donors, the plasma levels of ALB and APOH miRNAs were increased in all the hepatitis patients along with serum alanine aminotransferase (sALT) elevation ≥3×ULN (upper limit of normal (ULN)). Some patients with sALT elevation ≥3×ULN showed increased plasma levels of ALB and APOH miRNAs. The sensitivity for plasma GC and AMBP miRNAs were lower than that for ALB and APOH miRNAs. The plasma levels of the four target miRNAs significantly correlated with sALT level as an index for severity of liver injury. It was noteworthy that ALB and APOH miRNAs in plasma increased after TACE coincident with an elevation of sALT, further supporting the specificity of these miRNAs for liver injury. Our data demonstrated the potential application of plasma ALB and APOH miRNAs as clinical biomarkers for liver injury, and will promote the future evaluation of these miRNAs.

2028 Serum miR-206 As a Useful Biomarker of Skeletal Muscle Injury Compared with Conventional Biomarkers


Circulating miR-206 has been reported as a useful biomarker (BM) to detect skeletal muscle injury. However, the sensitivity of miR-206 compared with that of conventional BMs such as creatine kinase (CK), lactate dehydrogenase (LDH), myosin light chain 3 (My3) and skeletal troponin I (sTnI) has not yet been fully evaluated. Therefore, we investigated whether miR-206 could be a more sensitive BM than conventional BMs in rats.

In a 10×-methyl phenylene diamine (TMPD)-induced skeletal muscle injury model, male Sprague-Dawley rats were injected TMPD subcutaneously twice daily for 3 days. Blood samples were collected on Day 3 and Day 4, and serum levels of miR-206, My3 and sTnI, and plasma levels of CK and LDH were measured. Animals were sacrificed after blood collection and muscle tissues (femoral, crural, and cervical multidius muscles, and diaphragm) were histopathologically evaluated. In addition, in rats treated with in-house compounds in which muscle injury was observed, same BMs were also evaluated. In the TMPD-induced muscle injury model rats, histopathology showed slight to moderate muscle necrosis which was similar in severity at both sampling time points. The miR-206 and My3 levels were greatly increased at both time points. The CK and LDH levels were also elevated but the magnitude of change was much less than that of miR-206 and My3. The sTnI level was increased on Day 3 but returned to control levels on Day 4. In the rats treated with in-house compounds, histopathological severity of muscle injury was slightly than that was caused by TMPD in general. The miR-206 levels were increased but other BMs were slightly or not increased, indicating that miR-206 is more sensitive to detect muscle injury than other BMs. In conclusion, our investigation demonstrated that serum miR-206 could be a sensitive BM to detect skeletal muscle injury in rats, suggesting that miR-206 in nonclinical toxicity studies would be useful to identify the potential risk of skeletal muscle injury of compounds under development.

2029 MicroRNAs: Potential Tissue and Circulating Biomarkers of Structural Cardiotoxicity

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MicroRNAs play an important role in heart development and cardiac pathogenesis and are key regulators in human cardiac disease and animal models of heart disease. We have previously shown that PPARGamma-induced cardiac hypertrophy (secondary to hemodilution) altered the expression of heart microRNAs (e.g., miR-21 and miR-24), coincident with increased heart weight and increased expression of transcriptional markers of cardiac hypertrophy (e.g., fetal gene re-expression, induction of precursor proANP/BNP genes, and collagen gene expression). In this Bis(2-chloroethoxy)methane (CEM) 7-day rat study, we used transcriptomics (cardiac mRNA, cardiac miRNA, and circulating miRNA), NTP/ANP, eTnI, heart weight, and ultrastructural analysis to determine if cardiac risk “flags” can be detected prior to the onset of overt cardiotoxicity. As anticipated with this model compound, CEM caused lesions in the heart (multifocal necrosis) that correlated with increased serum cTnI. Although there was no increase in heart weight or NTP/ANP, there was a genomic signal (miRNA and mRNA) consistent with maladaptive cardiac hypertrophy, miR-206 and its target gene Myh6 showed reciprocally altered expression. A robust increase in plasma miR-133 was detected in 3/6 rats given CEM which generally correlated with increased troponin levels. This study shows a proof-of-principle of heart-selective miRNA detection in circulation surrounding the event of microscopic cardiac necrosis.
MicroRNA (miR) 208 is cardiac specific and miR1, miR133a and miR133b are enriched in cardiac and skeletal muscle in rats. Male Sprague-Dawley rats received a single dose of either isoproterenol (ISO); metaproterenol (MET); allylamine (AAM); mitoxantrone (MIT); acetaminophen (APAP) or vehicle. Blood (serum biomarkers) and tissues (histopathology) were collected from rats (n ≥ 3) in each group at 4, 24 and 48 h after dosing. ISO, MET, AAM induced cardiac muscle and tissue toxicity and APAP induced liver specific toxicity within 24 h. Serum levels of candidate miRs were compared to conventional serum biomarkers of skeletal muscle (aspartate transference [AST]; lactate acidenhpin protein 3 [FABP3], myosin light chain 3 [MyL3], skeletal muscle troponin [sTnl]) and cardiac (cardiac troponin [cTnl] and FABP3) toxicity. MT induced increases in cTnl and sTnl in the absence of correlating heart and muscle histopathology. Increases in cTnl were observed in all rats with cardiac toxicity (ISO, MET, AAM) but the magnitude of increases did not correlate with the severity of lesions. Increases in miR208 only occurred in rats with evidence of cardiac lesions. These results suggest that miR208 is more sensitive than cTnl in detecting cardiac injury in rats and more selective than cTnl as a biomarker of cardiac toxicity. ISO, MET and AAM induced increases in cTnl and cTnl in the absence of correlating heart and muscle histopathology. Increases in cTnl were observed in MIT-treated rats with cardiac toxicity (ISO, MET, AAM) but only sTnl was increased in MIT-treated rats. Increases in serum miR1, miR133a and miR133b occurred in ISO-, MET-, AAM- and MIT-treated rats but were absent following APAP treatment, while the muscle biomarkers AST, sTnl, and MyL3 were increased in APAP-treated rats in the absence of heart or skeletal muscle toxicity. Our results suggest that miR1, miR133a and miR133b are sensitive and specific markers of muscle/cardiotoxicity and that miR208 may differentiate cardiac toxicity from skeletal muscle toxicity. Used together these miRs are promising biomarkers of cardiac and skeletal muscle toxicity.

MicroRNAs (miRs) are promising biomarkers of drug-induced liver injury; however, the kinetics of release during injury and recovery are largely unknown. In this study, miRNA alterations in plasma and liver tissue were quantified in male Sprague Dawley rats administered α-naphthylisothiocyanate (ANIT) and were phenotypically anchored to the temporal progression of hepatobiliary injury and compensatory biliary hyperplasia which this compound is known to elicit. Rats received a single dose of ANIT (5 mg/kg, i.g.) or vehicle (corn oil) and were sacrificed 6, 24, 48, 72, 120, or 168 hours (h) post-dosing (n=6/treatment/time point). Significant ANIT-induced hepatobiliary injury occurred between 24h and 72h post-dosing; hyperplasia was observed from 48h post-ANIT-dosing through study termination. Profiling of plasma miRNAs demonstrated that species were primarily elevated in circulation during ANIT-induced hepatobiliary injury (n=162 of 163 and 86 of 87 significantly altered species at 24h and 48h post-dosing, respectively). Few miRNAs (n=3) were altered in liver tissue 24h following compound dosing, suggesting that passive leakage may account for the majority of elevations observed in circulatory miRNAs at this time. Conversely, at 12h following ANIT dosing, when hyperplasia predominated, a global reduction in circulatory miRNAs was observed (n=150 significant species). Concurrently, the expression of 31 miRNAs was altered in hepatic tissue, primarily representing elevated expression (n=30 of 31) of altered miRNAs. Half of these species (n=15) were also significantly reduced in the plasma, potentially indicating that select miRNAs are retained during hyperplasia to promote proliferation/repair. One miRNA, miR-182-5p, was significantly altered in both the tissue and plasma at 24h and 120h, potentially providing an early indicator of biliary hyperplasia in the rat.
collected before dosing and at various time points on day 1, at 48h, and at 72h. Hearts were also collected for histopathological examination at 24h, 48h and 72h post drug administration. Conventional BioM such as cardiac troponin T (cTnT), cardiac troponin I (cTnI) and myosin light chain 3 (My3) were assessed with the Meso Scale Discovery immunoassay. Regarding plasma miRNA detection, we used the latest high throughput miR profiling technology from Firefly Bioworks which assays the expression of a panel of 68 selected targets. Potential normalizers have been included in addition of any miR of interest in the setting of cardiac injury. While ISO and AAM both induced severe myocardial necrosis, most significant early changes in cTnT occurred in ISO treated rats. Similarly, My3 performed better as a predictive BioM after ISO dosing. Interestingly, data collected with the Firefly platform identified several miRs significantly increased after ISO and AAM administration and that peaked at 4h or 8h after exposure. These miRs, with miR 199a 5p, 3p miR-199a 3p, and miR 320 3p among others, were shown to be involved in human cardiac conditions such as myocardial infarction and cardiomyopathy, and were not previously expressed in vehicle treated rats. Further analyses using specific miR assays and absolute quantification are ongoing. Overall our results suggest that plasma miRs may serve as BioMs for early cardiotoxicity evaluation in rats.

**PS 2037 Glutamate Dehydrogenase and microRNA-122 Are More Sensitive Liver Injury Markers Than Alanine Aminotransferase in Acetaminophen-Treated Rats**

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Background & aim: There is an ongoing search for specific and translation biomarkers in drug-induced liver injury. Glutamate dehydrogenase (GLDH) and microRNA-122 (miR-122) were qualified as putative biomarkers in acetaminophen (APAP) treated rats and compared to standard alanine aminotransferase (ALT) measurements. Examining pathways of miR-122 release may provide an insight into whether this is an active or passive process. Methods: Rats were treated daily with APAP (500, 1000 or 1500 mg/kg). Plasma concentrations of GLDH, ALT, and miR-122 (in eososomal and protein-rich fractions) were analysed 2, 4, 6 or 24 h after a single dose, or 24 h after 2, 3, or 4 day treatment. In situ hybridisation (ISH) of liver miR-122 was performed. Results: After 24 h treatment, all biomarkers increased significantly in the 1500 mg/kg dose-group, however both GLDH and miR-122 were more sensitive (larger fold changes) than ALT (ALT: 260%. GLDH: 50%, miR-122: 180%). Peak levels for all biomarkers were obtained at day 2 and the highest elevation was observed for GLDH (ALT: 440%. GLDH: 4750%. miR-122: 800%). At day 3, miR-122 levels were back at baseline, whereas GLDH and ALT levels remained elevated at day 4. ISH confirmed that miR-122 was lost from centrlobular hepatocytes at peak toxicity. This coincided with miR-122 release in both eososomal and protein bound plasma fractions. Conclusions: GLDH and miR-122 are more sensitive biomarkers of APAP-induced liver injury than ALT in rats and could be used to complement ALT in future preclinical studies. Eososomal and protein-rich miR-122 profiles follow similar patterns during DILI, suggesting that miR-122 is a marker of necrosis and plasma membrane breakdown.

**PS 2038 The microRNA mir-708 Affects FXR-Dependent Regulation of Cholesterol Efflux in Hepatocytes, Macrophages, and Intestinal Cells**

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Certain microRNAs (miRs) are key regulators of cholesterol homeostasis in mammalian systems and are potential therapeutic targets for the treatment of atherosclerosis. Targets for these miRs include important lipid metabolism genes such as ATP Binding Cassette (ABC) transporters, specifically ABCA1, which in turn regulates cholesterol levels and HDL generation. The Nuclear Receptor (NR) Farnesoid X receptor (FXR) functions as a bile acid sensor that impacts the expression of genes involved in cholesterol, triglyceride (TG), and bile acid production. FXR regulates genes involved in cholesterol uptake and excretion in the liver, however the role of this NR in cholesterol metabolism in extrahepatic tissue is an area that is in need of further study. We have shown that several miRs, including mir-708, are regulated by the FXR ligand GW40064 and also effect cholesterol efflux in Macrophage Derived Foam Cells (MDFC). Hence, the objective of these studies was to examine the role of mir-708 in FXR-dependent cholesterol flux by examining its role on cholesterol transport in MDFC (THP-1), liver (Huh-7) and intestinal (Caco-2) model systems. Specifically, the effect of mir-708 transfection on GW4064-dependent cholesterol uptake and efflux as well as expression of key lipid metabolism and transport genes was determined in THP-1, Huh-7 and Caco-2 cells. Understanding of the mechanism behind the regulation of cholesterol efflux by FXR via Micro-RNAs will ultimately assist in discovering therapeutic approaches for the treatment of atherosclerosis.
Comparative data from Sprague Dawley IGS and Wistar (Hanover) rats group housed in a conventional non-barrier facility was reviewed. Data from animals housed in both polycarbonate (solid bottom) cages containing bedding and conventional metal (stainless-steel wire mesh or perforated floor) cages were included. Parameters of interest chosen for comparison included survival, body weight and body weight gain, food consumption, onset, progression and incidence of common neoplastic and non-neoplastic lesions. Dosage routes included oral gavage, subcutaneous injection, and nose only inhalation. Sprague Dawley rats were noticeably heavier than Wistar rats, where lower bodyweights were associated with increased survival rates and a lower incidence of spontaneously occurring tumors over a two year period. The incidence of clinical signs associated with foot lesions was higher in Sprague Dawley rats however a general reduction in the incidence and a delay in onset of these clinical signs were noted in rats housed in solid bottom cages, when compared to rats housed in metal bottom cages. The type of neoplastic and non-neoplastic lesions seen in the two strains differed principally in incidence, and spontaneously occurring tumors were seen in tissues including, pituitary, adrenal, and mammary tissue, there was no evidence of significant changes in incidence patterns in the data evaluated and the type of cage had no apparent influence on this data. It is concluded that differences in cage environment had minimal effect on the selected parameters examined. Survivability in Wistar rats was greater and associated tumor burden less than that seen in the Sprague Dawley rats.

Under ICH S1B, a 6-month carcinogenicity study in rasH2 (CByB6F1-Tg(HRAS)2JIC) mice is accepted by regulatory agencies as an alternative to a 2-year carcinogenicity study in mice. To determine if our facility’s background incidence of spontaneous findings in 6-month rasH2 mouse studies is comparable to previously published tumor incidence limits, we compared the incidence of spontaneous and induced tumors in multiple studies conducted in our facility with those published by Nambiar et al. (Pfizer) and Paranjpe et al. (BioReliance). For untreated or vehicle (negative) control mice at our facility, the most common spontaneous neoplasms in males and females were lung adenomas (7.6% in males; 5.3% in females), followed by splenic hemangiomas (2.5% in males; 3.9% in females), lung adenocarcinomas (2.2% in males; 2.8% in females), squamous cell papillomas of the stomach (1.4% in males; 0.7% in females), and Harderian gland adenomas (1.1% in males and females). For rasH2 mice administered N-nitrosomethyurea as a positive control at our facility, the most common neoplasms were lymphomas (68.0% in males; 74.7% in females) and squamous cell papillomas of the stomach (72.0% in males; 64.0% in females), followed by squamous cell papillomas of the skin (17.3% in males; 43.2% in females), adenocarcinomas of the small intestine (13.3% in males; 8.0% in females), lung adenomas (9.3% in males; 16.0% in females), squamous cell carcinomas of the stomach (10.7% in males; 13.3% in females), and adenomas of the small intestine (5.3% in males; 9.3% in females). These spontaneous tumor incidences in normal control rasH2 mice and induced tumor incidences in positive control rasH2 mice are within the tumor incidence ranges of the Pfizer and BioReliance databases.

Our previous studies indicated that decreasing visceral adipose tissue by surgical removal of the parametrical fat pads inhibited UVB-induced carcinogenesis in SKH-1 mice fed a high fat diet. In the present study, we tested the effect of an aqueous filtrate of the parametrical fat pad (fat tissue filtrate) on the in vitro transformation of mouse epidermal JB6 cells. Our results indicated that fat tissue filtrate stimulated JB6 cell proliferation and enhanced the conversion of JB6 cells from an epithelial-like morphology to cells with a fibroblast-like morphology. Fat tissue filtrate made from the parametrical fat pad of mice fed a high fat diet (HFD) had 160% more transforming activity than that from mice fed a low fat diet (LFD) (p<0.01) as measured by colony growth in soft agar. Cells that grew in soft agar formed tumors in SCID mice. Mechanistic studies indicated that the fibroblast-like cells had decreased levels of E-cadherin, increased levels of Twist as assayed by western blot. Immunochemical staining and adipokine array showed that parametrial fat tissue from animals fed a HFD had a higher density of macrophage-fused dead adipocytes (carcinoma-like structures) and more adipokines compared with parametrical fat tissue from animals fed a LFD. Flow cytometric analysis demonstrated that fat tissue filtrate stimulated the formation of reactive oxygen species in JB6 cells. These studies provide the first in vitro demonstration of a parametrical fat tissue-induced transformation of an epidermal cell.

Carcinogenicity studies enable us to predict the potential for a human carcinogenic risk when a chemical is used in humans. The National Toxicology Program (NTP), which is a national program co-ordinated by the National Cancer Institute of NIH, conducts rodent carcinogenicity studies to assess the potential human health risk. The majority of the data required by the regulatory agencies in the USA and Europe is derived from NTP studies. As such, the results of these studies are viewed by regulatory agencies as important in the process of assessing the human health risk associated with the use of a chemical. The potential for human risk is determined by comparing the cancer incidence observed in the rodent studies with the data collected from epidemiological studies. The comparison is based on the relative incidence of tumors observed in the rodent studies and those collected from human studies. The NTP’s statistical analyses of the rodent data, including the use of Bonferroni corrections and Fisher exact test, are designed to control the overall false positive rate. In recent years, however, the number of statistically significant findings far exceeds the number predicted by the false positive rate. We further show that the expected number of significantly positive results increased tumor incidences in negative studies is very low (e.g., 1 or 2), and do not lead to determinations of carcinogenicity, and illustrate this using several studies that the NTP concluded to be negative for carcinogenicity. Discussion of the details of NTP’s statistical methods and decision processes will help to resolve misunderstandings involving multiple testing and the role, and contribution, of false positive rates in carcinogenicity studies.

Colon cancer (CRC) treatment with 5-fluorouracil (5-FU) is the first line of therapy for this debilitating disease. However, treatment effectiveness is hampered by the development of drug resistance and toxicity at high doses. The naturally occurring phytoalexin Pterostilbene (PT) possesses antioxidant and anticancer effects. Estrogen receptor (ER-β) was reported to be essential for PT beneficial effects. Studies have shown that PT can play an important role in CRC development and possibly in its response to therapy. This study aimed to evaluate, in CRC cells, whether PT affects the expression/ activity of ER-β and can thus potentially sensitize cancer cells to chemotheraphy. The cytotoxicity of PT alone and in combination with 5-FU was tested and compared in HT-29, Caco-2 and HCT-116 cells. Our data indicate that PT exhibited a more potent cytotoxic effect in HT-29 and Caco-2 compared to HCT-116 cells (Half maximal inhibitory concentrations (IC50) 34, 40 and 85 μM). Co-treatment with PT/5-FU was more effective in HT-29 and Caco-2 cells as indicated by Calcusyn® Synergy analyses showing higher dose-reduction indices (12.9 and 4.5). Western blot indicated that ER-β is expressed only in HT-29 and Caco-2 cells and that its levels are boosted with PT treatment by about 1.5 folds (P<0.003). The impact of PT on the tumor suppressor FOXO-1 and the cell cycle inhibitor p27kip was also assessed since both are known to be downstream signals to ER-β activation. PT induced FOXO-1 (22 and 25%) and p27kip (36 and 33%) levels in HT-29 and Caco-2 cells, respectively which substantiates the potential role of ER-β in PT growth inhibition effects. These results provide a rationale for novel combination treatment strategies, especially for 5-FU-resistant CRC patients expressing ER-β protein.
Cancer is a principal cause of death worldwide. Breast cancer was by far the most common cancer diagnosed in women. Epidemiological studies indicate that populations consuming high levels of plant derived foods have low incidence rates of various cancers. Polyphenolic compounds are broadly distributed in the plant kingdom and have attracted considerable interest as potential chemotherapeutics. Puerarin (PE), Ursolic acid (UA) and Pycnogenol (PYC) are polyphenolic compounds and found in significant quantities in vegetables and fruits. In this study, we aimed to evaluate the cytotoxic activity of PE, UA and PYC by NRU and MTT assays at different incubation periods and compare the cytotoxicity assays. For this purpose, MDA-MB-231 cells were exposed to PE, UA and PYC in a concentration range of 25-300 μg/mL for 18 and 24 hours. A concentration dependent decrease was seen in the survival of cells exposed to polyphenolic compounds. IC50 value of PE was found to be 292.02 μM by NRU assay after 24 h incubation whereas IC50 value was not calculated by NRU after 18 h incubation. IC50 values of PE were found to be 296.58 μM and 293.16 μM by MTT assay after 18 h and 24 h incubation respectively. IC50 value of UA was found to be 282.65 μM by MTT assay after 24 h incubation whereas IC50 value was not calculated by NRU after 18 h incubation. IC50 values of UA were found to be 296.28 μM and 286.5 μM by MTT assay after 18 h and 24 h respectively. IC50 values of PYC were found to be 299.88 μM and 235.56 μM by NRU assay after 18 h and 24 h incubation respectively; 220.04 μM and 203.56 μM by MTT assay after 18 h and 24 h incubation respectively. According to IC50 values, the cytotoxicity of polyphenolic compounds ranked as follows: PYC > UA > PE. When comparing the cytotoxicity assays, MTT assay appears to be more sensitive in detecting loss of viability than NRU assay. Increasing incubation time resulted in increased cytotoxicity, which was observed more accurately with the MTT assay.

In the search for new therapies for castrate resistant prostate cancer, raloxifene (Ral), a second-generation selective estrogen receptor modulator has shown moderate activity. However, extensive first-pass metabolism results in low bioavailability of the drug. We have previously developed a delivery platform that exploits the amphiphilic nature of styrene co-maleic acid (SMA) in order to encapsulate highly hydrophobic drugs. This study was designed to determine if SMA-Ral would show superior activity in vitro compared to free Ral. Quantification of the intracellular content of Ral in PC3 cells treated with SMA-Ral (5 μM) or equivalent free Ral by high performance liquid chromatography demonstrated that SMA-Ral promoted a 30% and 75% higher accumulation of Ral in the cells compared to free Ral after 24 h and 48 h, respectively. This was explained by the different uptake mechanisms where SMA-Ral uptake occurred via caveolae-mediated endocytosis, whereas free Ral uptake was receptor mediated via the organic anion-transporting polypeptide OATP1B1 transporter family. To determine if this would translate to in vivo efficacy, male mice implanted with PC-3 xenografts were treated once a week for four weeks. SMA-Ral (1 mg/kg, iv) showed a similar reduction in tumor volume and weight compared to free Ral (5 mg/kg, iv), where tumor size was reduced by 40 and 39%, respectively, compared to vehicle control. Furthermore, mice treated free Ral (5 mg/kg, iv) or an equivalent dose of SMA-Ral showed the highest drug tissue concentrations in the liver, spleen, and tumors at 6 and 24 h post-injection, and these were higher in the mice treated with SMA-Ral. At 24 h tissue concentration of Ral remained higher in the mice treated with SMA-Ral compared to the free Ral treatment group, with 40% (p<0.05) higher concentration in the tumor compared to free Ral. In conclusion, in vitro and in vivo SMA-Ral elicited superior drug accumulation and efficacy compared to free Ral and thus micellar delivery of Ral warrants further investigation.
ability. To improve the anticancer activity of raloxifene, we encapsulated raloxi- 
fen e into a styrene co-maleic acid (SMA) nanocarrier. The amphiphilic property of 
SMA allows the encapsulation of highly hydrophobic drugs to improve their 
solubility, decrease their metabolism and ultimately improve their anticancer 
activity. One advantage shown in this study was more cytotoxic than the free 
drug toward three triple negative breast cancer cell lines in vitro. Specifically, 
EC50 values in MDA-MB-231, MDA-MB-468 and Hs578t cell lines were 11, 11.2 and 
11 μM for the free drug compared to the 7.9, 10.4 and 6.8 μM for SMA-raloxifene, respectively. 
Furthermore, treatment with SMA-raloxifene increased the number of cells in the G1 phase of the cell cycle by 
-10%, as well as the proportion of apoptotic cells by 2-fold, when compared to the 
equivalent concentration of free drug in both MDA-MB-231 and MDA-MB-468 
cells. The decrease in cell viability was also associated with both a reduced expres- 
sion and phosphorylation of proteins associated with cell proliferation and survival 
such as NFKB, AKT, EGFR and Src. In addition, treatment with free raloxifene 
and SMA-raloxifene induced cytochrome c diffusion in the cytoplasm and altered 
intracellular localization of several tyrosine kinase receptors including e-Met, 
epi- 
dermal growth factor receptor and focal adhesion kinase. SMA-raloxifene was also 
much more potent at reducing cell migration and cancer cell interaction with endothelial 
cells compared to free-raloxifene. In conclusion, encapsulation of raloxifene into 
a nanocarrier improved its anticancer activity against triple-negative breast cancer 
cells in vitro and warrants further investigation to identify the mechanisms involved 
as well as examine its efficacy in vivo.

### 2049 Investigating the Effects of the Dietary Carcinogens Benzo(a) pyrene and PhIP in LNCaP Prostate Cancer Cells

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Prostate cancer is the second most commonly diagnosed cancer in men worldwide, 
with dietary carcinogens posing an important risk for its development. Two im-
portant examples of dietary prostate carcinogens are benzo(a)pyrene (BaP) and 2-Amino-1-methyl-6-phenylimidazo(4,5-b)pyridine (PhIP): pro-carcinogens 
that require metabolic activation to their carcinogenic derivatives by the cytochrome 
P450 (CYP) family of enzymes. In the current study, the genotoxic and meta-

toblic effects of these two pro-carcinogens on LNCaP prostate cancer cells were 
investigated. LNCaP cells were incubated with BaP (1nM – 10μM) or PhIP (10nM-100μM) for a variety of time points up to 120h, and cell proliferation (al-
amarBlue® assay), genotoxicity (micronucleus assay), CYP activity (ethoxyresorufin 
assay). Antioxidant activities of MEXA were determined by DPPH, hydroxyl (OH) 
radical and inhibition of lipid peroxidation (LPO). Key findings: MEXA at 100, 
250 and 500 μg/mL scavenged DPPH by 48%, 62% and 70% and OH radical by 
39%, 58%, 67%, respectively. In addition, MEXA significantly (p<0.05) inhibited 
LPO in a dose-dependent manner. We showed that MEXA had an antiproliferative 
effect on androgen- insensitive (PC-3) and androgen-sensitive (LNCaP) prostate 
carcinoma cells. MEXA inhibited proliferation of PC-3 and LNCaP with IC50 of 
62.1 and 73.6 μM, respectively, at 96 h. LD10 assay showed that MEXA had 
low toxicity in vitro at its IC50 values. The extent of DNA fragmentation caused 
by MEXA in cancer cells showed higher values in PC-3 and LNCaP at 50 and 100 
μg/mL, suggesting possible induction of apoptosis. Treatment of cells with MEXA 
did not affect the network of vessels in CAM, thus lacking antiangiogenic property.

Conclusion: MEXA exerts antioxidant and antiproliferative activities in prostate 
cancer cells.

### 2050 Diindolylmethane and Its Halogenated Derivatives Induce ER Stress and Autophagy in Human Prostate Cancer Cells

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We have previously shown that 3,3′-diindolylmethane (DIM) and its halogenated 
derivatives (ring-DIMs) induce apoptosis in human prostate cancer cells. The mechanisms 
of action of ring-DIMs are not fully understood, but appear to be 
multifaceted and dependent on the halogen substitution pattern. Here, we show 
that ring-DIMs (≥ 30 μM) induce apoptosis in LNCaP and C42B cells. Treatment with 
ring-DIMs (≥ 30 μM) also induce autophagy by increasing the conversion of LC3B to LC3BI and LC3B punctuation. Pretreatment with the 
autophagy inhibitors bafilomycin or 3MA, as well as with LC3B siRNA 
exacerbated the cytotoxic effects of DIM and ring-DIMs. Pretreatment with CHOP + 
Apaf1, which increased basal autophagy, indicating a link between (ring-)DIM-induced ER stress 
and autophagy in human prostate cancer cells. Identifying the signalling pathway(s) 
targeted by DIM and ring-DIMs that result in prostate cancer cell death could help 
towards the development of novel drug therapies for this disease.

### 2051 Antiproliferative and Antioxidant Activities of Fruit Methanol Extract from Xylopia aethiopica on Prostate Cancer Cells

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Objective: Our previous studies have demonstrated that fruit methanol extract 
from Xylopia aethiopica (MEXA) exhibits strong antiproliferative activity in 
human cervical cancer (C-33A) cells via induction of apoptosis and cell cycle arrest. 
This study was designed to assess the antiproliferative, antiangiogenic and antiox-
dant effects of MEXA on prostate cancer cells (PC-3 and LNCaP). Methods: PC-3 
and LNCaP were cultured and treated with MEXA (10, 50 and 100 μg/mL). The 
XTT assay was used to evaluate the ability of MEXA to inhibit proliferation in 
cancer cells. Cytotoxicity of MEXA in cells was assessed by LD10 assay while DNA 
fragmentation was detected by cell death detection ELISA plus kit. MEXA was 
tested as inhibitor of angiogenesis using chicken chorioallantoic membrane (CAM) 
assay. Antioxidant activities of MEXA were determined by DPPH, hydroxyl (OH) 
radical and inhibition of lipid peroxidation (LPO). Key findings: MEXA at 100, 
250 and 500 μg/mL scavenged DPPH by 48%, 62% and 70% and OH radical by 
39%, 58%, 67%, respectively. In addition, MEXA significantly (p<0.05) inhibited 
LPO in a dose-dependent manner. We showed that MEXA had an antiproliferative 
effect on androgen- insensitive (PC-3) and androgen-sensitive (LNCaP) prostate 
carcinoma cells. MEXA inhibited proliferation of PC-3 and LNCaP with IC50 of 
62.1 and 73.6 μM, respectively, at 96 h. LD10 assay showed that MEXA had 
low toxicity in vitro at its IC50 values. The extent of DNA fragmentation caused 
by MEXA in cancer cells showed higher values in PC-3 and LNCaP at 50 and 100 
μg/mL, suggesting possible induction of apoptosis. Treatment of cells with MEXA 
did not affect the network of vessels in CAM, thus lacking antiangiogenic property.

Conclusion: MEXA exerts antioxidant and antiproliferative activities in prostate 
cancer cells.

### 2052 Apocynin, an NADPH Oxidase Inhibitor, Suppresses Rat Prostate Carcinogenesis

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Recently, there are considerable evidences suggesting oxidative stress contributes 
to the etiology and pathogenesis of the prostate cancer. Therefore, we focused on 
NADPH oxidase, which generates intracellular superoxide, and utilized its inhibi-
tor, apocynin for the suppression of prostate cancer carcinogenesis. In this study, 
we employed transgenic rats for adrenocarcinoma of prostate (TRAP) model. Male 
TRAP rats were randomized into 3 groups and given drinking water with apocynin 
(0, 100 and 500 mg/L) for 8 weeks. There were no toxic effects with apocynin 
treatment. The percentages and numbers of carcinomas in both ventral and lateral 
prostate were significantly reduced by apocynin treatment, with dose dependent 
manner. Reduction of reactive oxygen species (ROS) by apocynin was confirmed by 
immunohistochemistry of 8-OHdG and dihydroethidium staining. Positivity of 
Ki67 was significantly reduced by apocynin treatment. Down-regulation of clus-
terin expression and inactivation of the MEK-ERK1/2 pathway were detected in 
apocynin-treated group. In human prostate cancer cell line, LNCaP, apocynin 
also inhibited ROS production and blocked cell growth by inducing G0/G1 arrest 
with down-regulation of clusterin and cyclin D1. These data suggest that apocynin 
promotes a chemopreventive potential for prostate cancer.

### 2053 Penfluridol, an Antipsychotic Drug: A Treatment Option for Pancreatic Cancer

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Repurposing of old drugs as new anti-cancer drugs is important as it saves time and 
cost of drug development. Penfluridol is a first generation, highly potent antipsy-
chotic drug. In this study, we determined the anticancer effects of penfluridol in 
pancreatic cancer. Penfluridol treatment inhibited the growth of Panc-1, BxPC-3 
and AsPC-1 pancreatic cancer cells in a concentration-dependent manner. Panc-1, 
BxPC-3 and AsPC-1 cells treated with penfluridol exhibited apoptosis as 
evaluated by Annexin/FTTC assay and cleavage of caspase-3 and PARP. Our 
results showed that penfluridol treatment induced ER (Endoplasmic reticulum) stress 
in pancreatic cancer cells through the up regulation of ER stress markers like Bip/ 
Grp78, CHOP and IRE. Penfluridol treatment also induced autophagy in pan-
creatic cancer cells as observed by acridine orange assay. Western blot analysis of Panc-1, BxPC-3 and Apc-1 cells treated with penfluridol exhibited up-regulation of autophagy markers like LC3B, and p62. Microscopic analysis revealed punctate LC3B after penfluridol treatment in pancreatic cancer cells confirming autophagy. Interestingly, ER stress led to autophagy as ER stress inhibitors mitomycin and sodium phenylbutyrate or CHOP siRNA blocked penfluridol-induced autophagy. Furthermore, inhibiting autophagy by chloroquine, bafilomycin or LC3B siRNA significantly blocked penfluridol-induced apoptosis suggesting that autophagy leads to apoptosis in our model. Based on these observations, we conclude that penfluridol-induced ER stress leads to autophagy-mediated apoptosis in pancreatic cancer cells. To the best of our knowledge, our study for the first time demonstrates the anti-cancer effect of penfluridol against pancreatic cancer. Most importantly, penfluridol is already in clinical use with an established safety record; therefore any positive findings from our studies can be rapidly translated to the clinics for undertaking a clinical trial to treat advanced pancreatic cancer patients [Supported in part by R01 grant CA29038 awarded by NIH].

**2054 Chronic Oxidative Stress Induces Malignant Transformation of Human Kidney Epithelial Cells with Acquisition of Stem Cell Characteristics and EMT**

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Kidney cancer is highly fatal among all genito-urinary cancers and its incidence rate has been increasing since last decade. Multiple extrinsic risk factors have been reported for kidney cancer, but mechanisms associated with them are not fully understood. Oxidative injury to cellular macromolecules has been suggested as a common pathway shared by multiple risk factors, but there is no direct evidence for oxidative stress induced neoplastic transformation in human kidney cells. In this context, normal kidney epithelial cells (HK-2 cells) were exposed to non-cytotoxic (25 μM) and cytotoxic (250 μM) levels of hydrogen peroxide induced oxidative stress for 6 months and then evaluated for cell growth, survival and malignant transformation. The data of this study revealed that exposure to elevated but low level of chronic oxidative stress alone is sufficient to induce increased growth and neoplastic transformation of normal kidney epithelial cells. These changes were evaluated by gene expression changes in cell growth/survival and proliferation related genes using RT-PCR, cell cycle analysis by flow cytometry and anchorage independent growth by soft agar assay. Wound healing assay further revealed the increased migration potential of transformed cells. Up-regulation of cancer stem cell marker genes and colony formation of transformed cells in stem cell selective media also indicated acquisition of cancer stem cell characteristics during malignant transformation. Morphological changes like elongated spindle shaped appearance with up-regulation of mesenchymal markers and down regulation of epithelial markers also suggested epithelial to mesenchymal transition (EMT) in cells exposed to chronic oxidative stress. In summary, the findings of this study for the first time suggest that chronic exposure to elevated but low level of oxidative stress is sufficient to induce malignant transformation in kidney epithelial cells through acquisition of stem cell characteristics and EMT.

**2055 Exploration of Type I Interferon As a Novel Therapeutic Agent for Alveolar Rhabdomyosarcoma**


Bone and Soft Tissue Tumors (BSTT) are a group of neoplasms that preferentially target children, accounting for 15% of pediatric malignancies. Among BSTTs, rhabdomyosarcoma is the most common soft tissue sarcoma. Of the subtypes, alveolar rhabdomyosarcoma (ARMS) carries the worst prognosis, with a 5-year survival rate of 20% for metastatic cases despite multimodal therapy. Despite advances in understanding of the molecular and cellular aberrations underlying ARMS, this has not translated into new therapies. Our laboratory recently discovered that the TRE17/ubiquitin-specific protease 6 (USP6) oncogene is overexpressed in a highly restricted subset of neoplasms, which includes ARMS and other BSTTs. TRE17 encodes a TBC domain and a catalytically active USP. However little is known about how it causes transformation, how it is regulated, and whether its expression renders cells vulnerable to treatments. Addressing these questions was the goal of the current study. We identified a functional link between TRE17 and Type I interferon (IFN), a key physiological regulator of innate immunity. We found that overexpression of TRE17 rendered ARMS cells sensitive to apoptotic effects of IFN. Maximal death was observed after 24 hours of treatment. Death was not increased with longer treatment, suggesting a de-sensitization response. De-sensitization appears to derive from the post-translational downregulation of TRE17 by IFN: within 24 hours, expression of TRE17 protein was completely extinguished. Analysis of TRE17 mutants indicated that its USP activity, but not its TBC domain, was required for IFN-induced downregulation. Studies are currently in progress to find the mechanisms underlying TRE17 downregulation, with a focus on ubiquitin-mediated proteasomal targeting. Elucidation of this pathway may allow us to identify ways to sustain TRE17 expression, which would in turn enhance sensitivity to IFN-induced death. Since IFN is already approved for the treatment of a variety of other cancers, our studies will hopefully pave the way for testing it as a novel therapies for ARMS.

**2056 Diet Supplementation with Soy, but Not the Isoflavone Genistin, Protects against Alcohol-Induced Tumor Progression in DEN-Treated Male Mice**


In this study, DEN-treated male mice were assigned to 4 groups: a 35% high fat ethanol liquid diet (EtOH), an EtOH liquid diet with soy protein isolate as the sole protein source (EtOH/soy) an EtOH liquid diet supplemented with genistin (EtOH/GEN) and a chow group. EtOH feeding continued for 16 weeks. As expected, EtOH increased the incidence and multiplicity of basaloid lesions and adenomas compared to the chow group, p<0.05. Soy protein supplementation in the EtOH diet significantly reduced adenoma progression when compared to the EtOH and EtOH/GEN group, p<0.05. Genistin supplementation in the EtOH diet had no protective effect. Tumor reduction in the EtOH/soy group corresponded to lower serum ALT concentrations (p<0.05), decreased hepatic TNFα and CD-14 expression and decreased nuclear accumulation of NFκB protein compared to the EtOH group (p<0.05). EtOH consumption significantly impaired mitochondrial function through decreased expression of mitochondrial respiratory complexes and TFAM mRNA, which was partially reversed in the EtOH/soy group, p<0.05. Detection of sphingolipids using high resolution MALDI-FITICR imaging mass spectrometry revealed increased accumulation of long acyl chain ceramide species, sphingosine-1-phosphate, and glucosylceramide in the EtOH group that were significantly reduced in the EtOH/soy group. Chronic EtOH feeding also increased mRNA expression of β-catenin targets, including cyclin D1, MMP7 and glutamine synthetase, which were reduced in the EtOH/soy group, p<0.05. We conclude that soy prevents tumorigenesis by reducing pro-inflammatory and oxidative environment resulting from EtOH-induced hepatic injury, and by reducing hepatocyte proliferation through inhibition β-catenin signaling. These mechanisms may involve sphingolipid signaling. Supported in part by NCI R21 CA169389 (MJR).

**2057 Sunitinib Induces Growth Inhibition, Cell Cycle Arrest, Apoptosis, and Oxidative Stress in Human Breast Cancer MCF7 Cells**

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Sunitinib (SUN) is a new multi-targeted oral tyrosine kinase inhibitor that been recently approved against gastrointestinal stromal tumors and advanced renal cell carcinoma. Yet, the protective effect of SUN against breast cancer is poorly investigated. In this study, we have investigated the anti-cancer properties of SUN against breast cancer and the possible mechanism of action using MCF7 as an in vitro model. Treatment of MCF7 cells with SUN caused a dose-dependent cell growth suppression due to apoptosis. Apoptotic death by SUN in MCF7 cells was mediated by activation of caspase-3 and p53 mRNA and protein levels and increase in the percentage of apoptotic cells (40%) as determined by staining with annexinV-FITC and PI using flow cytometry. Mechanistically, blocking of de novo RNA synthesis by actinomycin D significantly inhibited the SUN-induced p53, but not caspase-3, mRNA indicating a transcriptional mechanism. This apoptosis-mediated inhibition of MCF7 cell growth is attributed to inhibition of the mRNA expression of cell cycle and proliferator genes (Cyclin D1, Cyclin E2, and E2F1) by SUN that significantly arrested MCF7 cells in the G2/M phase in the cell cycle machinery allowing upregulation of DNA repair gene expression such as x-ray repair cross-complementing protein 1 (XRCC1). In addition, quantitative polymerase chain reaction and Western blot analyses of MCF7 cells after treatment with SUN revealed a concentration-dependent induction of oxidative stress genes (heat oxygenase 1 and glutathione transerase, and Nr2) with marked inhibition of NF-κB at the mRNA and protein levels. These findings proposed that SUN suppressed the proliferation of MCF7 cells via cell cycle arrest and the induction of apoptosis through oxidative stress mediated pathways.

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2059 Impact of Long-Term Cigarette and Shisha (Water Pipe) Smoking on the Expression of Oxidative Stress and DNA Repair Genes in Healthy Subjects

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The present study was designed to evaluate the influence of long-term cigarette and water pipe (shisha) smoke on the expression of detoxifying and DNA repair genes. The study groups consisted of 45 subjects old who were divided into three groups; healthy non-smoker group (Control), cigarette-smoker group (Cigarette), and Shisha-smokers group (Shisha). A questionnaire and consent form was created according to the International Union of Pure and Applied Chemistry commission (IUPAC) and distributed to all groups. The study was approved by the College of Pharmacy Research Center Ethics Committee. Fresh blood samples were collected, and the total RNA was extracted from blood using the PAXgene Blood RNA Kit for isolation and purification (PreAnalytXQ, Qiagen) and mRNA expression levels of target genes were quantified by RT-PCR. The levels of several trace elements were measured using ICP-MS method. The demographic data showed that among the cigarette-smoker group, 80% smoke 20-39 cigarettes/day, whereas 12% smoke more than 40 cigarettes/day. With regard to water pipe smoke, the majority (46%) smoke more than 5 times/week. Blood analysis showed that a significant increase (50%) in the plasma levels of hydrogen peroxide was observed in cigarette-smoker group whereas no significant changes were demonstrated in shisha-smokers. In addition, the mRNA expression levels of DNA repair genes (OGG1 and XRCC1) were significantly inhibited in cigarette and shisha-smoker by approximately 30% and 60%, respectively. This was associated with a marked decrease (50%) in the expression of detoxifying genes such as GST. In addition, both cigarette- and shisha-smoker volunteers exhibited significant increase in the plasma concentrations of several toxic heavy metals such as Cd, Pb, and Ni. In conclusion: these findings clearly explore the genotoxic effect of cigarette and shisha smoking on human DNA.

2060 Exposure to Low-Dose Cadmium Enhances FL83B Cells Proliferation through Down-Regulation of Caspase-8 by DNA Hypermethylation

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Cadmium (Cd) is classified as a human carcinogen probably associated with epigenetic change. However, it’s underlying mechanism and role in epigenetic is still poorly understood. DNA methylation is one of epigenetic mechanisms by which cells control expression of various genes. Our previous in vitro experiment has shown that a Cd-stimulated FL83B cell malignant proliferation. When the FL83B cells were exposed to Cd at a low dose (0.085 μM) for just only 14 days, cell malignant proliferation, invasion and metastasis were significantly increased along with an elevation of DNA methyltransferase (DNMT) expression and activity, and significant decreases in mRNA and protein expressions of caspase-8 gene as well as cell apoptosis. Furthermore, caspase-8 gene promoter in Cd-exposed FL83B cells was hypermethylated, consistent with our in vitro experiment. A DNA methylation inhibitor, 5-aza-2’-deoxycytidine (5-aza-dC), prevented Cd-stimulated cell malignant proliferation, invasion and metastasis, along with the recovery of caspase-8 expression and activation. These results suggest that low-dose Cd may induce caspase-8 gene promoter hypermethylation, resulting in down-regulation of its expression and function, decrease in apoptotic cell death and, as a consequence, promoting cell malignant proliferation, invasion and metastasis, which may contribute to Cd-induced carcinogenesis.
Environmental exposure to carcinogens causes oncogene activation in many cancers and upregulation of growth signaling pathways. Targeting these pathways has proven to be an effective therapeutic strategy in many cancers, however metastatic prostate cancer prognosis remains poor. Combination treatment with amutavinitab, a receptor tyrosine kinase inhibitor, and erlotinib, an epidermal growth factor inhibitor, effectively decreases cyclin D1 protein levels, via different pathways, in LNCaP (PTEN-) and DU145 (PTEN+) human prostate cancer cell models. Current studies investigated 2+2 formalin fixation (2h 4°C, 2h 45°C) for the preservation of growth signaling proteins in response to amutavinitab and erlotinib treatment in LNCaP and DU145 mouse xenograft models. Results indicate 2+2 fixation significantly improves immunohistochemical (IHC) staining for cyclin D1, showing reduction due to drug treatment compared to traditional 24h (25°C) fixation. However, 2+2 fixation slightly decreased effective IHC for 4EBP1 pThr70 and pERK. Mechanistic studies to understand the modulation of cyclin D1 expression as a potential anticancer therapy revealed that pentoxifylline (PT, 1 mg/ml), a phosphodiesterase inhibitor, decreased cyclin D1 protein levels in LNCaP cells. Moreover PT reduced pAKT as well as downstream phospho-4EBP1, as indicated by western analysis. Interestingly, pAKT levels returned to baseline by 24h while phospho-4EBP1 and cyclin D1 remained decreased at 48h. In summary, 2+2 fixation is an effective technique to improve diagnostic IHC in prostate cancer. PT effectively inhibits the AKT pathway in prostate cancer cells although additional studies are required to determine its utility as an adjunct therapy in prostate cancer. (T32ES016652, T32ES007901, P50ES006949, AstraZeneca Studentship, Ventana-Roche).

Novel Phosphodiesterase 10 Inhibitor with Antitumor Activity in an Orthotopic Mouse Model of Lung Cancer


BACKGROUND: We recently reported that cyclic nucleotide phosphodiesterase 10A (PDE10) is overexpressed in colon tumors and essential for tumor cell growth. We are also studying the role of PDE10 in lung cancer and are developing a novel inhibitor designated as S-009, which we predict will have safety and efficacy advantages over currently available PDE10 inhibitors that are being developed for CNS disorders. OBJECTIVE: Evaluate pharmacokinetico, tolerance and anti-tumor efficacy of S-009 in mice. METHODS: C57BL/6 mice were orally administered with S-009 (100 mg/Kg) suspension. Free levels of S-009 were quantified in plasma and tissues by reverse-phase chromatography with tandem mass spectrometry detection. Anti-tumor activity was evaluated in athymic nude-Foxn1nu mice treated with S-009 (100 mg/kg/day) for 5 days before inoculating the left lung with 1x106 A549 (human alveolar adenocarcinoma cell line) or A549Luc (engineered A549 cells to express the reporter gene firefly luciferase) cells. Tumor growth was monitored by in situ bioluminescence using the In Vivo Imaging System. S-009 treatment continued for 4 more weeks. Anti-tumor efficacy of S-009 was evaluated by gross and histological grading of the lungs. RESULTS: S-009 displayed attractive oral bioavailability and reached high concentrations in lungs compared with plasma. S-009 treatment was well tolerated and reduced the incidence of lung tumors by 90% compared with vehicle treatment. CONCLUSIONS: This study supports a critical role of PDE10 in lung cancer and suggests that PDE10 inhibitors will be useful for the prevention or treatment of lung cancer.

Evidence on the Carcinogenicity of N-Nitrosomethyl-n-alkylamines


N-Nitrosomethyl-n-alkylamines (NMA) may form as a result of the reaction of nitrite with amine compounds. They have been detected in personal care and household cleaning products, such as shampoos, conditioners, dishwashing liquids and surface cleaners. N-Nitrosodiethylethylamine (NMA with a one carbon alkyl side chain, NMA-C1) and N-nitrosomethylethylamine (NMA-C2) are International Agency for Research on Cancer (IARC) Group 2A and 2B carcinogens, respectively. Other NMA have not been evaluated by IARC. The evidence on the carcinogenicity of NMAs as a group comes primarily from animal cancer bioassays conducted on thirteen NMA compounds. In total, more than 90 such studies were identified. These studies were conducted in rats, hamsters, mice, and guinea pigs via various routes of exposure. Tumors were observed following treatment with each of the thirteen NMA compounds in at least one species. Many of the observed tumors are rare, including tumors of the nasal cavity, tongue, oropharynx, esophagus, forestomach, kidney, and bladder in rats; of the nasal cavity, lung, liver, and bladder in hamsters; and of the nasal cavity, tongue, esophagus, and forestomach in mice. Additional evidence relevant to carcinogenicity comes from metabolism and genotoxicity studies, mechanistic data, and structure activity comparisons. NMAs are metabolically activated by cytochrome P450 enzymes, and are metabolized in a similar manner across chemicals and species. Several common metabolites have been observed amongst NMAs, including the carcinogenic metabolites N-nitrososarcoosine (for NMA-C2 and NMAs with longer alkyl chains) and formaldehyde. Positive findings from genotoxicity and DNA adduct studies indicate that NMAs are likely to operate through genotoxic mechanisms. Structure activity comparisons with carcinogenic N-nitroso-dialkylamines reveal common genotoxic and tumorigenic activities, with shared target tumor sites amongst chemicals and test animal species.
Sulfur mustard and nitrogen mustard (methylchlorohemate, HN2) are potent skin vesicants. As bifunctional alkylating agents, they cause oxidative stress and persistent tissue damage including blistering. In the present studies we determined whether activation of transcription factor nuclear factor-erythroid 2-related factor 2 (Nrf2) could mitigate HN2-induced cytotoxicity in mouse keratinocytes and if inhibition of multidrug resistance-associated protein (MRP) efflux transporters could suppress this protection. MRP1 and MRP2 export GSH-conjugated electrophiles from cells. HN2 causes injury by modifying biomolecules including GSH. Monofunctional GSH adducts, which contribute to cytotoxicity, can be exported by MRPs. Treatment of PAM212 mouse keratinocytes with HN2 inhibited cell growth (IC50 = 1.0 μM). Addition of sulforaphane (SFN) to cultured keratinoctyes activated Nrf2, which translocated from the cytoplasm to the nucleus and caused a marked increase in MRP functional activity. Pretreatment of the cells with 3 μM SFN for 3 hr protected against HN2-induced growth inhibition (IC50 = 13 μM). Protection was blocked by the MRP inhibitor, MK-571 (IC50 = 0.65 μM). SFN also protected wild type (WT) primary mouse epidermal keratinocytes from HN2 (IC50 = 1.4 and 5.2 μM without and with SFN, respectively), while SFN had no effect on keratinocytes from Nrf2−/− mice (IC50 = 0.33 and 0.14 μM without and with SFN, respectively). Protection of WT cells by SFN was inhibited by MK-571 (IC50 = 0.27 μM). These data show that MRP-mediated efflux is key in regulating HN2-induced growth inhibition in mouse keratinocytes. Enhancing MRP activity may represent a promising route to mitigate vesicant-induced cytotoxicity. Support: NIH AR055073; ES005022.
The calcium dependent serum hydrolase, paraoxonase 1, known for its ability to hydrolyze endogenous lactones, can hydrolyze some organophosphates (OPs) including some nerve agents and insecticides. This relatively inefficient detoxification requires an hydroxide ion from water as a nucleophile. A series of novel phenoxyalkyl pyridinium oximes have been synthesized and tested as more potent nucleophiles to potentially enhance the efficacy of PON1 in detoxifying toxic OPs. The OPs tested as substrates were sarin and VX, nitrophenyl isopropyl methylphosphonate (NIMP) and nitrophenyl ethyl methylphosphonate (NEMP), respectively. Novel nucleophiles were screened initially in commercially available pooled human serum with both NIMP and NEMP. Screening was done using a direct assay with 0.3mM NIMP or NEMP and 0.1mM nucleophile; the released 4-nitrophenol was monitored spectrophotometrically following a 15 min incubation. About 25 novel nucleophiles were tested. Additionally, an indirect screening assay with 120 nM NIMP or 30 nM NEMP was used to monitor residual anticholinesterase activity of undegraded surrogate as an index of PON1 activity. Enhancement of degradation was observed with 5 of the nucleophiles at high substrate concentration (direct assay) and with 8 of the nucleophiles tested with more realistic substrate concentrations (indirect assay) with both of the substrates. The indirect assay showed enhancement of NIMP degradation from 8-28%, and of NEMP degradation from 12-35%. Initial in vivo tests in rats with 3 nucleophiles administered immediately following exposure to NIMP or NEMP at sub-lethal dosages (0.2-0.3 mg/kg) indicate protection of brain and peripheral acetylcholinesterase from inhibition. These nucleophiles may have potential as a novel therapy for some organophosphates. (Supported by Defense Threat Reduction Agency HDTRA1-12-1-0043).
2075 Stability of Chloroethyl Ethyl Sulfide (CEES) and Mechlorethamine Hydrochloride (HN2) in DMSO, DMSO/Water Mixtures, and Complete Media


CEES and HN2 are commonly used surrogates for sulfur mustard (HD), a hydrophobic bifunctional alkylating agent. Like HD, CEES is quite hydrophobic – it cannot be added directly to aqueous media for testing in cell culture, necessitating the use of a non-toxic solvent. In the current study, we investigated the stability of 1.7 M CEES in DMSO under common laboratory conditions in order to develop best practices for its use in mammalian cell culture model systems. Using NMR, we found that CEES was stable in DMSO at room temperature and 37°C for at least 24 hours, whereas incubation at 70°C caused rapid degradation. Vortexing the CEES/DMSO mixture did not affect the rate of degradation. Because DMSO is highly hygroscopic and acquires atmospheric water over time, we measured the stability of CEES in a 10% D2O (v/v) DMSO solution. The addition of 10% D2O to a DMSO solution significantly increased the degradation of CEES. However, the amount of water found in benchtop deuterated DMSO was quite low (<0.5% m/m) and did not significantly affect the rate of degradation of CEES at room temperature. Finally, the stability of DMSO-dissolved CEES was tested in complete cell culture media using a cellular toxicity assay. CEES (10 mM) retained maximal toxicity for 10 minutes, after which time its toxicity decreased in a time-dependent manner. Similar studies were performed with 1.7 M HN2•HCl; HN2•HCl was found to be stable in D2O for more than 24 hours. Taken together, these data suggest that while CEES and HN2•HCl are relatively stable in DMSO and water, respectively, at room and physiological temperatures (37°C), they undergo rapid degradation once added to complete media.

2076 Potential Therapeutic Targets of Silibinin in Attenuating Nitrogen Mustard-Induced Skin Injury

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Exposure to chemical warfare agent sulfur mustard (SM) and its analog nitrogen mustard (NM) causes delayed blistering and incapacitating injuries to the skin tissue. The aim of the study was to evaluate the efficacy of silibinin against bi-functional alkylating vesicant Bis(2-chloroethyl)methylamine (NM)-induced skin injuries and associated potential targets in SKH-1 hairless mice. Topical application of silibinin (2 mg) 1 h after NM exposure caused a significant decrease in established NM-induced skin injury biomarkers in SKH-1 hairless mice. NM-induced lesions were assessed at 24, 72 or 120 h post exposure depending on their optimum injury. Silibinin treatment caused diminution of NM-induced increase in skin bi-fold thickness, epidermal thickness, microvesication, dead and denuded epidermis, and apoptotic cell death. Silibinin application also caused a 94% reversal in NM-induced increase in myeloperoxidase activity, which is indicative of neutrophil infiltration in inflammatory response. Furthermore, silibinin treatment caused a complete inhibition of NM-induced H2AX and p53 phosphorylation, indicating reversal of DNA damage. Similar silibinin treatment also reduced NM-induced increase in the levels of inflammatory and proteolytic mediators COX-2 and p38 (82% reduction). These findings in-duced increase in the levels of inflammatory and proteolytic mediators COX-2 indicating reversal of DNA damage. Similar silibinin treatment also reduced NM-induced increase in myeloperoxidase activity, which is indicative of the hyperplastic epithelium. Animals treated with Cx43aODN after NM exposure showed minimal K17 and continuous LM332 expression by IF at day 1. WB showed significant down regulation of Cx43 and pCx43 at days 3, 7 and 10. Dual labeling IF indicated that Cx43 aODN treatment successfully reduced Cx43 and pCx43 expression that was mostly restricted to the basal keratinocytes. Cx43 expression appeared more prevalent in both the wound margin and hyperplastic epithelium by day 3. The increased Cx43 expression may be associated with migratory keratinocytes to improve epitelialization. Overall, the use of Cx43aODN may alter the Cx43 expression profile to modulate gap junction communication and accelerate vesicant wound repair. Supported by ES009022, EY09056, and NIAMS U54AR055073.

2077 Connexin 43 Antisense Therapy Using a Nitrogen Mustard Hairless Mouse Skin Model

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Sulfur mustard [SM, Bis (2-chloroethyl) sulfide] and its analog nitrogen mustard [(NM) Bis(2-chloroethyl) methylamine] are vesicants which induces skin injury including edema, inflammation, separation of the dermal-epidermal junction (DEJ), prolonged wound healing, and scarring. Studies have shown that inhibition of the gap junction protein, connexin 43 (Cx43) impairs wound repair. Pharmacological inhibition of Cx43 (pC43) may disrupt gap junction communication during skin injury. We performed a time course study using NM-exposed SKH-1 mouse dorsal skin treated with Cx43 antisense oligodeoxyribonucleotides (aODN) and evaluated the wound healing response using several skin markers. Immunofluorescent (IF) studies on the NM-induced skin showed induction of K17 (a skin wound marker), a detached laminin 332 (LM332, basement membrane marker) at the DEJ, increased expression of Cx26 and pC43, and minimal expression of Cx43 at day 1. All markers were upregulated with time as the wounds progressed. Western blot (WB) analysis confirmed the increased levels of Cx43 and pC43 over time after NM exposure. Dual-labeling IF studies indicated strong expression of Cx26 near the leading edge of the wound. In contrast, Cx43 and pC43 were highly expressed in the hyperplastic epithelium. Animals treated with Cx43aODN after NM exposure showed minimal K17 and continuous LM332 expression by IF at day 1. WB showed significant down regulation of Cx43 and pC43 at days 3, 7 and 10. Dual labeling IF indicated that Cx43 aODN treatment successfully reduced Cx43 and pCx43 expression that was mostly restricted to the basal keratinocytes. Cx43 expression appeared more prevalent in both the wound margin and hyperplastic epithelium by day 3. The increased Cx43 expression may be associated with migratory keratinocytes to improve epitelialization. Overall, the use of Cx43aODN may alter the Cx43 expression profile to modulate gap junction communication and accelerate vesicant wound repair. Supported by ES009022, EY09056, and NIAMS U54AR055073.

2078 ROS-Mediated Induction of UPR Signaling Underlies Pathogenesis of Lewisite-Induced Skin Lesions


Lewisite (dichloro (2-chlorovinyl) arsine) is an potent arsenic-based chemical warfare agent known to induce painful cutaneous inflammation and blistering. It was developed during World War I as a potent chemical weapon. However, there is no effective antidote so far described. In this regard, the discovery of effective antidotes for lewisite was hampered by the lack of the exact molecular mechanism underlying its cutaneous pathogenesis. Here, we show that topical application of lewisite induced potent acute inflammation and microvesication associated with the generation of reactive oxygen species (ROS) and massive apoptosis in the skin of Pich/+/SKH-1 hairless mice. This murine model was found to be much more sensitive to arsenicals-induced cutaneous inflammation and blistering. We also found that lewisite-induced skin lesions in these mice were regulated through the activation of ROS-dependent UPR signaling pathway. A dose-dependent up-regulation of UPR signaling, inflammatory response and apoptosis was also observed in lewisite-treated human skin keratinocytes. Treatment of lewisite-exposed mice with chemical chaperone 4-phenylbutyric acid (4-PBA) or antioxidant N-acetylcysteine (NAC) attenuated lewisite-induced skin injuries. The inhibition in these pathobiological responses of lewisite was associated with the decreased production of ROS and reduction in inflammatory and UPR signaling pathways. This study unraveled a novel molecular mechanism involved in the cutaneous pathogenesis of lewisite-induced lesions and identified therapeutic targets for lewisite-mediated cutaneous inflammation and vesication

2079 Efficacy and Pharmacokinetic Studies of New Potential Cyanide Countermeasures in a Mouse Model

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The present Cyanide (CN) therapies of Nithiodote(TM) [sodium thiosulfate (TS) and sodium nitrite (SNI)]; and Cyanokit® [hydroxy-cobalamin (HCo)] have i.v. administration, and disposition limitations. Conversion of CN to the less toxic thiocyanate in the presence of a sulfur donor (SD) such as TS; scavenging of CN by scavengers such as HCo or SN-generated methemoglobin; and competition for enzymatic binding sites by SN-generated nitric oxide constitute major antidosal mechanisms. These investigations focus on formulation, in vivo efficacy, and PK studies of the reactive SD, (SDX), in combination with the HCo analogue cobinamide (Cbi). Both alone and in combination they overcome the limitations of the two present CN therapies. A 15% Polysorbate 80 formulation provided rapid i.m. absorption for the highly lipid soluble SDX, and it was also effective for the combination of SDX and Cbi. The in vivo efficacy studies on a mice model (Dixon Up and Down Method) showed over 2XLD50 protection against CN with SDX alone (100 mg/kg doses), and over 3XLD50 protection when applied with Cbi (20-250 mg/kg doses of various Cbi derivatives). There were also efficacy investigations on SOT 2015 Annual Meeting
various combinations of Cbi/SDX/TS/SN in a mouse model. The i.m. administered SDX and Cbi showed statistically significant improvements in antitodal efficacy over i.m. administered TS and SN. These results support the continued investigation of SDX and Cbi as next-generation cyanide countermeasures for mass casualty scenarios.

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2080 Hazard Assessment of VX Contaminated Remains

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The capability to adequately decontaminate and then transport chemically contaminated human remains from the battlefield to the United States is critical. Proof that the decontamination steps taken are adequate to allow the processing, preparation and return of decedents is vital to the safety of the mortuary affairs staff processing the decedents, both in theater and upon return to the United States. This study was designed to be a proof of concept study to determine the hazard remaining from a VX dermal contamination both with and without decontamination. Eight Yorkshire swine were grossly contaminated with VX and placed in cold storage 24 hours post-death (and post-decontamination, if applicable) for 1 week to determine the hazard. During the 1 week time period, head space (analyzed for VX) and wipe samples (analyzed for VX and EA2192) were collected at 24, 72, 120 and 168 hours post-death. Of the eight animals, four were decontaminated in accordance with the current Mortuary Affairs Contaminated Remains Mitigation Site (MACRMS) protocol, which consists of a soapy water wash and rinse and then a 60 minute bleach soak. The remaining four animals were control animals and did not receive decontamination. Significant hazard mitigation was required to perform this work safely, resulting in numerous dry runs and safe techniques practiced prior to study initiation. Overall, the decontamination steps taken did significantly reduce the contact and inhalation hazard when compared to the animals that were not decontaminated and both hazards decreased over time; however, a significant inhalation and contact hazard was still present one week post-death in both sets of animals. At one week post-death, the contact hazard of decontaminated animals was 40 times the no observable adverse effect level (NOAEL) and the inhalation hazard was 56 times the short term exposure limit (STEL) for VX. In comparison, control animals were 2400 times the NOAEL and approximately 19,000 times STEL at one week.

2081 Improved Methodology of Determination of Protein Adducts of Nerve Agents in Blood Plasma


Analysis of biomedical samples for biomarkers of exposure to chemical warfare agents is an integral part of activities associated with verification of implementation of the CWC. At present the OPCW is developing a system of designation of laboratories from OPCW Member States for biomedical sample analysis. To this end, Biomedical Confidence Building Exercises (BCBE) have been conducted since 2009 with the aim to compare different methods of analysis of biomarkers of exposure and develop recommendations on the most rational approaches. By present 4 exercises have been conducted, and tasks were complicated from exercise to exercise: from detection of hydrolytic metabolites of nerve agents and sulfur mustard in urine and plasma, spiked at 100 and 1-10 ng/ml, and nerve agents fluoride-regenerated from their plasma protein adducts to detection of adducts of nerve agents (GD, Vx) with butyrylcholinesterase (BChE) and albumin in plasma at spiking levels of 1-10 ng/ml. We present approaches used at our institute for analysis of samples in the framework of the BCBE with special focus on our developed or improved procedures. Vx-adducted BChE, as well as aged BChE adducts of GD and Vx (single analyte), were analyzed as nonapeptides FGES(ThylyMPA) AGAAS and AGES(MPA)AGAAS, respectively, by improved procedure, involving immunomagnetic bead separation, pepsinolysis, and detection by LC/ESI–HRMS/MS. The high sensitivity and selectivity of the developed procedures allowed detection of aged Vx-BChE adduct and Vx-Tyr in plasma samples spiked with Vx at 8 and 5 ng/ml about 30 days before analysis.

2082 Clinical Manifestations of Soman (GD) Poisoning following Dermal Exposure in the Large Pig

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Nerve agents are a group of highly toxic compounds that inhibit cholinesterase. Skin penetration studies involving the use of nerve agents to evaluate the efficacy of potential medical countermeasures use animal models as representative models for man. Traditionally, these models have included the rat, mouse and guinea pig. However, the pig is generally accepted as being the most representative model for human percutaneous exposures due to similarities in anatomical make up. Damaged skin offers a diminished permeability barrier against chemical penetration compared to undamaged skin. Nerve agent contamination on damaged skin will give rise to enhanced toxicity via the dermal route. The purpose of this study was to examine the clinical manifestations of the nerve agent soman (GD) after application to damaged ear skin in a terminally anaesthetised large white pig. Onset of signs could be grouped into early (mastication, fasciculations and tremors), intermediate (miosis, salivation and nasal secretions) and late onset (lacrimation, body spasms and apnoea) effects. There was a rapid depletion of whole blood cholinesterase in GD poisoned animals with animals decreasing to less than 5% of baseline values by 10 minutes post exposure. GD poisoning caused an increase in blood glucose, carbon dioxide, potassium, lactate, haematoctit and haemoglobin levels. This work was funded by the Health Protection Agency (HPA) and conducted in support of the “Haemostatic Decontaminants for Penetrating Injuries Contaminated with CW Agents” project (DTRA Project ID Number 2.F0026_08_RC_C) and carried out at the Defence Science and Technology Laboratory (Dstl). © Crown copyright 2014. Published with the permission of the Defence Science and Technology Laboratory on behalf of the Controller of HMSO.

2083 Reversing Acute Cyanide Lethality by Intranasal Delivery of Stabilized Isoamyl Nitrite


Acute cyanide (CN) poisoning can occur if CN is used as a chemical weapon and can prove lethal when not treated rapidly and effectively. Current U.S. approved antidotes require intravenous (IV) administration which limits fast multi-victim treatment. However, an antidote designed for nasal delivery in metered doses could rapidly treat and save many lives. SwRI, with funding provided by the U.S. Department of Health and Human Services, Office of the Assistant Secretary for the Preparedness and Response, Biomedical Advanced Research and Development Authority, under Contract HHSO100201100038, is currently developing an intranasal formulation of stabilized isoamyl nitrite (SIAN) as an antidote for treatment of CN poisoning. Beneficial effects of SIAN are related not only to methemoglobin (MetHb) formation but also to its vasogenic effects. In order to evaluate safety and efficacy of SIAN against a lethal dose of CN, a nasal SIAN instillation method and a potassium CN (KCN) challenge rat model were developed. KCN was administered by IV infusion over a 15-min period. KCN doses were successfully tested and lead to sub-lethal (low) and lethal (mid-high and high) effects. Typical of a steep CN dose response, at 3 mg/kg KCN 8/8 animals survived, whereas 8/8 and 7/8 animals in the 3.81 and 3.875 mg/kg KCN respectively, died 12-20 min after initiating the infusion. The clinical endpoints showed CN dose- and time-dependent relationships; a bioanalytical method was developed to measure CN in plasma and allowed for the selection of optimal time points for PK and endpoint analyses. SIAN, at a single dose of 375 mg/kg when administered at 1, 5, 10, or 12 min after the start of a lethal IV KCN infusion (3.81 mg/kg) in rats yielded ~80% efficacy, which was based on survival. Cardiopulmonary, blood gas, MetHb and PK endpoints were also measured. Studies are planned to develop an NHP model, define maximum tolerated dose (MTD), and no observed adverse effect level (NOAEL) in preparation for a Phase 1 clinical trial.
2084 Guinea Pig Cortex Proteome Pathway Analysis after Atropine and Pralidoxime Use Can Counteract Effects to Acute Sarin Exposure

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Sarin (O-isopropyl methylphosphonofluoridate; GB) is a toxic organophosphorous nerve agent developed during WWII that acts as a potent inhibitor of acetylcholinesterase (AChE). GB has been used in both military and civilian attacks. Our objective was to elucidate changes in the guinea pig cortex broad-spectrum proteome following acute GB exposure, and provide protein modulatory effects occurring after use of the current countermeasures atropine (ATR) and pralidoxime (2PAM). Male (n=16) and female (n=16) guinea pigs were equally divided into A) non-treated control group, and exposure groups B) GB(2×LD50) C) GB+ATR(4mg/kg), D) GB+ATR+2PAM(25.7mg/kg). Biometric analysis included clinical parameters and blood pathology. Proteomic analysis was applied to explore the response of the cortex after experimental termination or death at 30 min (groups B, C) and at 24h euthanasia of survivors (group D). Tandem Mass Tags (TMT) liquid chromatography mass spectrometry (LC-MS/MS) quantitative proteomics was applied for protein identity and quantitation and follow-up confirmation for key proteins was performed by Western blot. TMT LC-MS/MS quantitative proteomics detected 986 proteins (male samples) and 1013 proteins (female samples) of which identities of 575 and 487 proteins, respectively, were converted by the BioMart portal and Cytoscape 3.1.0 into GeneWiki identifiers. Use of cytoscape gene ontology pathway analysis using ReactomeF4.1.0. and ClueGo2.1.2 isolated distinct signaling pathway interconnections related to atropine vs. the higher effective combination of atropine and 2PAM. The gained information should aid in understanding of the complex cellular proteomic responses following high sarin dose for development of higher efficacious therapeutics against sarin poisoning.

2085 Inflammatory Cell Accumulation in Mouse Skin following Exposure to Sulfur Mustard


Sulfur mustard (SM) is a bifunctional alkylating agent that causes skin erosions and blistering. We characterized the early stages of leukocyte infiltration into mouse skin following exposure to SM. The dorsal skin of female SKH-1 hr mice was exposed for 6 min to SM or control. After 1, 3, 5, 7, 14 and 21 days, skin sections were analyzed for inflammation and injury. One day post SM, epidermal thickening, stratum corneum shedding and basal cell karyolysis was evident. Neutrophils (Ly6g+ cells) accumulated in the dermis, while macrophages (F4/80+ cells) were localized in the hypodermis. CCR2, a type 2-C-C chemokine receptor important in macrophage trafficking to sites of injury, was expressed in inflammatory cells within the hypodermis, in dermal fibroblasts, and in cells surrounding hair root sheaths. By 5 day post-SM, there was a 150% increase in wound thickness when compared to controls; this was correlated with the loss of epidermal structures and increased numbers of Ly6g+ cells within the eschar and F4/80+ macrophages beneath the eschar. CCR2+ dermal fibroblasts and inflammatory cells were scattered throughout the dermis. After 5 days, wound thickness increased to over 250% of control with epidermal hyperplasia, pyknotic and bird-eye nuclei in the basal layer and inflammatory cells in an edematous dermis. Seven days post-SM, a neo-epi-

2086 Attenuation of Nitrogen Mustard (NM)-Induced Pulmonary Injury, Inflammation, and Fibrosis by Anti-Tumor Necrosis Factor (TNF) Alpha Antibody

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NM (mechlorethamine) is a bifunctional alkylating agent known to cause acute injury to the lung which progresses to fibrosis. This is accompanied by a prominent infiltration of macrophages into the lung and upregulation of proinflammatory/proliferotic cytokines including TNFα. In these studies we analyzed the ability of anti-TNFα antibody to mitigate NM-induced lung injury and fibrosis. Treatment of male Wistar rats (250 g) with NM (0.125 mg/kg, i.t.) resulted in progressive histopathologic/fibrotic changes in the lung 3-28 d post exposure. Bronchoalveolar lavage (BAL) protein and cell content also increased after NM, along with expression of hemeoxygenase (HO)-1, indicating damage to the alveolar-epithelial barrier and oxidative stress. This was associated with increased numbers of inflammatory (CD11b+) macrophages in the lung. Whereas early after exposure (3-4 d), these cells were proinflammatory/cytotoxic, as reflected by expression of cyclooxygenase (COX)-2, inducible nitric oxide synthase (iNOS) and TNFα, subsequently they exhibited a phenotype of proinflammatory macrophages (YM-1+, galectin-3+, CD68+, CD163+, TGFB3). Treatment of rats with anti-TNFα antibody (15 mg/kg, i.v., every 9 d) beginning 30 min after NM significantly reduced lung injury, inflammation and oxidative stress. NM-induced fibrosis, assessed by trichrome staining, was also reduced. This was correlated with reduced numbers of proinflammatory and proliferotic macrophages in the lung. These data demonstrate that inhibiting TNFα is an important approach to mitigating acute and long term lung injury induced by vesicants. Supported by NIH Grants HL096426, AR055073, ES004738, CA132624 and ES005022.

2087 Confocal Raman Microspectroscopy: The Measurement of VX Depth Profiles in Hairless Guinea Pig Skin and the Evaluation of RSDL


The nerve agent VX is a potent organophosphorous compound that is extremely toxic. VX depth profiles were obtained using a confocal Raman microspectrometer (River Diagnostics) that used a specially designed microscope objective to focus low power laser light into the skin. Noninvasively and in real-time, the scattered light was collected to a depth of about 100 μm with an axial resolution of approximately 4 μm. The objectives of this study were to use confocal Raman microscopy to investigate the fate of percutaneously applied VX in the upper skin layers of hairless guinea pigs and to determine the ability of Reactive Skin Decontamination Lotion (RSDL) to remove VX from the skin surface and from a depot that forms below the skin surface. A total of 20 anesthetized hairless guinea pigs (337-456 g) were exposed to near VX (0.3 μl, 13-14 x LD50) using a specially designed template that allowed repeated Raman measurements on the same skin location. Animals were given a bioscavenger to protect against signs of VX toxicity. Raman depth profiles were recorded preexposure, at various times postexposure, and following decontamination with RSDL. Animals were euthanized no later than 10 hr. postexposure. In some animals, exposure site skin punches were collected after euthanasia and analyzed for VX. VX was observed to form a depot in the stratum corneum, 0 - 20 μm deep, in about 30 minutes postexposure. This depot decreased with time but was still detectable at 48 hr. postexposure. RSDL was observed to remove VX from both the skin surface and the depot below the skin surface. Our conclusions were that confocal Raman microspectroscopy is an effective tool to obtain real-time, non-invasive analytical data on the concentration of chemicals in the upper skin layers and that RSDL effectively removes VX from the skin surface and from the depot formed in the upper skin layers following percutaneous exposure.
Sulfur mustard (HD) is a powerful bi-functional vesicating chemical warfare agent. Inhalation is a highly toxic route of exposure for HD, for which there are currently no approved medical countermeasures. The mechanism of HD toxicity is not completely understood, but is believed to cause tissue injury through overproduction of reactive oxygen species resulting in oxidative stress. AEOL10150 is a manganese-containing porphyrin, possessing broad catalytic antioxidant activity. To evaluate the efficacy of AEOL10150 in suppressing HD toxicity, 1.4mg/kg HD was delivered directly into the lungs of anesthetized rats, with subcutaneous administration of either AEOL10150 (5 mg/kg) or saline beginning 1 hour post HD exposure (HPE). Upon euthanasia (6, 12, 18, or 48 HPE), blood samples were taken and lungs were either fixed or lavaged/frozen for analysis. In addition to tissue samples, data was also collected from noninvasive, live animal monitoring techniques including pulse oximetry, heart rate, and clinical scores to assess morbidity over time. Our results show that AEOL10150, when used as a medical countermeasure, was effective at improving markers of HD inhalation morbidity and mortality in a dose dependent manner. Survival was improved from 35% (controls) to 89% (AEOL10150, Q4H). AEOL10150 administration (Q4H) also demonstrated an improvement in clinical markers, including pulse oximetry (23% increase), heart rate (21% increase), and clinical scoring (62% improvement) at day 1. AEOL10150 was further evaluated against a lethal HD aerosol (1X LD50, intratracheal) dose with the most notable changes occurring at the 1 hr post-exposure time point. Fifty major functional classes of proteins exhibited changes in their phosphorylation status: 1) Ion channels/transporters, including ATPases, 2) Kinases/phosphatases, 3) GTPases, 4) Structural proteins, and 5) Transcriptional regulatory proteins. This study is the first quantitative phosphoproteomic analysis of HD toxicity in the brain. Understanding the toxicity and compensatory signaling mechanisms will improve the understanding of the complex toxicity of VX in the brain and aid in the elucidation of novel molecular targets that would be important for development of improved countermeasures.
PK studies. PK in miniswine strains as an important factor in selecting the relevant strain for importance of considering the impact of metabolic and dispositional differences on wine may be due to stereo-selectivity of protein binding. This work highlights the 2) versus ± 7) or Yucatan (9 ± to R,S-VER ratio was not different for Hanford (6 ± and Sinclair (18 and min/kg). N-demethylation of R,S-VER to form R- and S-Norverapamil (NOR) test). The clearance of R- and S-VER was significantly lower in Sinclair (18 and 10.1 mL/min/kg) as compared to other strains (8.7 to 10.1 mL/min/kg, p<0.0001 Dunnett’s ties were higher in intra-cranial tumor than healthy brain. Efficacy of capecitabine and its prodrug metabolites was observed in brain metastases compared to healthy brain. Integrated exposure of capcitabine and its nucleoside prodrugs in brain metastases were 12-39% of plasma level and 3.5-fold higher than healthy brain (P=0.05). The 5-FU levels in brain metastases were 6.7 times higher than in brain (P=0.05) with Cmax approaching values associated with antitumor activity in vitro. Cytidine deaminase and thymidine phosphorylase enzyme activities were higher in intra-cranial tumor than healthy brain. Efficacy of capcitabine in intra-cranial tumor was assessed by measuring inhibition of thymidine synthase enzyme and induction of apoptosis in tumors. These biomarkers of efficacy were compared with metabolite levels.

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samples was strongly associated with tissue macromolecules in male rats following gavage exposure. For these reasons, additional studies will be performed in adult mice and pregnant rats to assess potential toxicity following repeat exposure.

2100 The Disposition of 2-Ethylhexyl Tetrabromobenzoate (TBB) in Female Sprague-Dawley Rats after Administration of Single or Repeated Doses by Gavage


2-ethylhexyl tetrabromobenzoate (TBB) is an emerging brominated flame retardant (BFR) used to replace highly persistent and bioaccumulating BFRs, e.g. polybrominated dibenzyl ethers. TBB is readily absorbed as an additive BFR in polyurethane foam. As a consequence of use, TBB has been detected in household dust and in tissues of birds and marine mammals. The present study was designed to investigate the fate of TBB in female Sprague-Dawley rats, with emphasis on characterizing internal dose and the potential for accumulation in tissues. Single or repeated 14C-labeled doses of 0.1 μmol (0.06 mg) TBB/kg were administered in corn oil by gavage to rats. Results indicated that TBB was rapidly absorbed from the gut and excreted primarily as metabolites in urine and feces. TBB-derived 14C was detected in all assayed tissues one hour following a single dose. Blood contained 6% total dose at the 1 h timepoint, followed by muscle, liver, kidney, adipose, and skin, each containing 1-2% of the total dose. The C_{TBB} for 14C-TBB equivalents in these tissues was observed four hours after dosing. The major tissues contained approximately 36% of the total dose at the 4 h timepoint. The highest concentration (0.5 nmol TBB-equivalents/g) was observed in the kidney. The amount of TBB-derived 14C in the major tissues declined over time to approximately 26, 5, and <1% of the total dose at timepoints of 8, 24, and 72 h, respectively. The effect of repeated dosing on the disposition of TBB was investigated 24 hours following five consecutive daily doses of 14C-labeled 0.1 μmol TBB/kg. Results indicated minimal potential for accumulation in tissues in the rat at this dose. These data will be used for continued risk assessment of TBB. The present work was supported by the Intramural Research Program of the National Cancer Institute [Project ZIA BC 011476].

2101 Interspecies Pharmacokinetics of Atrazine and Its Chlorotriazine Metabolites

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Detailed pharmacokinetic studies have been conducted in rodents, non-human primates and humans in order to update a previously published physiologically based pharmacokinetic (PBPK) model for the rat. The model was revised with in vitro data, including partition coefficients and quantification of the metabolism of atrazine and the chlorotriazine metabolites, desipropylatrazine, deethylatrazine and diaminochlorotriazine. In vivo plasma pharmacokinetics in female Sprague-Dawley rats and cynomolgus monkeys were used to parameterize the PBPK model for the rat and monkey. The model successfully predicted rat plasma concentrations in both the 4-day repeat dose oral gavage study (3, 10 and 50 mg/kg/day) and the 4-day dietary exposure study (30, 100 and 500 ppm). The model also successfully predicted the monkey pharmacokinetics across dose, vehicle and route (oral and IV). Plasma pharmacokinetics of total chlorinated triazines (TCT) were qualitatively similar between the rat and monkey in both total exposure and elimination. The human model was used for prediction of human biomonitoring equivalents and exposure assessments. The PBPK model was used to predict TCT concentrations in human plasma and urine for the 99.97th-centric potential human exposure to atrazine from community water systems. When compared to TCT concentrations predicted at the EPA proposed BMDL of 2.56 mg/kg-day, margins of exposure were greater than 1,000. The similarities of atrazine pharmacokinetics across species provide confidence in the PBPK model for use in human health risk assessment.

2102 Using Toxicokinetic Data to Set Dose Levels for Regulatory Testing

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Advancements in scientific approaches and analytical techniques have allowed for a shift in the paradigm of regulatory toxicity testing using kinetically derived maximum doses (KMD) versus traditional limit or maximum tolerated doses (MTD).
by increased muscle, decreased plasma, and no change in biliary concentrations. Although Cyp3a and Cyp2c11 proteins were decreased in NASH, no alterations in SIM metabolism were observed. These data, in conjunction with previous data showing that human NASH causes a coordinated down-regulation of hepatic uptake transporters, suggest that NASH-mediated transporter regulation may play a role in altered SIMA disposition and the occurrence of myopathy.

2105 Impact of Major Efflux Transporters on the Disposition of Irinotecan in Rats in Different Metabolic Organs: An In Vivo Study

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Irinotecan (CPT-11), a semi-synthetic derivative of camptothecin, is currently used as a major component of the frontline therapy to treat advanced metastatic colon cancer. However, it elicits pronounced side effects such as late onset diarrhea and neutropenia that limit its efficacy. Different mechanistic studies indicate that excessive biliary excretion of SN-38, an active metabolite of CPT-11, is responsible for causing late onset of diarrhea in approximately 40% of patient population. In this study we investigated the contribution of different major efflux transporters in three different major metabolic organs (liver, kidney and intestine) on the disposition of CPT-11. We infused 5 mg/kg CPT-11 for 150 min through jugular vein of male wistar rats (n=4) along with cyclosporine (4 mg/kg i.v.), erlotinib (50 μM p.o.) and cimetidine (50 mg/kg i.v.) to assess the effect of P-gp, Mrp2, Bcrp and MATE transporters on CPT-11 disposition. Bile, urine, perfusate and plasma samples are collected at 0, 30, 60, 90, 120, 150 min and quantified by QTQRP 5500 UPLC-MS/MS, respectively. Our data showed that co-administration of cyclosporine, erlotinib and cimetidine decreases the biliary and intestinal excretion of CPT-11, SN-38 and its phase II metabolite SN-38 glucuronide significantly which implicates the involvement of P-gp, Mrp2, Bcrp and MATE on the disposition of CPT-11 in liver and intestine. However, compared to other inhibitors, only cimetidine decreases the urinary excretion of CPT-11 which signifies the role of MATE transporters on the efflux of CPT-11 in kidney. From these results, it is quite evident that modulation of P-gp and MRP2 transporters in liver and intestine of CPT-11 treated patients may be a viable strategy to reduce CPT-11 associated gastrointestinal toxicity.

2106 Electroencephalography (EEG) in Sprague-Dawley Rats and Cynomolgus Monkeys: Super-Intervals to Increase Model Sensitivity

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Introduction: Application of super-intervals to EEG data from rats and non human primates is not commonly used due to decreased sensitivity. Method: EEG data from 52 Sprague Dawley rats and 8 cynomolgus monkeys were analyzed to assess the effect of super-intervals on model sensitivity between day and night time. Results: In comparison, respiratory rate, tidal volume and heart rate in cynomolgus monkeys, increasing EEG data bins showed optimal variability reduction with a 60 min bin duration, with lower variability during night-time compared to daytime. In comparison, respiratory rate, tidal volume and heart rate in cynomolgus monkeys showed progressive improvements up to an interval duration of 480 min. In Sprague Dawley rats, EEG data bins also showed optimal variability reduction at 60 min without significant differences between day and night time. In comparison, optimal data bin durations for respiratory rate and tidal volume in rats was observed with bin duration of 5 min, with progressive improvements up to an interval duration of 480 min for heart rate. Discussion: Super-intervals decrease variability for respiratory, cardiovascular and EEG data. The benefit of increasing in bin duration should be weighed against the potential dilution of drug effects with unaltered data. The current analysis supports a bin duration of 60 min for EEG data in rats and non human primates when pharmacologically relevant.

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Introduction: Implanted EEG monitoring by telemetry was reported in non-clinical studies but non-invasive with freely moving EEG recordings by telemetry as used in humans was not previously reported in non-rodents. Methods: A system for non-invasive continuous EEG-video monitoring by telemetry was qualified in dogs and non human primates. EEG traces were obtained in Beagle dogs and cynomolgus monkeys using short electrophysiology needles in a Cz-Oz configuration from the 10-20 system connected to a telemetry transmitter. An intravenous infusion (1.7 mg/kg/min) of pentylenetetrazol (PTZ) was used to characterize the model for seizure detection. Results: The incidence of signal artifacts was low (<5%) of total EEG traces and adequate for EEG interpretation. Non-invasive EEG monitoring was associated with a higher incidence of signal artifacts than surgically implanted EEG telemetry. The power of the EEG spectral bands was lower than values obtained from surgically implanted EEG leads but with comparable ratios between each band (delta, theta, alpha, sigma and beta). EEG traces showed normal profiles for the species evaluated with circadian changes. Upon PTZ infusion onset, paroxysmal EEG activity was observed with the expected trace morphologies (e.g. isolated sharp waves, repeated sharp waves, increased synchrony). PTZ induced ictal activity showed expected characteristics with a dominant frequency at Fast Fourier Transform (FFT) ranging from 3-6 Hz in dogs and monkeys. Conclusion: Non-invasive EEG monitoring by telemetry in freely moving animals was confirmed to be adequate to monitor normal, pre-ictal and ictal CNS activity in conscious Beagle dogs and non human primates.

2108 An In Vitro Model for Probing Species-Dependent Microglial Activation and Toxicity

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Different species may show differential microglial activation and toxicity in response to compound treatment. An in vitro model has been established to assess the potential for species-dependent microglial activation using murine microglial cell lines and primary adult rat microglia culture to interrogate differences in inflammatory cytokine production, morphology and viability using known Toll-Like Receptor (TLR) agonists. Established EOC20 and C8B4 murine microglial cell lines were used to study cytokine production upon stimulation with LPS and the TLR7/8 agonist R848. EOC20 is known to be TLR4 deficient; however there are no such documented TLR deficiencies in C8B4. Both cell lines were cultured and stimulated with varying amounts of LPS and R848. Following treatment, supernatants from these cultures were collected at 2, 6, and 24 hour time points, and cytokine production was determined. Upon stimulation with LPS, EOC20 cells released smaller amounts of cytokines (such as TNF-alpha and IL-beta) than C8B4 cells. In response to R848, EOC20 cells, in general, produced greater amounts of inflammatory cytokines than C8B4 cells. Morphology and viability were not affected by stimulation of TLR agonists when tested at 24 hours after stimulation. To compare species differences in cytokine production, primary rat microglia were isolated and stimulated with LPS and R848. Primary rat microglia produced more IFN-gamma, IL-1-beta, and IL-12 than the EOC20 and C8B4 cells. However, viability was decreased and morphology changes were seen in primary microglia, which was not seen in the murine microglia cell lines. The variations in response to treatment observed in this work may be linked to cell lines, including differential expression of TLR receptors, versus primary cells as well as species differences. Therefore, additional primary cell culture of various species will be further developed for assessing species specificities.

2109 Microglia Mediate Diesel Exhaust Particle-Induced Cerebellar Neuronal Death

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Diesel exhaust particulate matter (DEP) is a complex mixture of numerous toxicants and is a major contributor to air pollution. While negative effects on the cardiovascular and respiratory systems are well-established, emerging evidence has linked air pollution to central nervous system disease. Limited studies have shown that microglia, the resident immune cells of the brain appear to play a role in mediating DEP-induced dopaminergic neuron dysfunction. Here we investigate whether microglia play a similar role in mediating cerebellar granule neuron (CGN) death. Primary mouse CGNs (PND7, 0.6 x 10^6 cells/2 cm^2) were grown in culture for 10 days, treated for 24 hours with DEP (25, 50, 100 μg/cm^2) and levels of lactate dehydrogenase were measured to assess cell death. DEP had no effect on the viability of CGNs at any concentration; however, in the presence of primary microglia, co-treatment with DEP (50, 100 μg/cm^2) induced a 2 to 3-fold increase in CGN death. When activated, microglia release factors which may contribute to neurotoxicity, such as inflammatory mediators and reactive oxygen species (ROS). To test the ability of DEP to activate microglia, primary microglia were treated for 24 hours with DEP (50 μg/cm^2), or size-filtered DEP (< 0.22 μM). Fluorescent imaging after immunocytochemically labeling for IBAl showed morphological changes indicative of microglia activation. DEP treatment (50 μg/cm^2) of microglia also resulted in a greater than 2-fold increase in ROS at 1 and 2 hours. Treatment and co-incubating microglia with datacline (20, 50 μM) attenuated this effect. Additionally, DEP-induced CGN cytotoxicity was attenuated in the neuron-microglia co-culture system by blocking microglia activation with minocycline (50 μM). Together, these results suggest that microglia activation by DEP is an important contributor to CGN neurotoxicity and that microglia may play a role in mediating the negative effects of DEP in the brain (Supp. in part by R01ES22949 and R2ES051264).

2110 Gender-Specific Multidrug-Resistance Transporter Expression in Choroid Plexus


Multidrug efflux transporters of the ABC-Binding cassette (ABC) family, ABC1 (Mrp1) and ABC4 (Mrp4) located on the basolateral membrane of the CP, play important roles in clearing the brain of unwanted substances and protecting it from potentially harmful material in the circulation. Previous research has shown gender-specific patterns and compensatory responses in liver and kidney. However, little is known about the gender differences and their function in brain. In the present study we examine Mrp1 and Mrp4 mRNA expression in CP using a Mrp4 knockout mouse (Mrp4+/−) model and also investigate whether Abcc transporters are expressed in a gender dependent pattern as in kidney and liver. We hypothesize that the female predominant Mrp1 and Mrp4 expression will remain in CP and that in the absence of Mrp4, the activity of Mrp1 will increase. qPCR and immunoblot analysis showed that Mrp4 mRNA and protein are expressed at much higher levels in the female than in male CP. Immunoblot and on CP of Mrp4+/− mice showed higher protein expression of Mrp1 in males supporting our hypothesis that the activity of the Mrp1 would increase. Abcc transporters share a wide variety of substrates: therefore, compensation by Mrp1 in the absence of Mrp4 and gender divergent transporter expression could manifest in differential disposition of endogenous substrates, toxicants, and therapeutic drugs, from the CSF to the blood. These sex-specific differences in the CP highlight the need to evaluate sex differences in neurological disorders, especially those that differ in prevalence and symptoms between men and women.

2111 Differential Impact of Tobacco Smoke Exposure at the Blood-Brain Barrier Endothelium: A Special Focus on the Nrf2-Dependent Antioxidant Mechanisms

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Tobacco smoke (TS) is a milieu of several thousand potentially toxic compounds (including nicotine) capable of inducing oxidative stress damage impacting both the peripheral and brain vasculatures. With respect to the blood-brain barrier (BBB) we have previously demonstrated that TS can impair BBB integrity via down regulation of tight junction proteins (such as ZO-1 and occludin) and concomitant vascular inflammation. Currently we are investigating the BBB endothelial impact of TS on the anti-oxidant response mechanisms. We performed a side by side response quantitative analysis of the antioxidant mechanisms triggered by a range of TS products including soluble nicotine extract (100ng/ml), full flavor (FF- conventional), and ultralow nicotine (ULN) cigarettes. With the exception of nicotine, our results have clearly shown activation of Nuclear factor (erythroid-derived 2)-like 2 (Nrf2) antioxidant response pathway in response to TS (FF and ULN) and increased expression of several antioxidant and detoxifying enzymes such as NAD(P)H dehydrogenase Quinone 1 (NQO-1), heme oxygenase (HMox-1); cytochrome P450s (CYP2S1 and CYP1A1). Further, Pgp efflux activity at the BBB was enhanced without notable alterations in its expression. TS effectively stimulated the Glutathione (GSH) based antioxidant system [including increased glutamate-cysteine ligase, catalytic subunit (GCLC)] and modifier unit (GCLM) activity at the BBB was enhanced without notable alterations in its expression.
Blood brain barrier is a complex network of specialized microvessels with tight intercellular junctions and high expression of membrane drug efflux transporters. P-glycoprotein (Pgp), multidrug resistance associated protein 2 (Mrp2) and breast cancer resistance protein (Bcrp) are major efflux transporters located on luminal side of brain capillary endothelium. The ability of Pgp and Bcrp to transport wide range of xenobiotic substrates poses a major challenge for CNS pharmacotherapy. Peroxisome proliferator activated receptor alpha is a master regulator of lipid metabolism. Along with β-oxidation of fatty acids, Ppar-α also plays a role in regulation of drug metabolism by inducing drug metabolizing enzyme and ATP binding cassette transporter expression in liver. However, the role of Ppar-τ in regulating transporters at the BBB is unclear. Rat brain capillaries were isolated and exposed to 25 and 50μM clotibrate, a Ppar-τ agonist. After 3h, capillaries were used for confocal microscopy based transport assay with a fluorescent cyclosporin A derivative, Texas red and BODIPY prazosin; these are substrates for Pgp, Mrp2 and Bcrp, respectively. Transporter protein expression was analyzed by western blot and immunostaining. A specific Ppar-τ antagonist GW6471 was used to demonstrate that observed effects are mediated through Ppar-τ. Clotibrate significantly increased protein expression and specific activity of Pgp, Mrp2 and Bcrp in the isolated rat brain capillaries. Moreover, environmental perfluoroalkyl compounds are also known to activate Ppar-τ. Our preliminary findings indicate that perfluoroctanesulfonic acid induces p-glycoprotein activity in rat brain capillaries. In summary, activation of Ppar-τ by clotibrate induced expression of three drug efflux transporters in rat brain capillaries. As fibrates are widely prescribed xenobiotics, our findings imply that blood-brain barrier efflux transporters are significantly induced in substantial portion of the population.
to hepatotoxicity. Thus appropriate mouse model is still lacking for neurotoxicity of 1-BP, limiting use of transgenic mice. The present study aims to build a mouse model for neurotoxicity of 1-BP, using 1-amino-benzoic acid (1-ABT), a cytochrome P450 inhibitor to reduce hepatotoxicity. Methods: 42 male C57/BL/6J/crl mice were randomly divided into 7 groups. 4 groups of mice were injected with 50mg/kg 1-BP were exposed to 1-BP at 0, 50, 250 and 1200 ppm. 8 hrs per day for 28 days. 3 groups of mice injected with saline were exposed to 1-BP at 0, 50 and 250 ppm. After exposure, all mice were dissected under anesthesia. Results: Serious hepatic pathological changes were found in the group of 250 ppm 1-BP without 1-ABT treatment, including massive necrosis, inflammation, and hepatocyte degeneration. No pathological changes were found in 1-ABT treated groups as high as 1200 ppm. The percentage of liver necrotic area at 250 ppm without 1-ABT treatment group is significantly higher than 1-ABT treated groups, even exposed to 1-BP at 1200 ppm. Discussion: 1-ABT can reduce liver injury induced by 1-BP exposure, confirming that cytochrome P450 has an important role in the metabolism and toxicity of 1-BP. Mice treated with 1-ABT may serve as a convenient model for studying neurotoxicity of 1-BP.

Energy drinks are a multi-billion dollar industry, and more than half of all energy drinks are consumed by adolescents and young adults. A key ingredient in energy drinks is the amino acid taurine. Taurine supports proliferation of neural progenitor cells and new synapse formation in brain networks involved in long-term memory. Although taurine can be neuroprotective and act as an antioxidant, recent studies indicate there is a risk of neurotoxicity at high doses. To determine if excess taurine could damage the maturing brain, we treated adolescent C57/BL/6J mice with distilled water or water containing 0.12% taurine (400 mg/kg) from postnatal day 30 to 60. Behavioral experiments began at P60. Here we report our findings on female mice. There were no significant differences in tests of anxiety (Zero Maze and Marble Burying) or sensorimotor gating (Acoustic Startle with pre-pulse inhibition) Taurine-treated mice spent more time in the periphery and reared more during Open Field Locomotor testing, but the differences were not statistically significant (P<0.05). Control mice showed greater improvement over five days of Rotarod acceleration testing, but the differences were not statistically significant. There was a trend for significance in Novel Object Recognition with control mice spending a greater percentage of time exploring the novel object compared with taurine-treated mice (P=0.067). There were no significant differences in the Acquisition Phase of Morris Water Maze (P>0.05); however, taurine-treated mice had significantly more zone crossings in the Probe trial compared with control mice (P<0.05). In the Reverse Phase, taurine-treated mice had significantly shorter path lengths on Days 1, 5, and 6 (P<0.05). When challenged with a stimulant (caffeine), taurine-treated mice showed significantly greater rearing activity than control mice. Together, these results show modest effects of early life taurine exposure that persist into adulthood. Supported by ES020053.

TETS is a potent convulsant rodenticide that is considered a credible chemical threat agent. Humans exposed to TETS can exhibit acute seizures, status epilepticus (SE) and death. Survival and toxicology of acute TETS-induced SE often exhibit persistent neurological sequelae, including spontaneous recurrent seizures. Emerging evidence suggests that persistent neuroinflammation contributes to the pathogenesis of epilepsy; therefore, in this study we assessed neuroinflammatory responses in an animal model of TETS-induced SE. Adult male NIH Swiss mice were implanted with a headmount for EEG recording from cortical screw electrodes. Following recovery from surgery, the animals were injected with rituxoluce (10 mg/kg ip) followed 10 min later with TETS (0.2 mg/kg ip). The animals exhibited >1 h of SE, consisting of repeated clonic seizures and electrographic seizure discharges. In the absence of rescue therapy, ~90% of the animals died by 24 h following TETS exposure. Administration of diazepam (5 mg/kg ip) or midazolam (0.73 mg/kg im) 40 min after the initiation of seizure activity increased survival at 24 h to 100% or >75%, respectively. However, TETS intoxicated animals rescued by diazepam or midazolam exhibited significant reactive astrogliosis and microglial activation in some brain areas as determined by GFAP and Iba-1 immunoreactivity. These changes persisted for at least 72 hours. TETS-induced SE could have long-term consequences as a result of the induction of persistent neuroinflammation. This mouse model provides a means of identifying neurotherapeutic agents that protect against the delayed and persistent neurological sequelae of TETS intoxication. This work is supported by the NINDS CountertACT Program (grant U54 NS097920).

The laboratory toxicologist frequently faces challenges of selecting the appropriate vehicle for testing in repeated dose oral toxicity studies. The objective of this study was to evaluate the effect of dosing with reverse osmosis water (ROW), 0.5% carboxymethylcellulose (CMC) and corn oil (CO) on motor activity, foot splay of Wistar rats following gavage administration of different vehicles. The age of animals at initiation of treatment was 5-6 weeks. Each group was to evaluate the effect of dosing with reverse osmosis water (ROW), corn oil (CO) and 0.5% carboxymethylcellulose (CMC) on motor activity, grip strength, and foot splay following 90-Day Oral Gavage Studies with Various Vehicles in Wistar Rats. Different Vehicles in 90-Day Studies

The objective of this study was to evaluate the effect of dosing with reverse osmosis water (ROW), 0.5% carboxymethylcellulose (CMC) and corn oil (CO) on motor activity, foot splay of Wistar rats when administered for ninety days via oral gavage. Each test group consisted of total 40 male and 40 female rats. Rats were 17-18 weeks of age at the time of motor activity and other parameters assessment. Motor activity measurements performed for total thirty minutes (three 10 minutes period) revealed 24% and 26% higher fine movement in CO treated male rats as compared to CMC and ROW treated male rats, respectively and for female rats increase was higher than 31% and 26%, respectively. In CO treated rats ambulatory activity was of 28% and 8% higher than CMC and ROW treated rats, respectively. Some small differences in forelimb and hindlimb grip strength as well as hindlimb foot splay were noted among the various vehicles. Higher hindlimb and forelimb grip strength was seen in male rats (7-14%) as compared to CMC and ROW treated male rats. The CMC treated female rats showed slightly higher (4-6%) hindlimb grip strength when compared to the CO and ROW treated female rats. Hindlimb foot splay measurement was decreased (14-15%) in CMC treated male and female rats compared to ROW treated rats and a similar decrease (14%) in hindlimb foot splay was seen for CO treated females compared to ROW treated females. The results from this study show that choice of vehicle may significantly affect motor activity measurements in neurotoxicity study. Minor influences of vehicle selection on forelimb and hindlimb grip strength and hindlimb foot splay were also noted.

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BFRs. Using PC12 cells we demonstrate that the majority of FRs induced negligible cytotoxicity, except zinc hydroxystannate (ZHS) and zinc stannate (ZS). Single-cell fluorescent Ca$$^{2+}$$-imaging revealed that aluminum trihydroxide (ATH), ZHS and ZS increased the basal intracellular calcium concentration ([Ca$$^{2+}$$]).

In the low mM range, many FRs, including tetrabromoisobiphenyl A (TBBPA), triphenylphosphine (TPP), ZHS and ZS reduced depolarization-evoked increases in [Ca$$^{2+}$$], due to inhibition of voltage-gated calcium channels. Next, using Xenopus oocytes expressing nicotinic acetylcholine receptors (nACh-R) we demonstrate that some FRs, including TBBPA, TPP and aluminium diethylphosphinate (AlPi), act as nACh-R antagonists. Based on the in vitro-derived neurotoxic potential we could identify suitable (e.g. AlPi) and less suitable (e.g. ZS) candidates for replacement. To substantiate this notion, we studied effects of neonatal exposure to TBBPA, AlPi or ZS on synaptic plasticity in mouse hippocampus ex vivo. These FRs did not significantly affect long-term potentiation and the expression of postsynaptic proteins. The FRs were absent from the brains, suggesting low bioavailability and/or rapid elimination/metabolism. Our findings demonstrate that several HFFRs could be suitable alternatives for BFRs. However, data on (in vivo) toxicity following prolonged (developmental) exposure is yet lacking.

Gulf War Illness (GWI) is a persistent, multi-symptom disorder with features characteristic of sickness behavior. Several exposures have been hypothesized as triggers of the recurring/chronic symptoms associated with GWI, including exposure to the nerve agent sarin that resulted from munition detonations at multiple sites. Here, we investigated the effects of exposure to the sarin surrogate, disopropyl fluorophosphate (DFP), on peripheral and CNS inflammation. Male C57BL/6J mice were pretreated with corticosterone (CORT) in the drinking water for 7 days to mimic high physiological stress, followed 1 day later by DFP (4 mg/kg, i.p.) exposure to model GWI. DFP exposure alone did not change inflammatory markers in serum and liver, however, increases in expression of inflammatory cytokines/chemokines were found in the brain. Further, CORT exposure for 7 days prior to exposure to DFP greatly augmented inflammatory responses in the brain. Pretreatment with anti-inflammatory antibiotic, minocycline, attenuated this neuroinflammatory effect. Subsequent investigation of neurodegeneration as indexed by GFAP protein content revealed no treatment-related increases following exposure to DFP or CORT+DFP. Immunohistochemical data revealed small, region-specific (e.g. hippocampus CA1) changes in microglia and astrocyte morphology (Iba-1 and GFAP, respectively); and no neurodegeneration was seen with silver or Fluoro-Jade B assessments of damage. These results suggest increases in neuroinflammation, findings consistent with sickness behavior, may be characteristic of GWI, potentially induced by exposure to nerve agents, and can be exacerbated by exposure to chronic stress conditions (e.g. extreme temperatures, daily threat of death/survival). While exposure to both stress hormones and nerve agents may be enough to produce increases in neuroinflammation, which likely contribute to sickness behavior seen in GWI, these conditions do not produce neural damage. Supported by USAMRCM W81XWH-09-2-0098.
2126 Altered Emotional Reactivity and Dopamine Turnover in Juvenile Rats Exposed Developmentally to Chlorpyrifos


Repeated developmental exposure to the organophosphorus (OP) insecticide chlorpyrifos (CPF) results in the inhibition of fatty acid amide hydrolase (FAAH), the enzyme that metabolizes the endocannabinoid anandamide (AEA), and leads to the accumulation of AEA in the forebrain. At lower dosages, this occurs without measurable inhibition of cholinesterase (ChE), the canonical target of CPF, suggesting that the endocannabinoid system may be an important target in the developmental toxicity of OP insecticides. However, in the absence of other biochemical changes, developmental exposure to CPF may also result in functional effects as the animal ages. The endocannabinoid system regulates emotional reactivity and this study investigated the persistent effects of CPF exposure on emotional reactivity. Following daily oral exposure to either 0.5, 0.75, or 1.0 mg/kg CPF from postnatal day 10–16, emotional reactivity was measured on day 25. The rats were placed into a dark container in a novel environment and the latency to emerge from the container was measured. In this test, rats that stay in the dark for a long time are considered emotionally reactive. All CPF treated groups spent significantly less time in the dark prior to emerging as compared to control suggesting a decreased level of emotional reactivity induced by CPF exposure. Immediately following behavioral testing, the levels of dopamine, serotonin, and their metabolites were measured in the amygdala and hippocampus. The levels of the dopamine metabolites were significantly elevated in the amygdala of all treatment groups suggesting that altered dopamine turnover plays a role in the decreased emotional reactivity.

2127 Neurotoxic Effects of Tri-Cresyl Phosphates (TCPs) and Cresyl Saligenin Phosphate (CBDP) In Vitro


We recently showed that the ortho-isomer of tri-cresyl phosphates (TCPs) ToCP impaired glutamate signaling in mouse primary cortical neurons (pCNs) at levels below its cytotoxic concentrations (Hausherr et al. 2014). Since TCPs are commercially used as mixtures of various isoforms and the ortho-isomer is metabolized into cresyl saligenin phosphate (CBDP) we performed additional experiments using EtCP,TpCP, a commercial TCP mixture and CBDP. We evaluated cell viability, neurite outgrowth, and the functionality of neurochemical processes. A 24 h exposure of mouse primary cortical neurons (pCNs) to the non-ortho TCPs and the mixture yielded EC50 values for cell viability above 100 µM. In contrast, CDPB was cytotoxic at concentrations as low as 10 µM. Using fluorescence-based life-cell Ca2+ imaging, we investigated TCP and CDPB effects on signals evoked by the main excitatory neurotransmitter glutamate which was significantly decreased after 24 h exposure to ToCP in concentrations of 100 nM. Glutamate-evoked signals in pCNs were none of the other TCP isomers, nor the mixture or the metabolite CDPB decreased the percentage of glutamate-responsive neurons and the mean response amplitudes at concentrations below 10 µM indicating a highly specific effect of the ortho-isomer on glutamate signaling. This specificity was confirmed by the simultaneous application of TOCP (100 µM) together with the glutamate stimulation resulted in a block of glutamate-induced responses by 70 %. Such a reduction was not observed when simultaneously applying CDPB (1 to 100 µM) to the glutamate stimulation. Even though CDPB was more cytotoxic to ToCP (EC50=89 µM) the impairment glutamate signaling seems to be a specific effect of the unmetabolized ortho-isomer of the tri-cresyl phosphates. Further research aims at investigating the mode of action of ToCP on the various glutamate receptors and effects of different ToCP amounts in TCP mixtures.

2128 Characterization of a Rat Model of Acute Diisopropylfluorophosphate (DFP) Intoxication

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Exposure to anticholinesterase organophosphates (OPs) can cause severe cholinergic crisis and death. Individuals that survive acute cholinergic crisis often develop persistent behavioral deficits for which effective therapeutics are not currently available. One of the challenges of developing therapeutic approaches to protect against the neurologic sequelae of acute OP intoxication is the limited characterization of OP-specific neurologic damage. Using DFP as a model OP, we characterized the long-term behavioral and neurochemical effects in rats. Adult male Sprague Dawley rats were exposed to a potentially lethal dose of DFP (4 mg/kg, sc) and rescued from death by atropine sulfate (2mg/kg, im) and 2-PAM (25 mg/kg, im), administered 30 min prior to DFP injection to reduce peripheral cholinergic symptoms. Behavioral assessments followed by immunohistochemical analyses of the brain were performed at 1 and 2 months post-DFP. DFP rats displayed hyperreactivity, aggressiveness and spontaneous recurrent behavioral seizures during this 2-month post-exposure period. In the elevated plus maze, DFP animals spent significantly more time in the open arms at both time points relative to the vehicle control indicating that DFP had a strong anxiolytic effect. Performance of DFP rats in the open field test for loco-motor activity and forced swim for depression-like behavior were not significantly different from vehicle controls; however, the DFP group exhibited a significant increase in climbing behavior during the swim test. DFP intoxication significantly impaired performance in contextual fear conditioning indicating cognitive deficits. GFAP immunoreactivity was significantly increased in the hippocampus and cortex at 1 but not 2 months post-DFP. Collectively, these data suggest that acute DFP intoxication causes persistent behavioral deficits that may be mediated by reactive astrogliosis. Supported by the NIH CounterACT program (NS079202) and by an NIH training grant (F32 GM099608).

2129 In Vivo Study of the Neuropathetic Potential of the Organophosphorus Compounds Fenamiphos and Profenofos: Comparison with Mipafox and Paraoxon

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Organophosphorus-induced delayed neuropathy (OPIDN) is a central-peripheral distal axonopathy that develops 8-14 days after poisoning by a neuropathic organophosphorus compound (OP). Several OPs that caused OPIDN were withdrawn from the agricultural market due to induction of serious delayed effects. Therefore, the development of in vitro screenings able to differentiate neuropathic from non-neuropathic OPs is of crucial importance. Thus, the aim of this study was to evaluate the differences in the neurotoxic effects of mipafox (neuropathic OP) and paraoxon (non-neuropathic OP) in SH-SYSY human neuroblastoma cells, using the inhibition and aging of neuropathy target esterase (NTE), inhibition of acetylcholinesterase (AChE), activation of calpain, neurite outgrowth, cytotoxicity and intracellular calcium as indicators. Additionally, the potential of fenamiphos and profenofos to cause acute and/or delayed effects was also evaluated. Mipafox had the lowest IC50 and induced the highest percentage of aging of NTE among the OPs evaluated. Only mipafox was able to cause calpain activation after 24 hours of incubation. Concentrations of mipafox and fenamiphos which inhibited at least 70% of NTE were also able to reduce neurite outgrowth. Cytotoxicity was higher in non-neuropathic than in neuropathic OPs while the intracellular calcium levels were higher in neuropathic than in non-neuropathic OPs. In conclusion, the SH-SYSY cellular model was selective to differentiate neuropathic from non-neuropathic OPs; fenamiphos, but not profenofos presented results compatible with the induction of OPIDN.

2130 Chlorpyrifos Oxon (CPFox) and 2, 2', 3, 5', 6-Pentachlorobiphenyl (PCB 95) Modulate Fc-Gamma Receptor Expression in Developing Neurons

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Fcγ receptors (FcγR) bind the constant (Fc) region of immunoglobulin G (IgG), and mediate uptake of IgG and cellular responses triggered by IgG binding in immune cells, including microglia in the postnatal brain. It has been shown previously that FcγR is expressed in the developing rat brain by cells other than microglia, specifically neurons and astrocytes and that binding of these receptors triggers...
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A fraction of the Organophosphorus-Sensitive Phenylvalerat Esterase Activity Shows Acetylcholine Hydrolyzing Activity in Soluble Fraction of Chicken Brain


Organophosphorus sensitive phenylvalerat esterases (PVases) have been studied in the nerve tissues of chicken, the experimental model for testing delayed neurotoxicity of organophosphorus compounds (OPs) looking for new toxic targets among the serine and tyrosine esterases. In soluble fractions of chicken brain, three enzymatic "components" of PVase called Et, Eγ and Eγ were discriminated, each one represents a fraction of esterases with similar sensitivities to OPs. Et-PVase is the activity resistant to 1500 μM PMSF for 30 min. It showed partial inhibition in the presence of acetylthiocholine with a kinetic fitting to a non-competitive inhibition model (Km=0.1-0.22 mM, Kt=6.6-7.6 mM). Otherwise, phenylvalerate showed competitive inhibition (Km=0.99-0.11 mM; Kt=1.7-2.2 mM) on the acetylthiocholine-hydrolysing activity resistant to 1500 μM PMSF. The Eγ-PVase activity (resistant 25 μM mifapox 30 min) was not affected by the presence of acetylthiocholine, while the acetylthiocholine-hydrolysing activity resistant to 25 μM mifapox showed a competitive inhibition in the presence of phenylvalerate (Km=0.05-0.06 mM; Ki=0.44-0.58 mM). The Eγ-PVase is the activity resistant to 25 μM mifapox; in this condition all cholinesterase activity is inhibited, however, the acetylthiocholine caused a non-competitive inhibition of the Eγ-PVase activity (Km=0.007-0.008 mM, Ki=0.20-0.26 mM). The interaction between both substrates suggests that part of PVase activity in the chicken brain soluble fractions might be due to protein(s) with acetylthiocholine-hydrolyzing activity and that acetylthiocholine can act in any way as a cholinesterase. The inhibitory kinetic characteristic of these fractions of esterase activity, its isolation and molecular identification is under study in order to understand its roles as target of OP toxicity.

2133 Novel Functions of Klotho during Paraoxon Exposure in Neuronal Cell Culture: Neurotoxicology and Proteome Pathway Analysis

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Paraoxon (PXN) represents an organophosphate oxon metabolite of parathion, an insecticide that is presently used in third world countries and has high toxicity in humans. Besides being a potent inhibitor of acetylcholinesterase (AChE), it triggers oxidative neuronal cell death. Recently, the anti-aging protein Klotho (KL), a well-characterized kidney humoral factor, was assigned a novel brain neuroprotective/neuro-regenerative role. Thus, the exact regulation of KL in response to oxidative neuronal damage remains to be determined. Our goal was to elucidate the complex molecular process of KL-dependent neuronal healing in vitro following paraaxon insult. Neuro-2a cells were exposed for 24h to PXN (65 to 100 μM) in the presence and absence of KL. Samples were analyzed by quantitative Tandem Mass Tags (TMT) liquid chromatography mass spectrometry (LC-MS/MS) including proteome pathway analysis and confirmation by Western blotting. TMT LC-MS/MS quantitative proteomics detected 510 proteins of which 347 were converted by the BioMart search engine into GeneWiki identifiers. Cytoscape 3.1.1 pathway analysis using Reactome Fl 4.1, Ibeta and ClueGo 2.1.2 applications demonstrated a KL-dependent reversal of the PXN observed cytotoxic signaling responses including gene ontologies for energy reserve metabolism, cell-death signaling, ROS defense, cytoskeletal rearrangement, DNA damage response, targeted protein degradation and apoptosis. The gained information will aid in understanding the complex cellular signaling pathways activated during KL-dependent neuronal healing that might be relevant for the development of novel counteractive therapeutics.

2134 Characterization and Inhibition of Guinea Pig Acetylcholinesterase

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Organophosphates (OPs) are highly toxic insecticides and nerve agents that have been designed to inhibit the hydrolysis of the neurotransmitter acetylcholine by irreversibly binding to the active site serine of acetylcholinesterase (AChE). Historically, the guinea pig (Cavia porcellus) has been believed to be the best non-primate model for OP toxicity and therapeutic development because, similarly to humans, guinea pigs have low amounts of OP metabolizing carboxylesterase in the blood. To explore the hypothesis that guinea pigs are the appropriate responder species for OP toxicity and therapeutic development, guinea pig AChE (gpaAChE) was cloned into pENTR/D-TOPO, LR recombined into pT-REDEST30 and transiently expressed in Human Embryonic Kidney (HEK) 293 cells using Cell Line bioreactors. Recombinant gpaAChE was purified on the General Electric AKTAExplorer using size exclusion and Nickel affinity chromatography. Western blots and coomassie staining were used to confirm expression and purity. The Ellman assay was used to enzymatically characterize the protein. IC50's for disopropylfluorophosphate (DFP), dicrotophos, paraoxon and tacrine were found to be 9.9 ± 1.1 μM, 303 ± 60 μM, 1.21 ± 0.22 μM and 301 ± 15 μM. Inhibition constants (Ki) for DFP, dicrotophos, paraoxon and tacrine were found to be 8350 ± 590, 4520 ± 270, 295 ± 12 and 458 ± 64 nM respectively. Lineweaver Burke plots confirmed tacrine as a mixed inhibitor and paraoxon, dicrotophos and DFP as non-competitive inhibitors of recombinant gpaAChE. In conclusion, significant differences in enzyme kinetics can be seen across various species, including human. To further elucidate these differences, X-ray crystallography can be performed to obtain the 3 dimensional structure of recombinant gpaAChE. These results may be used to develop novel therapeutics against OP intoxication.
Neuroprotective Effect of Calcium Channel Blockers on Organophosphorus-Induced Delayed Neuropathy (OPIDN) in SH-SY5Y Cells

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Organophosphorus-induced delayed neuropathy (OPIDN) is characterized by a central-peripheral distal axonopathy and Wallerian-type degeneration that develops 8–14 days after poisoning by a neuropathic organophosphate (OP). To generate OPIDN it is necessary the inhibition and aging of at least 70% of the neuropathy target esterase (NTE). The mechanism that has been proposed is that NTE inhibition and aging by neuropathic OPs is associated with a decrease in extracellular calcium and increase of calpain activity that is an intracellular cysteine protease. As calcium may be involved, several studies have attempted to alleviate the signs and symptoms of OPIDN by controlling the balance of calcium. This study aimed to evaluate the neuroprotective effect of calcium channel blockers on organophosphorus induced delayed neuropathy (OPIDN) in SH-SY5Y human neuroblastoma cell. Nimodipine and amiloride, calcium channel blockers, were used as possible neuroprotective agents, trying to avoid the increased levels of intracellular calcium and activation of calpains. The relation between the inhibition and aging of neuropathy target esterase (NTE) by the trichlorfon, which is an OP often utilized in Brazil, was evaluated as a possible indicator of the compound ability to induce OPIDN. Mipafos was used as inducer of OPIDN and paraaxon was used as non-inducer. The compounds were diluted in ethanol and the cells were incubated with the OPs for 24 hours. Considering the esterases inhibition and aging results, the Trichlorfon exhibited inhibition and aging of at least 70% NTE, being a possible neuropathic agent. It also increased levels of intracellular calcium but did not increase the activity of calpains. The amiloride and nimodipine were able to avoid the increase of intracellular calcium caused by neuropathic OP (mipafos), and only nimodipine was able to avoid the increased of the activity of calpain induced by a neuropathic OP (mipafos).

Subchronic and Repeated Exposure to Insecticides Inhibits the Depolarization-Evoked Increase in Intracellular Calcium Concentration in PC12 Cells


Insecticides are well-known neurotoxictants that are not species specific and may thus pose a risk for human health. Previously, we demonstrated that different classes of insecticides acutely inhibit voltage-gated calcium channels (VGCCs) at (sub)μM concentrations in vitro. However, since human exposure is usually chronic and repeatedly, we investigated if selected pyrethroids, organophosphates, organochlorines, carbamates, and neonicotinoids also disturb calcium homeostasis after 24h (subchronic) exposure and in combination with a second (repeated) acute exposure. Effects on calcium homeostasis were investigated in PC12 cells with single cell fluorescence (Fura-2) imaging. Cells were depolarized with high-K+ saline and endosulfan inhibit the K+-evoked increase in [Ca2+]i after 24h of exposure (IC50: ~0.1-6 μM) and after repeated exposure (IC50: ~0.1-6 μM). Repeated exposure induced a larger inhibition of the K+-evoked [Ca2+]i compared to 24h exposure. Carbaryl and imidacloprid did not inhibit the K+-evoked [Ca2+]i. In conclusion, insecticides inhibit VGCCs following 24h of exposure. The potency of insecticides to inhibit VGCCs increased if cells were exposed repeatedly. The potency of insecticides to inhibit VGCCs after repeated exposure was comparable to acute exposure, with the exception of chlorpyrifos. Compared to acute exposure, chlorpyrifos inhibits VGCCs 5-fold more potent after repeated exposure. This suggests that repeated exposure scenarios should be considered in risk assessment. Funding: European Union [DENAMIC project; FP7-ENV-2011-282957] and the Faculty of Veterinary Medicine of Utrecht University. 1 Meijer et al., (2014) Tox. Sci. 141; 103-11

Actions of a Type II Pyrethroid Mixture on Oxidative Stress Could Be Predicted by a Dose-Additive Using an In Vitro Model

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Pyrethroids are synthetic neurotoxic insecticides structurally based on the pyrethrins which are widely used today in agriculture, home pest control and as medical and veterinary products. Pyrethroids have been classified as Type I or Type II based on acute high-dose biological effects and chemical structure. Type II compounds contain an α-cyano group on the phenoxybenzyl moiety, and acute exposures produce a syndrome characterized by choreoathetosis and salivation (CS syndrome). In the present study, we tested the hypothesis that Type II pyrethroids, with a common mechanism of toxicity, could act in a dose-additive manner. In this study we used a mixture of 6 pyrethroids to test the hypothesis of dose additivity in SH-SY5Y, HepG2 and Caco-2 human cells. Oxidative-stress is a key factor in neuronal cell death, herein we evaluate changes in the production of nitric oxide (NO) and lipid peroxides measured as malondialdehyde (MDA) in SH-SY5Y, HepG2 and Caco-2 human cells. We determined: (1) dose-response curves for MTT and LDH assays for the 6 Type II pyrethroids (deltamethrin, α-cypermethrin, λ-cyhalothrin, cyfluthrin, cyphenothrin and esfenvalerate) individually; (2) IC50 values to compare the relative potencies of the 6 pyrethroids; and (3) NO and MDA levels individually for each pyrethroid and for a mixture of IC50 of the 6 pyrethroids (100%, 66%, 50%, 33%, 10%, 5%, and 1% of IC50). When IC50 dose of individual pyrethroids were combined in the mixture, we found significant dose-related decreases in NO and MDA production. This data suggest that Type II pyrethroids act in a dose-additive manner; both SH-SY5Y and HepG2 human cell lines could be used as in vitro models to test additivity for pyrethroid. Work supported by projects Refs. GR35/10-A UCM-BSCH, S2009/AGR-1460 (CAM) and Consolider-Ingenio 2010 No.CSD2007-00063 (MEC), Madrid, Spain.

Neuropathic and behavioral effects of lambda-cyhalothrin and lambda-cypermethrin in Sprague-Dawley rats

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Neurotoxins are a family of growth factors crucial for growth and survival of neurons in developing and adult brain. Reduction in their levels is associated with reduced neurogenesis and cognitive deficits in rodents. Recently, we demonstrated that long-term exposure to relatively low levels of the pyrethroid pesticide deltamethrin causes hippocampal apoptosis and learning deficits in mice. Here, we examined the gene expression of several neurotrophic factors in the hippocampus of deltamethrin-treated animals. No changes in the mRNA expression of brain-derived neurotrophic factor and neurotrophin-3 were observed in the hippocampus of deltamethrin-treated animals. However, nerve growth factor (NGF) mRNA expression was significantly decreased by 30% in deltamethrin-treated animals. This was accompanied by a similar reduction (39%) in NGF protein. In primary hippocampal neurons, deltamethrin significantly decreased NGF levels, which was accompanied by increased levels of activated caspase-3. Inhibition of voltage-gated sodium channels with tetrodotoxin (1μM) reversed the depletion of NGF and prevented the apoptotic cell death. Finally, co-treatment with NGF prevented deltamethrin-induced apoptosis. Collectively, these results demonstrate that the loss of NGF may contribute to deltamethrin-induced apoptosis in the hippocampus, and that this may subsequently impair learning and memory. Supported by NIH P30ES005022, R01ES015991, and R01ES021800.

Influence of Stressors in Lambda-Cyhalothrin-Induced Brain Dopaminergic Dysfunctions in Rats

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There are substantial evidences demonstrating that physical, psychological or environmental stress may lead to physiological changes and psychiatric illness. The present study has been carried out to understand the effect of immobilization stress (IMS), a psychological stressor and forced swim stress (FSS), a physical stressor on the neurobehavioral toxicity of lambda-cyhalothrin, a new generation, type-II synthetic pyrethroid with extensive uses in controlling wide range of insects and public health programs. Marginal changes in motor activity, rotarod performance, plasma

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2138 Nerve Growth Factor Protects against Deltamethrin-Induced Apoptosis in Primary Hippocampal Neurons

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cortisol levels and blood brain barrier permeability were observed in rats subjected to IMS (15 min/day) or FSS (3 min/day) for 28 days or exposed to LCT (3.0 mg/kg body weight, p.o.) for 3 days as compared to rats in the control group. Changes in DA-D2 receptors, activity of mitochondrial complexes and stress marker proteins in corpus striatum were also not significant in rats subjected to IMS or FSS as those exposed to LCT alone as compared to controls. Pre-exposure to IMS or FSS for 28 days followed by LCT treatment for 3 days in rats resulted in increased plasma cortisol levels, blood brain barrier permeability and decreased motor activity as compared to rats exposed to IMS or FSS or LCT alone. It was interesting to note that pre-exposure to IMS or FSS followed by LCT treatment also caused a marked decrease in DA-D2 receptors, activity of mitochondrial complexes and stress marker proteins in corpus striatum as compared to rats exposed to IMS or FSS or LCT alone. The results exhibit that both psychological and physical stress contributes in the LCT induced dopaminergic dysfunctions. Further, alterations in behavioral and neurochemical end points were more marked in LCT treated rats pre-exposed to IMS as compared to those pre-exposed to FSS.

2140 Responses of Invertebrate Crayfish to Systemic Insecticide Fipronil
Systemic neonicotinoids and fipronil disturb nervous system function by targeting receptor-mediated neurotransmission. Global declines of amphibians and honeybees have not been linked to single causes, but effects by systemic pesticides have been implicated. In the early 2000’s, fipronil-coated rice seed was permitted in LA to control rice water weevils (Lissorhoptrus oryzophilus), however non-target crayfish Procambarus clarkii co-cultured with rice crop were exposed. Crayfish in mesocosms were exposed to coated seed at 0.65 oz/100 lb (seed treatment), water with fipronil at 0.38 – 4.32 ppb (water treatment), or well water (control) (N = 10 animals/treatment). Hemolymph was analyzed for cellular phenoloxidase activity (PO), and hemocytes for viability and count. A response to stressors is activation of PO. Both PO and viability in the seed treatment were significantly lower (P<0.05) than controls, and hemocyte count was positively associated with PO (r2 = 0.6). To observe and measure potential agonistic behavior following consumption of a coated seed, males were socially isolated for 7 days and paired to document interactions. Within 24 hr of eating a seed or not, crayfish encounters were videotaped for 15 min and scored for duration (sec) and intensity (ranked 1-5) per described behaviors (i.e., meral spread, restrained claw use, clawlock, unrestrained combat, and appendage removal). Ten pairs per group (Group 1: Control vs. Control; Group 2: Control vs. Treated; Group 3: Treated vs. Treated) were scored. Discriminant, multivariate, and univariate analyses were applied. Behaviors among groups were significantly different (P<0.0095). The highest intensity was in Group 3, in which encounter duration was highest (i.e., 220 sec), as compared with 71.2 sec and 21 sec for Groups 2 and 1 respectively. These data indicated an agonistic display by crayfish that consumed one fipronil coated rice seed. Nerve cell damage was quantified by fluorescence microscopy with 6-Methoxy-N-ethylquinolinium iodide binding to chloride ions.

2141 Dysregulated Glucose Homeostasis and Impaired Locomotor Activity of Male C57BL/6 Mice Caused by Acute Oral Atrazine Treatment
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There are multiple reports for neurotoxicity associated with short-term or chronic exposures to the herbicide atrazine (ATR), with locomotor activity alterations being a major adverse outcome. Limited, mainly chronic exposures data, suggest that ATR is capable of perturbing glucose homeostasis leading to metabolic syndrome-like abnormalities. The objectives of the present study were to determine the acute effects of a dose range of ATR (0, 1, 10, and 100 mg/kg BW; 5 mice per group) given by oral gavage on locomotor activity and glucose handling. Retired C57BL/6 male breeders were used in a Latin-square design study (5-days between experiments) for 4 experiments; (i) acute effect of ATR on blood glucose, (ii) effect of ATR pre-treatment on oral glucose tolerance (2 g/kg BW, administered 30 min post ATR), (iii) effect of ATR pre-treatment on insulin sensitivity (0.5 IU/kg BW, administered to min post TR), and (iv) acute effect of ATR on locomotor activity in an open field test (60 min testing, initiated 10 min post ATR). Acute ATR administration led to: (i) hyperglycemia (10 and, especially, 100 mg/kg), (ii) only a trend for exaggerated response to the oral glucose challenge caused by ATR pre-treatment, (iii) significantly impaired insulin resistance (10 and 100 mg/kg), and (iv) markedly decreased total distance traveled in the open field test (all doses) that only at the highest dose of ATR (100 mg/kg) was also associated with apparent increased anxiety (increased corner time). These data indicate that acute ATR treatment dysregulates glucose homeostasis, with insulin sensitivity being more sensitive to ATR than glucose overload. Importantly, acute ATR administration causes decreased locomotion that is apparent even at the lowest (1 mg/kg) dose employed in the study. Together, the study suggests that locomotor activity is highly sensitive to the acute effects of ATR and that ATR’s effect on the brain, especially at lower doses, might be independent of its effects on circulating glucose.

2142 Mancozeb-Induced Cell Cycle Arrest and Senescence via RTP801
Mancozeb (MZ), one of the ethylene-bis-dithio-carbamates (EBDCs), is widely used as fungicide. It is a polymer chemical complex containing zinc and manganese ions. Study showed that MZ induces the expression of RTP801 and this induction is in response to MZ-triggered cell death. Humans are constantly exposed to various chemicals or stresses. Cellular senescence is an irreversible cell cycle arrest in response to DNA damage and oxidative stress. Activation of NF-kappa B signaling pathway has a causal role in promoting senescence. The cross talk between RTP801 and NF kappa B activation is associated with MZ-induced cell death. This study was to reveal the role of RTP801 in MZ-triggered senescence. The biomarker of senescence is based on beta-galactosidase activity at pH 6 (senescence-associated beta-Gal; SA-beta-Gal), which is only present in senescent cells. Cell cycle analysis was conducted by using Attune Acoustic Focusing Cytometer. The results showed that cell cycle was halted at G1/G0 phase when SH-SY5Y cells were treated with MZ (10-100 μM) for 1.5 hours. However, when cells were treated with MZ for only 1 hour, cell cycle was arrested at G2/M phase at the lowest tested dose (10 μM) and at G1/G0 phase at higher tested doses (20-100 μM). An increase in SA-beta-Gal staining was observed from PC12 cells that were given 4 hours of MZ treatment (10-50 μM) and 24 hours of recovery. The increase in SA-beta-Gal staining was more perceptible in cells treated with low dose (10 μM) of MZ as compared to the cells treated with higher doses (20 and 50 μM). The quantitative data of SA-beta-Gal were coherent with SA-beta-Gal staining results. In order to study the role of RTP801 in MZ-induced senescence, short-hairpin RNA for RTP801 (sh RTP801) was used to knock down the expression of RTP801. After introducing sh RTP801 to the cells for 48 hours, the SA-Beta-Gal activity showed a smaller increase than in cells with normal RTP801 expression. This study demonstrates that MZ can trigger cell cycle arrest and cellular senescence and that RTP801 is partially involved in this toxic response.

2143 Manganese-Containing Dithiocarbamates Increase the Expression of Amyloid Precursor Protein and the Level of Phosphorylated PKR
Environmental factors, such as pesticides, play a critical role in the pathogenesis of neurodegenerative diseases including Alzheimer’s disease (AD). The pathogenesis of AD is not fully understood. Accumulation of amyloid β peptides (Aβ) in the brain is a hallmark of AD. The neurodegeneration of AD brains has been linked to activation of the RNA-sensitive double-stranded RNA dependent protein kinase (PKR). Studies demonstrated that PKR is able to phosphorylate p53, which can hinder activity of mammalian target of rapamycin (mTOR) in response to amyloid β peptide 42 (Aβ42). The study was aimed to elucidate the effect of manganese-containing dithiocarbamates, maneb (MB) and mancozeb (MZ), on the expression of amyloid precursor protein and Aβ42 and further investigated the involvement of PKR in the elevations of APP and Aβ42 in PC12 cells and SH-SYSY cells. The cells were treated with MB and MZ at the different concentrations for various exposure times. The cells were lysed and the total proteins gathered were subjected to Western blot analysis for APP, Aβ42, phosphorylated p-T446-PKR, and total PKR expressions. The level of Aβ42 was also quantitatively determined by ELISA. Western blot data indicated that AβPP and Aβ42 expressions increased in a dose-dependent manner after 24 hours of MZ exposure. For MB, AβPP levels significantly increased only after 48 hours of exposure. ELISA data confirmed the Western blot data. Aβ42 expression dramatically increased after 24 hours of MZ treatment, but only slightly increased after 24 hours MB treatment. MZ showed to be a stronger inducer for Aβ42 expression. MB increased the phosphorylation of PKR at T446 when cells were treated with low doses, 10 μM and 20 μM, for 8 hours. MZ increased the phosphorylation of PKR as early as 4 hours when cells were treated at doses of 10 μM, 20 μM, and 50 μM. The highest tested dose, 100 μM, was too toxic to see a response.
2144 Developmental Coexposure to γ-Radiation and Paraoxon Can Exacerbate Cognitive Dysfunction in Adult Mice

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Investigation of neurotoxic effects following fractionated low dose ionizing radiation (IR), resembling a series of CT scans or dose delivered to non-target tissue during radiotherapy, and possible interaction effects with other agents is of great importance for risk and safety evaluation. In previous studies we have shown that low dose exposure to IR during neonatal brain development in the mouse, can interact with agents that affect the cholinergic system e.g. nicotine and PBDE 99, to exacerbate cognitive dysfunction at an adult age. The present study was conducted to evaluate developmental neurotoxicity following co-exposure to IR and an agent affecting the dopaminergic system, the herbicide paraquat. Neonatal male C57Bl/6 mice were exposed on PND 10 and 11 to 1) external γ-radiation (100 mGy or 300 mGy); 2) Paraoxon (0.02 or 0.2 mg/kg b.w.); 3) Co-exposed to 100 mGy and 0.02 or 0.2 mg/kg b.w. paraquat; 4) Co-exposed to 300 mGy and 0.02 or 0.2 mg/kg b.w. paraquat. Control animals were exposed to vehicle and sham irradiated. At 2 and 3 months of age animals were tested for spontaneous behaviour in a novel home environment (60 min observational period) and tested in a radial arm maze test. A pronounced increase in incorrect arm entries was observed in the 300 mGy+0.2 mg/kg b.w. paraquat group compared to controls and the sole agents. In the radial arm maze test, 2 and 3 months of age animals were tested for spontaneous behaviour in a novel environment (60 min observational period) and tested in a radial arm maze test. A pronounced increase in incorrect arm entries was observed in the 300 mGy+0.2 mg/kg b.w. paraquat group compared to controls and the sole agents.

2145 Assessment of Cross-Chemical Predictability for Changes in Blood Clinical Bioindicators and EEG Produced by Pesticides with Different Modes of Action


Electroencephalography (EEG) is often used as an apical measure of multiple types of central nervous system (CNS) changes, while biomarkers in blood may serve as predictors for adverse outcomes. Correlation between these two measures would suggest that certain changes in biomarkers may be related to altered CNS function. Changes in brain cytokine levels have been reported to alter neuronal function and peripheral cytokines may alter peripheral nervous system function, which could in turn alter EEG activity. The EEG of awake Long-Evans rats was recorded after exposure to pesticides with different modes of action (permethrin, deltamethrin, fipronil, imidacloprid, carbaryl, triadimefon) at the time of maximum behavioral change. Animals were sacrificed after EEG testing and serum and plasma samples collected and processed by Myriad RBM using RodentMAP® and RAT MetabolicMap® assays as well as a Meso Scale Discovery ® (MSD) 7plex ultra-sensitive assay, for blood levels of cytokines, hormones, and other biologically active proteins. For a given chemical, some biomarkers had significant within-subject correlations with EEG changes. Out of about 80 biomarkers that were monitored, 27 were altered by one or more pesticides, and of these, 22 were significantly correlated with alterations in EEG parameters. However, there were not consistent changes in the biomarkers that were associated with unique alteration(s) in the EEG parameter(s), reducing confidence in an association or causal relationship. These data do not demonstrate cross-chemical predictability for changes in serum clinical bioindicators and EEG. This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

2146 Neuroprotective Effect of Acetyl-L-Carnitine in Peripheral Nervous System (PNS) following a Prolonged Exposure to Rotenone

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Chronic or prolonged exposure to the environmental toxicant rotenone, a potent mitochondrial toxin, leads to degeneration in striatal nerve terminals and nigral neurons (CNS) as well as peripheral sympathetic and parasympathetic nerves (PNS). Rotenone-induced behavioral, neurochemical and neuropathological changes in rats mimic those observed in Parkinson’s disease. Here, we aimed to investigate the effect of acetyl-L-carnitine (ACL) - known for its anti-inflammatory, anti-apoptotic, and anti-oxidant effects - on rat peripheral nerve conduction parameters following prolonged exposure to rotenone. Adult male rats were injected i.p. with rotenone (1 mg/kg) either alone or with ACL either 10 or 100 mg/kg, ACL10, ACL100, respectively) once daily on days 1, 3, 5, 8, 10, 12, 15, 17, 19, 22, 24, 26, 29, 31, 33, and 37. Control rats received either ACL at 100 mg/kg or vehicle (30% Solujet HS 15 in 0.9% saline) injections. Animals were weighed on injection days and monitored daily. Motor latency (ML) in response to stimuli [distal (S1) and proximal (S2)] and motor conduction velocity (MCV) were assessed under isoflurane anesthesia using action potential detected from the tail muscle through surface receiver electrodes. In rats exposed to rotenone and rotenone/ACL10, a significant increases in both S1 and S2 ML and a decrease in MCV were detected in tail nerves (p<0.05). The conduction parameters in rats co-treated with rotenone/ACL100 were not different from control. Data indicate a neuroprotective effect of ACL in rotenone-evoked peripheral motor nerve dysfunction in the rat.

2147 Toxicity and Safety Assessment of Fipronil, S-Methoprene, and Amitraz in Dogs following Topical Certifect® Application

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Currently, the use of ectoparasitocides on dogs to control ticks and fleas is inevitable, and the safety data for dogs, dog owners, and veterinary personnel are scarce. This investigation was therefore undertaken with two objectives: (1) to determine the residual of fipronil, s-methoprene, and amitraz in dog blood and (2) to determine the transferable residues of these insecticides in gloves worn while petting experimental household dogs after the topical application of Certifect®. Certifect® (4.28 mL) contains 9.8% fipronil, 8.8% s-methoprene, and 22.1% amitraz. Certifect® kills ticks, fleas and chewing lice of all stages. All three insecticides produce their toxicity by different mechanisms. The blood samples (approximately 4-5 mL from each dog) were collected into EDTA tubes. The glove samples were collected by petting each dog for 5 min while wearing a different glove per dog. Blood and glove samples were extracted in methylene chloride and petroleum ether, and the extracts were assayed for residues of fipronil, s-methoprene, and amitraz using GC/MS. Blood analysis revealed the presence of only amitraz (0.42±0.16 μg/mL) 48 hr post Certifect® application. In gloves, significant residues of fipronil, s-methoprene, and amitraz were detected after 24 hr, with maximum transferable residue at 72 hr (432.16±79.18; 301.84±52.51; and 398.37±112.19 μg/g, respectively). After day 7, the concentrations of these insecticides in glove extracts were about 100 μg/g glove wt. While fipronil and s-methoprene were detected until day 28 (0.87±0.55 and 2.66±1.22 μg/g, respectively), the residue of amitraz was detectable only until day 14 (6.01±1.75 μg/g). In conclusion, Certifect® appears to be safe for dogs and their owners following once a month exposure, but veterinary personnel can be at risk following daily exposure to transferable residue of fipronil, s-methoprene, and amitraz, if not properly protected.

2149 Safety Evaluation of Permethrin and Indoxacarb in Dogs Treated with Activyl® Tick Plus

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The use of ectoparasitocides on pets is incredibly important since pets are commonly infested with fleas, ticks, and other external parasites. Unfortunately, there is very little data on the safety of these products for dogs, their owners, and veterinary personnel who come into contact with the animals on a daily basis. Therefore, this investigation was undertaken to determine the residue of permethrin and indoxacarb in the dog’s blood and to determine the transferable residues of these insecticides to gloves worn while petting six adult dogs after the topical application of Activyl® Tick Plus. Samples were collected on days 0, 1, 2, 3, 7, 14, 21, 28, and 35. At these time intervals, the dogs also underwent physical examination. The blood samples (approximately 4-5mL) were collected into EDTA tubes. The glove samples were obtained by using 100% cotton gloves and petting each dog for 5 minutes, using a different glove each time. Blood and glove samples were extracted in methylene chloride and petroleum ether, and the extracts were assayed for residues of permethrin and indoxacarb using GC/MS. Blood analysis did not reveal the presence of permethrin or indoxacarb at any time during the experiment. In the gloves, the highest concentrations of permethrin and indoxacarb were determined at 24 hours (89.18±253.22; 90.80±35.10 μg/g, respectively). Residues of both ingredients were found in significant concentrations in the gloves until day 7 (174.85±46.98; 7.63±2.83 μg/g, respectively). Permethrin residue was found in the gloves in detectable amounts until day 35 (28.12±11.59 μg/g). Indoxacarb
residue was found in the gloves in insignificant amounts until day 21 (0.65 ± 0.45 mg/g). In conclusion, Activyl® Tick Plus appears to be safe for dogs, as no adverse reactions occurred and the residue was never found in the blood. Owner’s and veterinary personnel can be exposed to significant levels of permethrin and indoxacarb following daily exposure if the proper precautions are not taken.

2150 Chemoproteomic and Metabolomics Platforms Reveal Organophosphorous Flame Retardants Inhibit Liver Carboxylesterases and Cause Metabolic Alterations


Organophosphorous flame retardants (OPFRs) are widely used chemicals based on a reactive chemical scaffold with a high prevalence of consumer exposure. However, despite their use and potential for human exposure, comprehensive mechanistic toxicity information is lacking. Traditional toxicity testing using indirect assays that broadly characterize phenotypes or examine predetermined potential targets are limited in revealing the direct interactions between a chemical toxinant and a biological target. We wished to explore a clear and potentially novel mechanism of toxicity behind the most widely used OPFR triphenyl phosphate (TPP), and thus needed to identify the direct targets of TPP in a complex system. To accomplish this, we used a tandem chemoproteomic and metabolomic platform to first identify in vivo targets of TPP and then characterize the functional metabolic consequences of target inhibition. Using a clickable bioorthogonal TPP probe, we identified specific liver carboxylesterase enzymes involved in lipid metabolism are targets of TPP, but not of all OPFRs. Next using functional metabolomics, we studied the biochemical and pathophysiological consequences of inhibiting metabolic enzyme off-targets of OPFRs, and showed widespread alterations in lipid metabolism and striking dyslipidemia in vivo, suggesting a potential for these highly prevalent compounds to act as environmental obesogens. Overall, this combined chemoproteomic and metabolomics platform shows promise in comprehensively revealing the direct biological targets of environmental chemicals and understanding their toxicities.

2151 Birth Defect Rates in High and Low Atrazine-Use States

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Atrazine is a broadleaf and grassy weed herbicide with widespread agricultural use in the US, primarily on corn and soybean crops. Observations that it can disrupt reproductive hormones at relatively high exposure doses raised concern that atrazine might cause birth defects in humans exposed via drinking water. This seemed to be supported by several ecological epidemiology studies that reported associations between various indicators of atrazine exposure and birth defects. Based on studies in laboratory animals, however, birth defects in humans would not be expected at the relatively low exposure levels associated with atrazine in drinking water; this discrepancy could be due to inter-species differences in response to atrazine or methodological limitations inherent in epidemiology studies. Using statewide data regarding atrazine use and birth defects for the 48 contiguous US states between 1998 and 2004, we evaluated whether birth defect rates were associated with atrazine usage. We also evaluated whether birth defects were more likely to occur for births in which the mother’s last menstrual period (LMP) prior to conception occurred when atrazine usage was heaviest (April to July). We found that total annual birth defect rates were not correlated with average annual atrazine use, and birth defect rates were not higher for women whose LMP occurred during the heavy atrazine-use months. Although our analysis shares limitations with ecological epidemiology studies showing an association between atrazine exposure and birth defects, the difference between our results and those of previous studies raises logical epidemiology studies showing an association between atrazine exposure and birth defects, the difference between our results and those of previous studies raises logical epidemiology studies showing an association between atrazine exposure and birth defects, the difference between our results and those of previous studies raises logical epidemiology studies showing an association between atrazine exposure and birth defects, the difference between our results and those of previous studies raises logical epidemiology studies showing an association between atrazine exposure and birth defects, the difference between our results and those of previous studies raises logical epidemiology studies showing an association between atrazine exposure and birth defects, the difference between our results and those of previous studies raises logical epidemiology studies showing an association between atrazine exposure and birth defects, the difference between our results and those of previous studies raises logical epidemiology studies showing an association between atrazine exposure and birth defects, the difference between our results and those of previous studies raises logical epidemiology studies showing an association between atrazine exposure and birth defects, the difference between our results and those of previous studies raises logical epidemiology studies showing an association between atrazine exposure and birth defects, the difference between our results and those of previous studies raises logical epidemiology studies showing an association between atrazine exposure and birth defects, the difference between our results and those of previous studies raises logical epidemiology studies showing an association between atrazine exposure and birth defects, the difference between our results and those of previous studies raises logical epidemiology studies showing an association between atrazine exposure and birth defects, the difference between our results and those of previous studies raises logical epidemiology studies showing an association between atrazine exposure and birth defects, the difference between our results and those of previous studies raises logical epidemiology studies showing an association between atrazine exposure and birth defects, the difference between our results and those of previous studies raises logical epidemiology studies showing an association between atrazine exposure and birth defects, the difference between our results and those of previous studies suggests that the mammalian immune system may be particularly sensitive to IMI (Badgurar et al., 2013; Gatne et al., 2006; Gawade et al., 2013). Here we present a critical review and evaluation of these studies, followed by a weight of evidence assessment regarding IMI immunotoxicity. Although each study has strengths and weaknesses, they all demonstrate evidence of immunomodulation, including effects on functional and observational immune assays and immune organ cellularity and mass. The weight of evidence suggests that IMI is a direct mammalian immunosuppressant, with further support from biological plausibility. Specifically, IMI and its desmethyl metabolite bind and activate the mammalian nAChR subtype, a critical component of the endogenous cholinergic anti-inflammatory pathway; this metabolite is as potent at the G7-nAChR as nicotine, a known immunosuppressant.

2152 Mammalian Immunotoxicity of Imidacloprid: Review and Weight-of-Evidence Evaluation

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Imidacloprid (IMI) is a best-selling insecticide and one of several neonicotinoid insecticides recently implicated in honeybee Colony Collapse Disorder. IMI exerts its effects via binding to the nicotinic acetylcholine receptor (nAChR). While IMI is a potent agonist of the insect nAChR, differences between insect and mammalian nAChRs make IMI only a weak agonist in mammals. Recent peer-reviewed studies conducted in rodents suggest that the mammalian immune system may be particularly sensitive to IMI (Badgurar et al., 2013; Gatne et al., 2006; Gawade et al., 2013). Here we present a critical review and evaluation of these studies, followed by a weight of evidence assessment regarding IMI immunotoxicity. Although each study has strengths and weaknesses, they all demonstrate evidence of immunomodulation, including effects on functional and observational immune assays and immune organ cellularity and mass. The weight of evidence suggests that IMI is a direct mammalian immunosuppressant, with further support from biological plausibility. Specifically, IMI and its desmethyl metabolite bind and activate the mammalian nAChR subtype, a critical component of the endogenous cholinergic anti-inflammatory pathway; this metabolite is as potent at the G7-nAChR as nicotine, a known immunosuppressant.
Chlorpyrifos Promotes the Growth of Colorectal Adenocarcinoma H508 Cells through the Activation of EGFR/ERK1/2 Signaling Pathway

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Apart from the neuronal effects on cholinergic system, the epidemiological study shows the association of chlorpyrifos (CPF) pesticide exposure and cancer risk, especially colorectal cancer. This in vitro study examined the effects of CPF and its toxic metabolite, which is a chlorpyrifos oxon (CPF-O), on the growths of human colorectal adenocarcinoma H508, normal colon epithelial CCD841, hepatocellular carcinoma HepG2, and normal hepatocyte THLE-3 cells. The results showed that CPF (0.1-100μM) concentration dependently increased the viability of H508 and CCD841 cells in serum free condition, and this effect was not found in HepG2 and THLE-3 cells. Meanwhile, CPF-O (50-100μM) reduced the viability of all cell lines. The cell cycle analysis showed the induction of cells in the S phase, and the EDU incorporation assay revealed the induction of the DNA synthesis in CPF-treated H508 cells. Even though, the inhibitory effect on the acetylcholinesterase activity was observed, atropine, which is a muscarinic receptor antagonist, did not antagonize the growth promoting effect of CPF. In addition, pretreatment either with a specific aryl hydrocarbon receptor antagonist, CII22319, or a high affinity estrogen receptor antagonist, IC131785, failed to attenuate the CPF-induced H508 cell growth. Furthermore, CPF increased the phosphorylation of the epidermal growth factor receptor (EGFR) and its downstream effector, extracellular signal regulated kinase (ERK1/2) in H508 cells. AG-1478, a specific EGFR tyrosine kinase inhibitor, completely inhibited the phosphorylation of EGFR and ERK1/2 induced by CPF. Moreover, the growth promoting effect of CPF was completely mitigated by the AG-1478 treatment. In conclusion, these results suggest that CPF promotes the growth of H508 cells through the activation of EGFR/ERK1/2 signaling pathway.

Short-Time Exposure to Thiram Induces Developmental Toxicity and Affects Deiodinase3 Gene Expression in Zebrafish Embryos

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Thiram, a pesticide of the dithiocarbamate chemical family, is widely used to prevent fungal disease in seed and crops in China. To date, little toxicological information is available and its residual toxicity is of great public health concern. In our previous studies, we found exposure to thiram, caused developmental toxicity (e.g., edema, delayed hatching, and curvature of the notochord) and may it affect the thyroid system. In the present study, zebrafish embryos (AB line) were used as a whole animal model to investigate Thiram developmental toxicity with short term exposure (1 hour) at different stage periods and observed up to 72 hpf. Embryo survival rate was decreased at 10 μM and 100 μM, and hatching rate was delayed at 0.1 μM and 1 μM. The notochord curve rate, which was a common biomarker in previous studies with thiram, was significantly increased at 0.1 and 1 μM by 1 hour thiram exposure for embryos at the stage of 2, 4 or 8 hpf. In contrast, no obvious changes were observed for the embryos exposed at the stages of 24, 48, 60, and 72 hpf. Deiodinase 3, an important enzyme in embryonic development and metamorphosis, was significantly increased at 24 hpf by 0.1 and 1 μM thiram exposure for embryos exposed at 2 or 4 hpf. These results demonstrate that the toxicity of thiram is stage-dependent and that early life stage exposure could cause more adverse effect later in life. Furthermore, this study confirmed that zebrafish embryo is a sensitive model to thiram toxicity and can become a useful model for future pesticide environmental toxicity assessment.

The Toxicity of Mancozeb in Human Colon Cells May Be Related to an Alteration of Metal Homeostasis

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Ethylendibisdithiocarbamate (EBDC) pesticides are used primarily as broad range contact fungicides on a wide variety of crops. A subset of the metal containing EBDC pesticides includes Mancozeb (MZ) which is complexed with the transition metals manganese (Mn) and zinc (Zn) and Zineb (ZB), which is complexed with Zn alone. While these agents are reported to possess low human toxicity, previous testing in our laboratory has established the toxicity of these compounds to transformed colon cells, HT-29 and Caco2. Significant decreases in viability were observed with MZ in HT-29 (80-200μM) and Caco2 cells (40-180μM). ZB exposure however, produced no significant decrease in cell viability in both cell types up to 800μM. Recent data from our laboratory suggests that exposure to the EBDC pesticide Maneb results in increases in intracellular Mn, Zn, and copper (Cu) levels within HT-29 and Caco2 cells. Therefore, the purpose of the present study was to determine if MZ and ZB exposure results in increases in Mn, Zn, and Cu levels within HT-29 and Caco2 cells. Cells were exposed to MZ (100-200μM) and ZB (20-60μM) for 24 h. The lack of toxicity observed following treatment with ZB within the same concentration range as MZ, suggests that the increased intracellular accumulation of Mn, Zn, and Cu may play a role in the generation of toxicity. The rapid increase in metal concentrations within the cell may alter metal homeostasis, producing a metal overload which may result in the toxicity seen following exposure to MZ in human colon cells.

Assessment of Oxidative Stress and Genotoxic Potential of Triazophos in Laminellus marginalis

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Genotoxic studies evaluate the effects of pollutants on edible organisms and consequently, their implications on human health. Triazophos, an organophosphorous insecticide is widely used to control various pests in several countries including India. This study is focused on the assessment of oxidative stress and genotoxicity bio markers in Laminellus marginalis (Lamarck, 1819) exposed to sub lethal concentration (0.1 mg/l) of Triazophos for 14 days. After the treatment, animals were allowed to recover for 4 days in pesticide-free water. The comet assay, micronucleus (MN) test and the frequency of cells with double nuclei (DN) in gill cells were used as indicators of DNA damage, chromosome aberrations and abnormalities in cell divisions, respectively. Cellular antioxidant defenses i.e. anti-oxidant enzyme activities (catalase, superoxide dismutase, glutathione reductase and glutathione-S transferase) and oxidative damage, i.e. lipid peroxidation (measured as thiobarbituric acid reactive substances) were used as bio markers of oxidative stress. The results demonstrated that mussel gill cells showed significant genotoxicity at 0.1mg/l (2-fold increase for DNA strand breaks). Tissue specific alterations in antioxidant enzymes were observed. Triazophos caused elevation of LPO, CAT, and SOD however, inhibition of Protein and GR in all tissues. GST showed tissue specific response like induction in Mantle, Gill and Hepatopancreas, while inhibition in Foot and Adductor muscle. Oxidative damage observed after acute exposure indicates that, mussels faced an oxidative challenge but were able to counteract, as values of antioxidant enzymes could recover to some extent after recovery for four days. Our findings suggest that oxidative stress may, in part, be contributing to triazophos-induced genotoxic damage to gill cells in L.marginalis.

Health Evaluation of the Consumption of Citrus Fruit, Leaves, and Flowers from Trees Treated with Imidacloprid for Asian Citrus Psyllid Control at Residential Sites in California

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Imidacloprid (IMI) is a neonicotinoid insecticide used by the California Department of Food and Agriculture at residential sites in California to control the Asian Citrus Psyllid (ACP), an invasive pest that is the vector of a bacterium fatal to citrus trees. IMI is applied as a soil drench to the base of citrus trees, where it is taken up by the tree roots and distributed throughout the plant. Residents could be exposed to IMI through the consumption of fruit, leaves or flowers of treated trees. The US EPA and the Department of Pesticide Regulation (DPR) of the California Environmental Protection Agency have derived oral reference dose (RfD) values for IMI using animal toxicity studies. The RfDs are based on adult and developmental neurotoxicity endpoints for acute exposures and on liver and thyroid morphological changes and decreased maternal body weight gain endpoints for subchronic exposures. There is no evidence for IMI carcinogenicity. This health evaluation uses data of IMI levels in fruit sampled from ACP treated trees together with data on citrus fruit consumption from the National Health and Nutrition Examination Survey (1999-2004) to estimate IMI exposure. Acute and subchronic
oral exposures are estimated for three subpopulations: young children (who are highly exposed on a body weight basis), women of childbearing age (as a surrogate for the fetus who may have increased susceptibility to neurotoxicity from IMI), and adults. High-end exposure estimates of the three subpopulations are found to be many-fold below the oral RfDs. We also estimate IMI exposure from the consumption of citrus leaves and flowers; the estimated doses are much lower than that from citrus fruit. Thus, adverse health effects are not expected to residents from IMI exposure via the consumption of citrus fruit, leaves or flowers from ACP treated trees.

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Attention deficit/hyperactivity disorder (ADHD) is a neurodevelopmental disorder which affects about 5% of children. Prenatal pesticide exposure has been suggested as one of the environmental risk factors affecting ADHD. We recently reported that developmental exposure to deltamethrin (DM) resulted in behavioral and neurochemical changes that are associated with ADHD. The current study evaluated the effects of developmental exposure to DM, 3 mg/kg given orally every 3 days from GD6 through PND21 on sex-specific gene expression patterns in mouse midbrain. Ingenuity Pathway Analysis (IPA) was performed on the microarray data obtained from a male and female mouse selected from each individual litter (n=5) on PND60. In female mice, a total of 113 genes were significantly changed that were associated with the functional categories of movement disorder, behavior, and cognition. In male mice, 88 genes involved in these same categories were differentially expressed. At total of 13 genes were significantly altered in both male and female mice. Three genes, PDE4B, BCL2L11, and ITSN1, were significantly up-regulated in both sexes, and four genes, DTNA, APOA4, KCNQ2, and HOMER1, were significantly down-regulated. In contrast, six genes, SDRSA4, LYNX1, TLR4, PAX6, NTRK3, and NFA1TC4, were significantly altered in opposite directions in the two sexes. Four of the significant genes, GABRA3, ADCYS, CNR1, and CHRNA2, were evaluated by quantitative RT-PCR (qPCR) for validation. However, the changes observed by qPCR did not quite reach statistical significance. Further studies with more power on earlier time points may better elucidate the sex-based differences in gene expression in mouse midbrain following developmental exposure to DM. Supported in part by NIH Grants P30ES005022 and R01ES015991.

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Exposure to organophosphate pesticides (OPs) has been associated with neurodegenerative problems in children. Chlorpyrifos (CPF) affects neurodevelopment in young individuals even at levels below toxicity guidelines due to their susceptibility and immaturity. However, data on low levels of CPF exposure experienced by the child population is lacking. The objective of this study was to investigate the relationship between urinary metabolite 3, 5, 6-trichloro-2-pyridinol (TCPy) related to exposure to CPF, chlorpyrifos-methyl (CPF-methyl), and/or TCPy and neurodevelopmental scores in 2-year-old children. Child neurodevelopment was assessed using the Gesell Developmental Schedules (GDS) from 364 infants. Participants who lived in an agricultural area of Jiangsu Province (China) were enrolled into the study from June 2011 to January 2012. Multiple linear regression and logistic regression analysis were used to estimate the association between TCPy levels and GDS outcomes. Generalized additive models (GAMs) were used to evaluate the shape of the TCPy-effect relationship by fitting splines. No statistically significant associations were found in linear regression models. However, using GAMs, decreasing trends of DQs with relatively high TCPy concentrations (>10μg/L) were observed in motor, adaptive and language area. Also, the nonparametric trends were significantly associated with TCPy levels in adaptive (p=0.0056), language (p=0.0297) area and average score (p=0.047) among girls. The findings provided suggestive evidence that the postnatal exposure to CPF, CPF-methyl and/or TCPy may adversely affect the neurodevelopment of infants living in the agricultural area.


The QuEChERS method is widely used as pesticide multi-residue method by many governments and organization laboratories. However, the main disadvantage of the QuEChERS method is that with r-DSPE it can’t achieve effective cleanup like SPE, especially for some difficult matrices like tobacco. In this study, a kind of new applicable material, multi-walled carbon nanotube, was tested to replace PSA/GCB/C18 as r-DSPE sorbent, with the purpose of achieving better cleanup performance and thus to minimize chromatography maintenance and to meet the MRM analysis for tobacco matrices. A multi-pesticide residue method based on modified QuEChERS sample preparation with multi-walled carbon nanotubes (MWCNTs) as r-DSPE material was validated for 37 representative pesticides in tobacco. The detection was performed by LC/MS/MS with multiple reaction monitoring (MRM) mode. Three major types of tobacco, blue-cured tobacco, burley tobacco, and oriental tobacco were studied and compared. Three factors, which could influence the MWCNTs’ performance, including extraction time, external diameter, using amount of MWCNTs were investigated. Average recoveries of all of the compounds in tobacco were in the range of 70.8 - 114.8% with relative standard deviations lower than 20.0% at three spiking levels of 0.02, 0.05 and 0.2 mg/kg. The limits of quantification (LOQs) and the limits of detection (LODs) for 37 pesticides ranged from 0.46 to 28.57 μg/kg and 0.14 to 8.57 μg/kg at the signal-to-noise ratio (S/N) of 10 and 3, respectively. The validated method was successfully applied to the analysis of 30 real tobacco samples, and 4 pesticides were detected. Triadimefon had the highest detected frequency, and this was followed by acetamiprid, azoxytrobin and oxadiazon. All of the residues were lower than the GRLs set by the CORESTA ACAC. These results demonstrated that the developed method could be applied to the analysis of pesticides in tobacco samples.

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The objective of this study was to determine the background exposures to pesticides as detected in urine from from 21 healthy companion dogs in Northern Colorado. A panel of 301 pesticides was used to screen urine samples collected from dogs using an established ultra-performance liquid chromatography-mass spectrometry (UPLC-MS/MS) platform. Canine food intakes were controlled for one month on diets that were also screened for pesticide contents. Fifteen distinct pesticides were detected in urine. The most frequently detected compounds in canine urine samples collected over a one-month period were atrazine, fuberidazole, imidacloprid, terbuthalin, and clopyralid. Fuberidazole was the only pesticide detected in both the diets and urine. Companion dogs develop many similar chronic diseases as humans and represent an advanced model for biomonitoring combinations of environmental pesticide exposures, and for evaluating the potential relationships between environmental exposures and disease risk.

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OPIDN (Organophosphorus-ester induced delayed neurotoxicity) is a neurodegenerative disorder characterized by ataxia progressing to paralysis with a concomitant central and peripheral, distal axonopathy. A single dose of DFP (Disopropylphosphorofluoridate) produces OPIDN in the chicken that results in mild ataxia in 7-14 days and severe paralysis. White leghorn layer chickens were treated with DFP (1.7 mg/kg, sc) after atropine (1mg/kg, sc) and eserine (1mg/kg, sc) treatment. Control chicken were treated with vehicle propylene glycol (0.1 ml/kg, sc), atropine in normal saline and eserine in dimethyl sulfoxide. The chickens were sacrificed at different time points such as 1, 2, 5, 10 and 20 days, and the tissues from cerebrum, midbrain, cerebellum, brainstem and spinal cord were quickly dissected and frozen for mRNA (northern) studies. Northern blots were probed with Tau, beta actin, and 28S RNA to study their expression pattern.
Separate set of chickens was treated for a series of time points and perfused with phosphate buffered saline and fixative for histological and immunohistochemistry (using CaMK II and β monoclonal antibodies) studies. Various staining protocols such as Hematoxylin and Eosin (H&E); Sevier-Munger; Cresyl echt Violet for Nissl substance; and Gallocyanin stain for Nissl granules were used. Increased cell death and other degenerative changes noted in the susceptible regions (spinal cord and cerebellum) than the resistant region (cerebrum), accompanied by altered protein (CaMK II β and β subunits) and mRNA (Tau) expression of above mentioned molecules, may cause the homeostatic imbalance between cell survival and cell death mechanisms. Thus activation of cellular processes involving the above mentioned molecules may play significant role in the clinical progression and syndromic clinical feature presentation of OPIDN.

### PS 2165 Application of an In Vitro Tiered Testing Approach for Predicting Eye Irritation Potential of Agrochemical Formulations

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Evaluation of chemicals for eye irritation potential is an important aspect of safety assessment. Although the rabbit Draize test has served as the conventional approach, recent global regulations and responsible stewardship programs are driving the development of non-animal approaches. Cytotoxic potential is one of the primary modes of action that can result in eye irritation. Herein, we describe our application of two cytotoxicity-based in vitro approaches, the neutral red release (NRR) and EpiOcular(TM) (EO) assays, for the evaluation of the eye irritation potential of prospective agrochemical products. The NRR has been shown to have good performance for identification of strong irritants, while the EO performs well with moderate and mild irritants. Therefore, the assays were implemented in a tiered manner starting with the higher-throughput 2D culture NRR assay to predict severe irritants and utilization of the 3D culture EO assay to identify moderate and mild irritants. In the first-tier assessment, 79 compounds representing agrochemical co-formulants (20), and ten types of formulations (59) containing 1-3 active ingredients were tested in the NRR assay and the results were compared to Draize data. The assay exhibited good performance in correctly predicting all the tested 16 severe irritants; however, it over-predicted the irritation potential of moderate (33.3%) and mild non-irritants (38.1%). To address this limitation, the EO assay was used as the second-tier test for 14 of the NRR-over-predicted formulations. The EO assay demonstrated good performance with 86% concordance for correctly classifying the NRR-over-predicted moderate, and mild/non-irritants. Overall, these data highlight the strengths and limitations of the assays as well as present a potential tier-based approach for assessing the eye irritation potential of a wide variety of agrochemical formulations.

### PS 2166 Endosulfan Exposure Is Associated with Prostate Cancer in Mexico

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It is important to assess the extent to which a chemical in the systemic circulation gains access to the brain, especially for neurotoxic compounds such as pyrethroids. We evaluated BBB permeability and uptake of DLM into the brain as a function of its free fraction in plasma, as well as the potential role of membrane transporters. 14C-DLM in HBSS buffer with different human serum albumin (HSA) concentrations (0.01-4%) was infused through the left common carotid artery @ 500 µl/min for 2 min, using a Harvard infusion pump. The left half of the brain was then collected, processed and analyzed for DLM by liquid scintillation counting. Infusion of DLM in 0.01% HSA resulted in left brain levels of 258.3 pmol/g, whereas levels of 248.6 and 133.4 pmol/g were measured following infusion in 0.1% and 4% HSA, respectively. Cyclosporine A (CSA) was coinfused with 14C-DLM to examine the potential role of transporters in brain uptake. CSA reduced the uptake of DLM from 258.3 to 175.1 pmol/g when infused in 0.01% HSA, whereas in 0.1% and 4% HSA uptake was not significantly altered. These data suggest that unidentifed influx transporters may play a role in the transport of high concentrations of free DLM across the BBB. The similar extent of DLM brain uptake in the presence of 0.01% and 0.1% HSA may be attributable to non-specific binding of DLM at low HSA concentrations. Prior infusion of mannitol increased the uptake of DLM (100 µg/ml) from 39 to 251 ng/g. The BBB thus appears to play some role in opposing the entry of DLM into the brain. In summary, these data show the novel finding that DLM uptake into the brain is dependent on its free fraction in plasma. The role of transporters in uptake may only be important at high concentrations of free DLM. Supported by the Council for the Advancement of Pyrethroid Human Risk Assessment.
Pyrethroid insecticides are used extensively in the U.S. and Europe. Low-level exposure occurs frequently in humans. Empirical toxicokinetic data for construction and validation of refined physiologically-based toxicokinetic (PBTK) models are quite limited, particularly for low doses closer to “real life” exposure levels. This study was undertaken to determine the plasma and tissue time-courses of deltamethrin (DLM) after giving an oral bolus dose of 0.05 to 5 mg/kg in corn oil (5 ml/kg) to male Sprague-Dawley rats. Serial plasma samples were obtained from 4-7 cannulated rats/dose to characterize DLM plasma kinetics, and to assess the effect of vehicle/volume on kinetics. Additional groups of uncannulated rats were dosed to obtain plasma, brain, muscle, liver, and fat at selected time points from both adult male rats and 21-day-old pups. Larger volumes of corn oil delayed and decreased the absorption of DLM from the GI tract. DLM was slowly absorbed, yielding peak plasma concentrations after 5-7 hr. At DLM doses ranging from 0.05-5.0 mg/kg, the peak concentrations and AUCs from 0-24 hr increased proportionately with dose, while the effective half-life was constant. A slow terminal portion of the time course was observed and the effective half-life was determined by the terminal slope. These parameters were fitted to the model for DLM release from body fat. In adults, liver concentrations were 20-40% higher than plasma concentrations. Brain concentrations were significantly lower than plasma concentrations. Brain concentrations were significantly lower than plasma levels, which was unexpected since DLM is a highly lipophilic compound. Extensive plasma protein binding (~90%) may limit distribution of DLM to the central nervous system. Plasma and brain levels of DLM were generally higher in 21-day-old pups than in adults at dosages of 0.1-0.5 mg/kg, while liver levels were lower in pups. This indicates age-dependency of DLM kinetics, which may be important in risk assessments in this dosage range. Supported by the Council for Advancement of Pyrethroid Human Risk Assessment.

Chlorpyrifos (CPF) and profenofos (PFF) are organophosphorus (OP) insecticides that are applied seasonally by the Egyptian Ministry of Agriculture to cotton fields. Urinary trichloro-2-pyridinol (TCPy), a specific CPF metabolite, and 4-bromo-2-chlorophenol (BCP), a specific PFF metabolite, are biomarkers of exposure, while inhibition of blood butyrylcholinesterase (BuChE) and acetylcholinesterase (AChE) activities are effect biomarkers. Urinary TCPy and BCP and blood BuChE and AChE activities were measured over a 10 month period in 2010 in adolescent pesticide applicators (n=57; 12–21 years of age) and age-matched non-applicators (n=38) prior to, during and after 28-31 days of CPF application followed by 9-14 days of PFF application. Applicators demonstrated significantly higher TCPy and BCP levels and greater BuChE depression than non-applicators throughout the OP application period. Urinary TCPy remained elevated for 4-7 weeks after the cessation of agricultural spraying, while BCP levels readily returned to baseline. While large interindividual differences in exposure were observed throughout this longitudinal study (peak urinary BCP and peak TCPy levels ranging from 1.3 to 1,726 and 10.8 to 5,715 μg/g creatinine, respectively), these OP exposure biomarkers were highly correlated within applicators (r=0.8, p<0.0001). The highly variable exposures support the need for exposure biomarker data when assessing neurobehavioral and other health outcomes associated with pesticide exposures. While lower levels of pesticide exposure were found to be associated with showering or bathing and changing clothes immediately after work, and wearing clean clothes to work, it is necessary to identify other work practices that may contribute to the high degree of variability in exposures, with the goal to reduce exposures. (Supported by the Fogarty International Center and NIEHS R21 ES017223 and R01 ES022163).
cancer bioassays. Two studies were excluded due to the presence of epilochlorohydrin. Of the six remaining studies, one oral study demonstrated increases in benign rat liver adenomas, with the highest incidence of 18% (4% in control), and one inhalation study demonstrated increases in male benzine lung tumors with the highest incidence of 44% (18% in control). Both tumor types were observed only at the 2-year terminal necropsy and were not present in the interim examinations. The other four studies revealed no neoplastic responses. Next, we examined the extensive set of genotoxicity studies and excluded those studies that utilized epilochlorohydrin. Based on the in vivo data-set, it was concluded that 1,3-D is not a genotoxicant. Lastly, analysis of mode of action (MoA) studies and temporal onset of tumors demonstrated that long-term 1,3-D exposure likely operates by promoting growth of spontaneous lesions/tumors rather than by initiating cancer de novo. The slight increases in late onset benign portal of entry tumors combined with no evidence of genotoxicity in vitro indicate an indirect, nongenotoxic MoA for 1,3-D induced rodent tumors. Therefore, the data support a threshold-based approach for risk assessment and re-classification as Suggestive Evidence of Carcinogenic Potential.

2174 Formulations of Seeds from Cannabaceae Inhibit Feeding, Reproduction, and Normal Development of Tenebrio Beetles

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Stored-product pests are a major problem globally, with losses exceeding millions of dollars per year. One of the principal pests that cause damage are the adult and larval stages of beetles. All may be a problem by their presence, either alive or dead, in grain that is to be processed for food. Extracts from the plant family Cannabaceae, in particular, have been shown to affect insect development. As a result, we investigated formulations made from seeds of Cannabaceae. All developmental stages of Tenebrio beetles were examined following exposure to the formulations made from Cannabaceae. Three groups of Tenebrio beetles were mated and raised on wheat flour (controls) and two formulations of Cannabaceae. After measuring the amount of flour each group ingested for seven days, we observed that beetles ingested significantly less flour when formulations were present compared to the controls. Approximately 61% of flour in controls was eaten compared to 30% of flour ingested in treatment groups containing Cannabaceae formulations. When beetles were mated, beetles that fed on the Cannabaceae formulations laid fewer eggs (less than 45%) compared to the controls in which 80% of couples laid eggs. Beetles, which were raised on Cannabaceae formulations (produced fewer eggs to hatch and 60-70% of surviving larvae were either deformed or died by the time they reached the adult stage.

2175 Genotoxicity and Mutagenicity Study of the Herbicides Trifluralin and Tebuthiuron

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Herbicides are a heterogeneous category of chemicals made to control weeds. Trifluralin (O,O-dihydroxy-O-(3-methyl-2-trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine) is used, among other crops, to soybean and sugarcane, and chlortoluron (1-(5-tert-butyl-4-thiazolidaz-2-yl)-1,3-dimethylurea) is widely used in crops of sugarcane. Despite some herbicides are described as selective in its mechanism of action, the study of these compounds is of extremely important. The objective of the present work is to analyze the potential of trifluralin and tebuthiuron to cause genotoxic or mutagenic effects. The comet assay and the micronucleus test were performed in HepG2 cells exposed to trifluralin and tebuthiuron at concentrations from 1 through 100 µM of each herbicide dissolved in dimethylsulfoxide. Statistical analysis were done using ANOVA test followed by Dunnett. The Salmonella/microsome test, developed by Ames, was performed in Salmonella strains TA 98 and TA 100 exposed to trifluralin and tebuthiuron at concentrations from 0.01 through 1000 µg/plate of each herbicide dissolved in dimethylsulfoxide. Statistical analysis was done through the software Salanal, using the Bernstein model. The comet assay and the micronucleous did not show difference in treated cells compared to the control. A similar result occurred in the Salmonella/microsome test, which there were no difference between treated and control group. Therefore, neither trifluralin nor tebuthiuron presented genotoxicity or mutagenicity on tested concentrations. Supported by: FAPESP - Proc. 2012/15220-3. “The opinions, assumptions, and conclusions expressed in this material are those of the authors and do not necessarily reflect the views of FAPESP.”

2177 Extraction of Highly Lipophilic Pyrethroid Insecticides from Adipose Tissue for Analysis by GC-MS

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Pyrethroids are highly-lipophilic compounds derived from the naturally-occurring pyrethrins. They are widely used in household and commercial applications, and are thus of concern for their potential health effects. As these compounds accumulate in adipose tissue, an efficient method for their extraction was necessary. An improvement on current protein precipitation methods was achieved using QuEChERS (Quick Easy Cheap Effective Rugged Safe; Agilent, Santa Clara, CA) extraction. The method was validated by FDA guidelines for bioanalytical method validation. Adipose tissue was homogenized in hexane at a ratio of 1:2, respectively. Protein precipitation was performed using acetonitrile with 1% phosphoric acid and 50 ng/mL cis-permethrin as internal standard. Supernatant was decanted into QuEChERS kits designed for high lipid content samples, which contain MgSO4, 50 mg octadecylsilyl (C18), and 50 mg primary secondary amine (PSA). The supernatant was filtered through a 0.2 µm nylon membrane into autosampler vials and evaporated in a vacuum oven. Samples were reconstituted with 10 µL toluene and vortexed prior to analysis. Deltamethrin (DLM) was quantified using an Agilent model 6890N gas chromatograph equipped with a model 5973 quadrapole mass analyzer. Method accuracy and precision had less than 20% bias at the LLOQ of 1 ng/mL and less than 15% over the remaining linear range of 1 ng/mL to 1000 ng/mL. The concentration of DLM recovered from adult rat adipose following oral dosing with 30 mg/kg DLM was found to be ~1000 ng/mL. This enhanced lipid-extraction process resulted in increased analytical sensitivity and decreased accuracy and precision bias. This work was supported by the Council for Advancement of Pyrethroid Human Risk Assessment.

2178 Using California Pesticide Information Portal (Cal/PIP) to Assess Use Patterns: Four Applications, with Caveats


The 2012 Cal/PIP use patterns for mosquito control indicate best practices. Control techniques and agents used in California reflect two key patterns: larvicides predominate over adulticides, and very few treatments rely on acetylcholine (AChE) inhibitors. Water treatments include (as highest to lowest frequency) Bacillus-based microbialis > juvenile hormone growth regulators > surface film agents > spinosad (synthetic analog of bacterial insecticide). One AChE inhibitor still used in water bodies in 2012 was temephos. Adult treatments were mainly ultra-low volume (ULV) treatments with pyrethroids and natural pyrethrins. Year 2012 citrus crop treatments were mostly recent-generation pesticides with reduced

2176 Predictive MOAs of Uterine Adenocarcinoma Development Induced by Pesticides in Rats

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Endometrial adenocarcinomas in the uterine corpus have been increasing with westernized life for menopausal women. In women, estrogen plays an essential role for the endometrial adenocarcinoma development, and there are several estrogen-mediated pathways in carcinogenic processes. Long-term treatment of xenobiotic chemicals including pesticides sometimes increased uterine adenocarcinomas in rodents mainly rats. Estrogen is also important for rodent uterine carcinogenesis, and three pathways are considered major mode of actions (MOA) of uterine carcinogenesis process in rodents: 1) estrogenic activity, 2) serum level of ratio of 17beta-estradiol (E2) to progesterone (P4), and 3) modulation of estrogen metabolism in the liver. In the present study, 7 pesticides with uterine carcinogenicity potential of over 300 pesticides evaluated in Japan since 2003 were nominated, and their MOAs were predicted based on their toxicological profiles including mechanism studies for the carcinogenicity such as estrogen metabolism in the liver or uterus, drug metabolism enzyme induction in the liver, hormone assay, or analysis for estrogenic activity. MOAs in cycoxygenase and benzyliccarb-isopropyl were predicted the modulation of estrogen metabolism, pyriminobac-methyl and spirodichlofen being increased E2 to P4 ratio. MOAs in isopropyrazam and sedaxane suggest a possibility to modulate the estrogen metabolism due to chemical structure/mechanistic similarities to cycoxygenase, but couldn’t be defined. Metazosulphon has not estrogenic activity but its MOA was not predicted. In many cases, the increases in endometrial adenocarcinoma were observed at the highest dose tested. However, since uterine carcinogenesis based on estrogen and/or metabolite driven pathway in rats is considered to be common to menopausal women, mechanism studies are useful for MOA prediction in risk assessment.
off-target potential, such as pyrethroids, neonicotinoids, microbial-derived agents, and growth hormone analogs. If the Asian citrus psyllid threatens major California citrus areas, the IPM model of today may radically change to aggressive suppression, greatly changing the patterns of insect control. Cal/PiP results for agents used to control soil-borne rodents shows little year to year change from 2004 to 2012. Second-generation anticoagulants such as brodifacoum and brodifomide are of particular environmental concern. These two compounds have consistently comprised about 20% of applications over the time frame of years 2004 to 2012. Continued use despite the legitimate concern appears to reflect the difficult trade-off between high efficacy vs. high potential for off-target kills. Groups interested in limiting pesticide exposure often seek simple metrics such as total pounds applied in CA per year for assessment of stewardship. An examination of the influence of spring weather patterns on use of fungicides, particularly high volume ingredients such as sulfur, indicates the need for caution in interpreting year to year trends.

2179 Effects of Organophosphorus Pesticides (OPs) on Airway Physiology
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OPs are well known neurotoxotants that cause toxicity via acetylcholinesterase (AChE) inhibition. OPs are also implicated in human asthma, and we previously demonstrated that the OP parathion causes airway hyperreactivity in guinea pigs independent of AChE activity. In non-sensitized guinea pigs, OP-induced airway hyperreactivity is mediated by TNF-α. In this study, we determined whether a single s.c. administration of parathion similarly influenced airway physiology as well as the local and systemic immune response of male Brown Norway rats. Airflow function was measured through controlled methacholine ventilation challenge by Flexivent 24 h after subcutaneous injection of 0.1, 1, or 10 mg/kg parathion. After mechanical ventilation studies were completed, lungs, cerebellum, and peripheral blood were collected and assayed for AChE activity, while peritoneal and bronchoalveolar lavage (BAL) samples were collected for cytokine expression profiles by ELISA and mast cell count. Parathion caused a dose-dependent increase in airway resistance. AChE activity was significantly decreased only at the highest dose of 10 mg/kg of parathion. IL-1β, TNFα, TGF-β and TGF-β were not altered in BAL or peritoneal lavage of parathion-treated animals relative to vehicle controls. The number of mast cells increased significantly at 0.1 mg/kg of parathion and decreased significantly at 10 mg/kg of parathion, indicating recruitment of mast cells at low doses and possibly degranulation at the highest dose of parathion tested.

2180 Analysis of Acetonitrile/Hexane-Extracted Biological Samples for Multiple Pesticides by HPLC UV-Vis
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Exposure to chlorinated pesticides continues to be of great public health concern. They are extremely persistent, accumulate in sediments, plants, and animals and have high potentials of bioaccumulation in human possibly contributing to chronic health conditions; neurological diseases, reproductive toxicity and cancer. Consequently, effective and comprehensive methods to determine concentrations of multiple pesticides in biological samples are desirable in controlling exposure. We have determined the concentration of different pesticides in different organs of rats exposed to multiple pesticides (endosulfan 1 and 2, Endrin, Dieldrin, 4-DDT, and Aldrin; 1:100, of LD50, in a mixture orally) for 2 weeks in corn oil. Rats’ livers, hearts, kidneys, and brains were collected, soaked and homogenized in acetonitrile or hexane to determine better extraction media and effects on detection. Homogenates was centrifuged, supernatant collected and dried. Samples were reconstituted in 1 mL hexane or acetonitrile, standards of individuals and pesticides mixtures were ran to obtain standard calibrations and retention times using HPLC UV-Vis. The results indicates that the standards elutes at 12.90, 14.90, 15.77, 15.90, 20.06, and 29.20 minutes for (endosulfan 1 and 2, Endrin, Dieldrin, 4-DDT, and Aldrin). Acetonitrile extracted samples showed significantly higher yield of the chlorinated pesticides compared to hexane extracted samples with a ratio (Acetonitrile: Hexane) of 1:3 (brain); 1:8 (liver); 366:1 (heart) and 1,1586:1 (liver) for the detection of endosulfan 1. While endosulfan 2 showed 1:1 (brain); 39:1 (kidney); 161:1 (liver). Apart from dieldrin that was not detected in these samples, the other pesticides were detected with lower ratios. Thus, these results indicate that the media used to extract chlorinated pesticides samples could have significant effects on the yields and hence quantification probably leading to under estimation of concentration chlorinated pesticides in biological samples.

2181 Bacterial Biodegradation of Organophosphorus Pesticides in Agricultural Soils Contaminated
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The increasing use of organophosphorus pesticides in agricultural practices in Colombia over the past few years has generated a number of environmental problems; these compounds tend to bioaccumulate through food chains showed high levels of toxicity triggering potential health risk for species that are exposed to these substances. In this research were used the soxhlet and solid phase micro extraction in headspace (HS-SPME) methods for the extraction of organophosphorus pesticides in agricultural cattle soils. The presence of demeton-S-methylsulfone was determined by gas chromatography coupled to mass spectrometry detector at concentrations between 272.9 and 1793.3 ppm in cropland. Besides, native soil bacteria were isolated degrading capacity of these pesticides, Bacillus sp and Pantoea agglomerans gave results of degradation of 73.5% and 68.67 %, respectively in the concentration of chlorpyrifos, showing that these microorganisms are a possible solution for improving soils contaminated by this class of pesticides.

2182 Some Toxicological Studies on Two Commercial Herbicides from the Egyptian Market on Catfish (Clarias gariepinus)
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This study was designed to evaluate the toxicological effect of two commercial herbicides from the Egyptian market on the Nile catfish. The first herbicide (herbicide-1) contains the active ingredient Glyphosate. The active ingredient of the second (herbicide-2) is Pendonethalin. The LC50 of both compounds was determined during 96 hrs. 120 fish was randomly divided into four equal groups with three replicates each. One group served as control. A group exposed to half LC50 of herbicide-1. Another group exposed to half LC50 of herbicide-2 while the fourth group exposed to combination of the two herbicides at the same doses. All groups were exposed for 15 days. The sera was separated for estimation of 8-hydroxy 2-deoxy guanosine (8-OHdG),then the fish were sacrificed and specimens from gills of all groups were obtained and kept at -20°C for Comet essay. Additional sets of gills samples were fixed in 10% neutral buffered formalin for histopathological examination. The results indicated that both herbicides cause significant increase in DNA damage that was highly obvious in group exposed to the combination of both herbicides. The gills showed histopathological changes. Further studies is undergoing on lower exposure doses. Also, to determine the active ingredient or other commercial additives could be the source of these toxicological effect.

2183 Biochemical Effects of Some Commercial Pediculicides on Some Mammalian Targets
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The biochemical effects of some pediculicides, namely Quick lotion (0.5% malathion), Neocid shampoo (1% permethrin) and Llidic lotion (0.6% tetramethrin with 2.4% piperonyl butoxide) on male mice were investigated. Pediculicides were applied on the whole body hair of mice two times with three days intervals then were sacrificed 24 hrs after the last treatment. All the tested pediculicides induced signs of neurotoxicity without mortality. The biochemical results indicated that the “Quick” treatment significantly inhibited the activities of brain acetylcholinesterase (AChE), liver Aspartate aminotransferase (AST) and liver and serum alanine aminotransferase (ALT), while brain gamma aminobutyric acid (GABA) and liver adenosine triphophatase (ATPase) activities were significantly increased. In the case of “Llidic” treated mice, significant inhibition of brain AChE, serum and liver ALT and liver AST were recorded, while significant increase in serum AST and liver ATPase activities were recorded. “Neocid” treatment significantly inhibited the activities of brain GABA and glutamic acid, and serum and liver ALT, however, liver ATPase activity was increased. The alteration shown in some of the tested biochemical parameters from the control values denote biochemical impairment and
reflect the possibility of side effects of some lotions and shampoo based pesticides to mammals. Since there are similarities between different vertebrate groups, disorders observed in mice may indicate risks to humans. More studies are necessary to evaluate the risks of these pesticides to human.

2184 Subchronic Exposure of Dicrotophos Worsens Hepatic Injury in Diabetic Rats


Background: Organophosphorus pesticides are one among the heavily and widely used pesticides. Incidence of diabetes has been also reported to increase alarmingly throughout the world. Exposure of pesticides like organophosphorus compounds have been seldom addressed under pathological conditions. Objectives: The aim of the study was to screen the effect of subchronic exposure of dicrotophos (DCTP) on STZ-induced diabetic rats and compared with non-diabetics with emphasis on liver. Methods: There were four groups of Wistar adult male rats with five rats in each group. Group 1 (G1); untreated control, G2; non-diabetic rats treated with 75μmol/rat DCTP (1/10 of LD50), G3; diabetic untreated control and G4 diabetics rats treated with 75μmol/rat of DCTP. Animals were treated intraperitoneally daily for six weeks. Blood glucose was checked before treatment and collection of samples. Liver function test and lipid profile were carried out from blood serum and AChE measurement was done from venous blood samples. Liver was collected and processed for transmission electron microscopy. Results: The treatment over six weeks did not suppress AChE which is a toxicity marker enzyme for OPC poisoning. However, all animals showed about 14% inhibition of enzyme; indicates a mild toxicity of DCTP. Liver function test (direct albumin, alkaline phosphate, ALT, AST, GGT, LDH total protein, and albumin) were found to be raised in both DCTP treated groups but only LDH was statistically significant than the corresponding control. Lipid profile (Cholesterol, HDL, LDL, triglycerides) were elevated in DCTP treated groups but statistically not significant. Transmission electron microscopy provided evidences of steatosis in both treated groups but more in diabetes treated specimen. Conclusion: The study concludes that even the non-lethal/sub-lethal dose of dicrotophos is detrimental to liver, which may be more pronounced in diabetic condition. Further studies with more prolonged exposure is suggested for a tangible conclusion.

2185 Terbufos Sulfone Toxicity Is More Severe in Diabetic Rats

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Background: Environmental exposure to organophosphorus compounds (OPC) has been reported to induce diabetes mellitus (DM) but exposure of diabetic subjects to OPC is scarcely investigated in spite of the fact that the prevalence of DM is increasing globally. Objectives: Terbufos, an OPC, is a widely used insecticide/pesticide. The present study was aimed to investigate the toxicity of terbufos sulfone (TBS) on streptozotocin (STZ) induced diabetic rat model. Materials & Methods: The animals were treated with sublethal doses (1/10) of TBS viz. 100 nmol/rat. Kaplan-Meir survival analysis, in vivo RBC-AChE activity, biochemical tests from serum (lactate dehydrogenase, creatinine kinase, blood urea nitrogen, GGT, ALT, AST), and urine analysis by dipsticks were performed. Electron microscopy was employed to examine the effect of TBS on the morphology of red blood cells (RBC), heart and kidney. Results: TBS produced 100% mortality in three weeks in diabetic rats compared to non-diabetes where all animals survived even after six weeks of daily injection. RBC-AChE decreased by significantly in diabetes group. Creatinine, BUN, Creatinine kinase (CK) and Lactate dehydrogenase (LDH) serum levels were altered in the treated groups. There was no evidence of morphological aberration in RBC. EM studies demonstrate severe renal and cardiac damage. Conclusion: TBS is highly toxic under diabetes condition. Significant depression of AChE, nephrotoxicity and cardiac infarction, are possible causes of death in diabetic rats. The study provided the first evidence of the elevated toxic effects of an OPC after the onset of DM.

2186 LactMed: A Critical Online Resource on Drugs and Breastfeeding

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Numerous professional health related organizations including the American Academy of Pediatrics, the American Academy of Family Physicians, the American Congress of Obstetricians and Gynecologists, and the World Health Organization, strongly support breastfeeding, the prevalence of which continues to rise dramatically nationally and worldwide, and which has documented health benefits for both mother and baby. Stoked by media reports of chemical hazards of all kinds, nursing mothers as well as their health care providers are concerned, sometimes justifiably but other times needlessly, about the consequences of medication use on their infants. Although caution is warranted, the risk to an infant or nursing mother of exposure to a maternal drug must be balanced against the benefit of that drug to the mother. LactMed (Drugs and Lactation Database) is a National Library of Medicine (NLM) online resource offering guidance in this area as part of its free online TOXNET system (http://toxnet.nlm.nih.gov). LactMed contains over 1100 records representing a variety of drugs, herbs, and diagnostic agents, and receives about 80,000 user queries per month. LactMed content can be searched by substance names, CAS Registry numbers, pharmacologic categories, and MeSH subject terms. Records include information on drug concentrations in breast milk, effects on breastfed infants, possible effects on lactation and, if recommended, alternative drugs. LactMed is an evidence-based, peer reviewed resource containing data drawn from the scientific literature to ensure scientific accuracy and validity. Updated monthly, the database can be searched separately or simultaneously with other TOXNET databases, such as the Hazardous Substances Data Bank which contains peer reviewed toxicology data for over 5,000 hazardous chemicals, and TOXLINE, which is a bibliographic file of 4 million references to the broad toxicological literature, to obtain a more complete profile of the substance in question. LactMed apps are available for both iPhone and Android devices.

2187 MyAlternaMed: Digital Aggregation and Organization of Phytochemical Data from Traditional Medicines and Dietary Supplements and Potential Interactions with Western Therapeutics

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The biochemical, toxicological and safety data of traditional medicines and dietary supplements is oftentimes in question by both professionals and consumers due to limited research sources and difficulty in accessing these data. MyAlternaMed is an easy-access tool for finding aggregated data about frequently used and toxicologically relevant phytochemicals found in various forms of traditional medicines and dietary supplements. The web interface is initially available in both English and Japanese. This platform can be used by health care practitioners, researchers, as well as the general public, and consists of multiple content sources which are persisted by the platform into a searchable form that can then be interpreted through the web app or as part of custom tools, such as a virtual medicine box. The personal medicine box allows a professional or consumer to “drop-in” a series of current and potential medications and it quickly determines both positive and potentially negative interactions. The data in MyAlternaMed have been sourced from curated monograph data from sources such as WHO and Health Canada and then summarized in the system with citations linking back to both monograph sources as well as additional literature and database sources. Applications from additional tools such as T3DB, OpenTox, Lhasa Derek and Meteore, PharmMapper, CTD, and Stitch, provide users of MyAlternaMed an understanding of how natural products, herbal preparations, and medicinal compounds interact with human physiology and disease. As an example of the way computational toxicology and aggregated data are used together in the MyAlternaMed platform, a case study is presented with the traditional medicine Kang Ai Pian (KAP) used for various types of cancer. KAP consists primarily of 8 herbs where constituent phytochemicals have been shown to have interactions with typical Western therapeutics used for the same cancer treatments.
2188 A Bioinformatics Pipeline—ArrayTrack for Genomics-Based Molecular Characterization of Foodborne Pathogens

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Genomic technologies (e.g., microarrays and next-generation sequencing) provide enhanced capability and sensitivity at the molecular level over conventional means such as Pulsed-Field Gel Electrophoresis (PFGE) for pathogen identification and traceback investigations. However, the value of genomic technologies cannot be fully realized without a robust bioinformatics infrastructure that can quickly process large volumes of genomic information in real-world applications. The NCTR/FDA has developed a genomics tool, ArrayTrack, which is an integrated system to manage, analyze, and visualize microarray data. Recently, the microbial genomics functionality was developed in ArrayTrack for foodborne pathogen identification and genomic characterization. Once the microarray data of unknown samples from field labs are imported into ArrayTrack, a “One-Click” summary report is generated to provide a variety of key information for the molecular characterization of pathogens. The report includes specific molecular typing (e.g., O-type, H-type, eae alleles, shiga toxin genes) of unknown samples and an overview dendrogram showing how similar or different the unknowns are compared to known reference strains. In addition, a suite of visualization and data exploration tools is available to display the gene signatures and patterns specific to outbreak strains. In this poster, the application of ArrayTrack to the CFSAN/FDA’s ECID (Minimal Signature E. coli Array Strip) array data is presented to highlight its utility to analyze microbial genomic data. The results demonstrate that ArrayTrack microbial genomic tools can be valuable in rapidly and accurately identifying bacteria and their genetic traits that are potential threats to the US food supply.

2189 Screening of Information on Drug-Induced Liver Injury from the US FDA-Approved Drug Labels

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Drug-induced liver injury (DILI) is a significant concern for drug development and regulatory application, which has led a broad range of investigation with emerging technologies to develop molecular biomarkers and predictive models for an enhanced assessment and understanding of DILI. These efforts usually start with a list of drugs whose DILI liability is reliably defined. The FDA drug labels with consensus regulatory safety information contain DILI-related annotations and have been used to define a drug’s DILI potential. However, drug labels are not static, with some 400 to 500 new or updated labels added weekly. This rapid pace of change poses a significant challenge to read the labels for a consistent DILI annotation. Here, we report a comprehensive list of terms from drug labels that enable automatic identification of drugs with DILI potential. We applied 1452 lower-level terms relevant to liver injury in the Medical Dictionary for Regulatory Activities along with 245 customized terms to three safety sections of drug labels: boxed warning, warnings and precautions, and adverse reactions. When applied to 1488 labels for single-active-ingredient prescription drugs, we identified a subset of terms (412 terms) that can effectively annotate DILI liability of a drug. For example, when 485 (32.6%) non-DILI drugs were screened with these terms, there were no false positives. To further assess the utility of these terms, we developed a DILI terminological tree by mapping terms to the Systematized Nomenclature of Medicine-Clinical Term (SNOMED-CT). The mapped terminological tree, in turn, can be used to mine DILI information from other textual sources, many of which undergo constant update such as PubMed. Our result suggests that the 412 DILI terms can be used to screen new labels for potential DILI drugs, and that counterpart terminological tree from SNOMED-CT can be used to identify drugs with DILI potential from various document collections.

2190 An Integrated Analysis of Gene and microRNA Expression Profiles in the Rat Liver Exposed to Two Structurally Similar Drugs: Amiodarone and Benzbramide

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Drugs sharing similar chemical structure are often expected to lead to similar outcomes. However, the exception to this expectation is also observed, which provides an opportunity to have an understanding of underlying mechanisms in drug toxicity. To this aim, we conducted an integrated analysis of gene and microRNA (miRNA) expression profiles in the rat liver treated by two structurally similar, but phenotypically different compounds, amiodarone (AM) and benzbramide (BBR) that induce severe liver injury. In order to identify molecular factors associated with these different phenotypes, we analyzed both miRNA and mRNA expression data from the same liver samples that were generated for four time points (8, 15, 29 days) and three dose levels (low, medium, high). We calculated differentially expressed miRNAs (DEMs) and genes (DEGs) and compared the two at gene expression level and over-expressed pathways. It was observed that the number of DEMs followed different trends over time for any given dose level as seen in DILI potential. For BBR showed a clear dose-dependent expression up to 29th day, AM did not come with a dose pattern until the 29th day. Similarly, the number of DEGs for BBR correlated with the dose level across time, but AM did not have a significant change except at the high dose. The number of pathways also confirmed the dose pattern of BBR and demonstrated the low impact of AM at early stage. Enriched toxicological functions by DEMs and pathways by DEGs indicated there exist common functional components involved in their toxicological mechanisms. We conclude that an integrated analysis of both miRNA and mRNA expression profiles can explain differences for drugs that are structurally similar. In that regard, our findings indicate that AM and BBR not only show different pathological manifestations such as steatosis and necrosis, but also engage in diverse toxicological functions by altering certain miRNAs and genes.

2191 Molecular Determinants of Pulmonary Lesions

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Microvascular lesions are observed in Group I pulmonary arterial hypertension (PAH) that restrict blood flow in pulmonary arteries. These lesions make it difficult for the right ventricle of the heart to push blood into the lungs, and eventually cause the heart to fail. Molecular and environmental risk factors associated with the development of pulmonary vascular lesions are largely unknown. Bioinformatics, related to publicly available microarray data are readily available to understand gene regulatory networks involved in toxicity pathway function. Therefore, the overall aim of this study was to meta-select microarray data from studies of PAH in order to identify molecular determinants of microvascular lesion formation in PAH patients that can be used to predict molecular risk factors of pulmonary lesions in the population. We performed the following: (i) a meta-analysis was conducted to select common up- and down-regulated genes across several studies; (ii) an enrichment analysis of significant genes; (iii) a network analysis of significant genes; and (iv) investigation and validation of the network analysis. Genes found to be most influential to PAH were ACHE, ADRA2A, AT2, BCLA1, TMF1, CYCS, DDX18, FGA, LDHA, MAP7, MAPK6, NRAS, NRP1, PDX5, PPIA, PTGS1, CD40, GP1BA, and HAAO. MAPK6 and ATP2 were the best molecular predictors of PAH. These findings suggest that modified expression of several ‘key Markov genes’ may be required for the development of pulmonary lesions and its application may be useful in identifying individuals who are susceptible to pulmonary microvascular lesion formation.

2192 StemCellQC™: A Smart Detection Multiplexing Toolkit for Toxicological Evaluation of Stem Cell Processes and Health


There is a need for rapid, high content methods to evaluate the effects of environmental chemicals and drugs on embryos and fetuses, the most sensitive stages of development. Human embryonic stem cells (hESC) are a powerful model for assessing prenatal health. This study presents a smart detection multiplexing toolkit, StemCellQC™, which non-invasively extracts cellular features from time-lapse phase-contrast video data to identify processes affected by treatment. To validate the software, control and cigarette smoke-treated hESC videos were analyzed over 48 hours, and 23 dynamic and morphological features were extracted. The features affected by treatment were determined using Bhattacharyya statistics and visual inspection of graphed data. 34 colonies were analyzed and colonies were classified as healthy, unhealthy, or dead. Features related to colony growth showed that: 1) healthy colonies grew substantially; 2) unhealthy colonies grew less; and 3) dead colonies decreased in size at critical times. Motility of healthy colonies decreased over time, while the unhealthy group had elevated and erratic, sub-diffuse motility. Analysis of total area revealed that the healthy group maintained their size, whereas the unhealthy group had decreased clone area over time. A shape descriptor, solidity, revealed a characteristic peak at 12hr for dying colonies, which can be used to predict colony death. The software’s classifier distinguished colony health with 96% accuracy using single or combinations of features. StemCellQC™ reduced the time and resources needed to track hESC colonies and eliminated false classification due to human bias. The software can
be used to distinguish key differences in treated colonies that are not visible or intuitive, and offers versatility by providing user-specified and classifier-determined analysis. StemCellQC™ can serve as an automated, label-free cell health evaluation technology in research facilities, clinics, and industry where knowledge regarding stem cell quality, health, response and functions is indispensable.

2193 A Comparative Analysis of Estradiol and PPT Treatment Effects in MCF7 Cells As Part of the Human Toxome Project Using Agilent's Genespring Multi-Omics Analysis and Integration Solution

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MCF7 cells are a widely used in vitro system to study estrogenic effects. Using Agilent Gene Expression and Comparative Genomic Hybridization (CGH) microarrays we are building a transcriptional network of 17β-estradiol (E2) and propyl pyrazole triol (PPT) responsive genes (RGs) including time and dose dependencies and interactions of estrogen RGs with other signaling pathways. Cell culture work is performed at two independent sites (Johns Hopkins University and Brown University) with extensive protocol synchronization. CGH profiling of MCF7 cells from each site allows for “customization” of network inputs including available transcription factors (TFs) and transcription factor binding sites (TFBSs) present in the genome of MCF7 cancer cell line. We previously presented differences in responsiveness in a subset of differentially expressed (DE) genes between cells cultured at different sites and are working on producibility issues. We also reported estrogen response “sentinel” genes, a subset of RGs, which are DE in E2 and PPT treated cells relative to vehicle controls in several experimental conditions including comparisons with relevant publicly available datasets re-analyzed in this study. We created gene lists based on correlated E2 responsive- and are testing whether their correlation is associated with predicted transcriptional regulators. Using GeneSpring Multi-Omics Analysis (MOA) software we integrated gene expression microarray, RNA-seq and metabolomics datasets and identified common RGs between E2 and PPT induced cells. Currently we are in the process of extending estrogen ligand network with this additional information.

2194 Flavonoids As Chemopreventive Agents by Interacting with ERBB Receptors

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ErBB or epidermal growth factor (EGF) transmembrane tyrosine kinase receptors comprise four related members: EGFR (HER1/Erbb1), ERBB2 (HER2), ERBB3 (HER3) and ERBB4 (HER4), capable to regulate critical cellular processes through Akt, MAPK, and other signaling pathways. ErBB overexpression has been associated with tumorigenesis in breast, ovaries, lung, brain, and prostate gland. Additionally, EGFR has been identified as prognostic indicator in head and neck, ovarian, cervical, bladder and esophageal cancers. All these aspects have led to consider this receptor family as targets for cancer therapy. The search for more potent anticancer compounds has been a scientific priority in recent times, when natural compounds, such as flavonoids, could act as anticancer drugs, as suggested by several epidemiological studies. In this work, in silico calculations were performed to evaluate the interaction between flavonoids and ErBB in order to identify new promising natural anticancer compounds. In total 105 flavonoids were obtained from literature and optimized by DFT B3LYP/6-31G using Gaussian 09 program. This structure was then submitted to molecular docking protocols on four different optimized ErBB structures, HER1/ITW, HER2/3P90, HER3/3LMG, HER4/2R4B, using AutoDockVina. Docking results showed natural compound such as vicenin 1, neodiosmin, hesperidin and eriocitrin presented favorable binding affinity values, with HER3 (r=0.5 ± 0.1 kcal/mol), HER4 (r=-0.4 ± 0.1 kcal/mol), HER2 (r=0.6 ± 0.1 kcal/mol) and HER1 (r=-2.1 ± 0.0 kcal/mol), respectively. In silico results suggest these flavonoids could be potential anticancer drugs and serve as scaffolds for designing new and more potent anticancer molecules via ErBB interaction. Maldonado-Rojas is sponsored by Colciencias, 528-2011.

2195 Chemical Effects in Biological Systems (CEBS) Database: Advanced Histopathology Search Applications

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One of the central components to the design of the Chemical Effects in Biological Systems database (CEBS: http://cebs.niehs.nih.gov) is the ability to capture not only a comprehensive set of toxicity endpoints, but also study related protocols that enhance a user’s ability to evaluate study outcomes. CEBS serves roles as a curated public repository for all toxicoology data arising from the National Toxicology Program’s (NTP) test program, and as a portal for users to query this data by test article, CAS number, NTP study type and assay endpoint. Data captured in CEBS extends beyond standard toxicological testing e.g. PCR and microarray, which when combined with the additional study protocol information makes CEBS a valuable resource for toxicity assessments. The ability to search data in CEBS has recently been extended to include additional query options for reviewing histopathology data. Currently, CEBS has histopathology findings for 512 test articles. Using workflows (guided search modules), users can access a summary table for site specific neoplasia or customize their search criteria to review specific subsets of histopathology data. Queries of the database can provide information on the test articles associated with different NTP defined levels of evidence of carcinogenic activity, tumor morphologies and sites (Sites and Association workflow). The user also has the ability to query the data for statistically significant tumor findings or individual animal neoplastic and non neoplastic findings (NTP Pathology Data workflow). Data can be filtered on the basis of study date, morphology, organ, severity grade, species, sex, route and treatment group type. All data in CEBS including workflow results can be downloaded.

2196 Exposure Science Data and the Comparative Toxicogenomics Database

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Human exposure to chemicals and other environmental stressors can contribute to disease and related phenotypic outcomes. Exposure science characterizes the interactions between human or ecological receptors and environmental stressors, and contributes to the assessment of human health risks and prioritization of toxicological research. We sought to curate and integrate exposure science data with core information in the Comparative Toxicogenomics Database (CTD; http://ctdbase. org) framework, to provide a centralized, freely available resource that facilitates the identification of connections between real-world exposures, chemicals, genes, proteins, diseases, and molecular pathways. CTD is a manually curated database containing over 24 million toxicogenomic relationships for chemical-gene-disease interactions, Gene Ontology (GO) annotations, and molecular pathways that are integrated with analysis tools to promote understanding of the molecular mechanisms underlying environmental diseases. In our exposure curation paradigm, the peer-reviewed literature is manually curated using several controlled vocabularies and free text to capture details in more than 50 data fields characterizing four Exposure Ontology (ExO) concepts: Stressor, Receptor, Event, and Outcome. To date, over 1000 articles have been curated, resulting in more than 41,000 exposure statements for 550 unique chemicals, 197 distinct receptor populations from 100 countries, 231 diseases, and 128 phenotypic outcomes. Here, we describe the design of our user-friendly web portal for incorporating exposure data within the CTD framework, and how this integrated platform will help promote mechanistic understanding of environmental influences on human health.

2197 The Comparative Toxicogenomics Database: Ten Years in the Making

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The Comparative Toxicogenomics Database (CTD; http://ctdbase.org) was originally developed out of a need to formalize, harmonize, and centralize the numerous genes and proteins responding to environmental toxic agents across diverse species. Now celebrating our ten-year anniversary, CTD remains a free scientific resource that informs hypothesis development about environmental effects on human health. CTD core content includes a triad of chemical, gene, chemical-disease, and gene-disease interactions manually curated from the scientific literature, using controlled vocabularies and structured formats. These data are then integrated with functional information and canonical pathways to infer relationships between chemical exposures and biological events. Utilizing enhanced curation practices
(including targeted journal curation and text mining), CTD continues to address data completeness and currency in providing information to the toxicology community. Here, we present new updates and tools for CTD, including our increased data content (>24 million toxicogenomic connections), our new "Pathway View" visualization tool (to generate novel toxicogenomic interaction modules), an expanded chemical-phenotype strategy (to generate meaningful links between chemicals, early cellular phenotypes, and diseases). CTD continues to expand in depth and functionality to meet the evolving needs of the environmental health research community.

2198 ToxEvaluator: An Integrated Computational Platform to Aid the Interpretation of Study-Related Findings


Attempts are frequently made to find adverse findings from preclinical and clinical studies in order to better understand underlying toxicity mechanisms. These efforts often begin with limited information, including a characterization of the finding, knowledge of the structure of the chemical associated with its cause, and the intended biological target. ToxEvaluator was developed jointly by Pfizer and NCsu as an in silico platform to facilitate hypothesis generation for toxicity findings. Through the integration of a diverse set of in silico tools and leveraging a number of public and private databases, ToxEvaluator streamlines the process of aggregating information. The user enters compound and target identifiers, and selects adverse event descriptors from a safety lexicon and mapped MeSH disease terms. ToxEvaluator provides a summary report with five distinct areas organized according to which target or structural aspect has been linked to the adverse event, including: 1) predicted unintended pharmacology, 2) known unintended pharmacology, 3) structurally similar proprietary compounds, 4) structurally similar external compounds, and 5) intended pharmacology. Similar proprietary compounds and their associated in vivo toxicity findings are reported along with a link to relevant supporting documents. For similar external compounds and interacting targets, ToxEvaluator integrates relationships curated in the Comparative Toxicogenomics Database (CTD; http://ctdbase.org), returning all direct and inferred linkages between them. As an example of its utility, ToxEvaluator rapidly identified an off-target of cerivastatin, SLCO1B1 (OATP1B1), with a direct literature linkage to myopathy, the adverse finding input to the query. Several use cases will be included that demonstrate how ToxEvaluator could serve as a model for data integration and a user-friendly, in silico tool to efficiently identify information for deriving testable mechanistic hypotheses.

2199 Big Data to Knowledge (BD2K)—A Graphical Approach for Data Coordination and Integration

J. F. Reichard, M. Medvedovic and S. Sivasag.

Big data has the potential to transform toxicological risk assessment by supporting identification of early effect biomarkers, key events, pathway-based points of departure and adverse outcome pathways. Such data includes gene expression signatures resulting from disease processes, chemical perturbations, gene knockout/down, DNA binding (e.g. ChIP-seq data) as well as protein interaction networks. However, leveraging such data requires extensive expertise to mine genomic data, proficiency with sophisticated software required to evaluate data, and an appropriate application of statistical methodologies to identify true signatures without resorting to ad hoc methods. The Big Data 2 Knowledge (BD2K) project (www.bd2k.nih.gov) is addressing these challenges in order to make big data more accessible to toxicologists and risk assessors. Big data projects funded through NIH Common Fund include major data generation efforts, such as Library of Integrated Network Cellular Signatures (LINCS) and BD2K Centers, which are developing methodologies and computational infrastructure for analyzing such data. As one of these centers, we are focused on developing statistical algorithms to integrate multidimensional data at the intersection of the LINCS and BD2K initiatives. Our method is based on a diffusion graphical kernel approach, as is used in machine learning and physics, rather than ascribing arbitrary ontology relationships. The diffusion kernels approach captures long-range relationships between graph vertices (e.g. proteins), increasing the sensitivity to identify active pathways. This approach will be part of a web-based interface, such as the current iLINCWS web-based portal, which enables the identification of functional connections between drugs, genes and diseases (http://eh3.uc.edu/GenomicsPortals/viewILincs.jsp). Supported by: U54 HL127624

2200 A ToxBank Integrated Data Analysis of SEURAT-1 Reference Compounds


The SEURAT-1 Safety Evaluation Ultimately Replacing Animal Testing-1 research cluster is comprised of seven EU FP7 Health projects and is co-financed by cosmetics Europe. The SEURAT-1 strategy is to adopt a mode-of-action framework to describe repeated dose toxicity to derive predictions of in vivo toxicity responses. ToxBank is the cross-cluster infrastructure project which provides a web-accessible shared repository of research data and protocols. The ToxBank data warehouse makes use of the ISA-Tab standard to describe experimental metadata and OpenTox services supporting interoperable data integration and analysis. In addition, a curated data standard foromics and assay data is incorporated in supporting the meta analysis. We present here a workflow for pathway analysis using Open TG-GATEs, ToxCast and other data. A summary of the 14 compounds present in both the SEURAT-1 common set of reference compounds (details available at wiki.toxbank.net) and the Open TG-GATEs will be presented. Information derived from the Open TG-GATEs has been uploaded into ToxBank for these compounds. Using this information a multiple KEGG pathway enrichment analysis of TG-GATEs and gene targets from the ToxCast assays has been performed for Valproic acid. The addition of the CYP19A1 gene from ToxCast data promotes the significance of the steroid hormone synthesis pathway inferring the impact of VPA on the conversion of cholesterol to hormonal steroids. Characterization of the SEURAT-1 reference compounds’ differential expression patterns lends support to the idea that they activate highly variable biological mechanisms, which nevertheless are compound-specific and may represent viable biomarkers for toxicity.

2201 Development of a Framework for an Environmental Health Science Language


Language standards, from simple glossaries to more complex controlled vocabularies, thesauri and ontologies, enable data sharing, integration and analysis across diverse research, clinical and regulatory settings. One component of the NIH Big Data to Knowledge (BD2K) initiative is to establish community-driven frameworks for developing and using standards for data and metadata. Language standards are of particular importance to environmental health science (EHS), where there is a need to work across very diverse disciplines and an expanding landscape of data types (e.g., exposures, genotypes, phenotypes, and the microbiome). To address this need, we employed a multi-pronged strategy to support development of an EHS language framework. We systematically identified existing resources and sought input from diverse stakeholders (e.g., research domain experts, publishers, curators, database managers, research funders) through Requests for Information (RFI) and workshops. These activities indicated the need to build communities around specific research interests and needs, enable integration across the spectrum of EHS data, and provide a low barrier of access for researchers to use or build a language standard. Specific recommendations included: (1) enabling discovery and evaluation of existing language standards; (2) assisting early collaborations in developing standards; (3) proactively engaging collaborations in identifying and resolving inconsistencies in language standards; (4) supporting citation and attribution of language standards; (5) planning for sustainability; (6) capitalizing on existing projects to pilot a language framework approach. Collectively, these activities created a community of practice to develop and refine use cases for language standards that target critical EHS research questions. This poster will provide an overview of existing EHS language resources and gaps, use cases for new language standards and standards development activities within the larger NIH BD2K initiative.

2202 Using the Reactome Pathway Database and Bioinformatics Tools for the Visualization, Interpretation, and Analysis of Toxigenomic Data

M. E. Gillespie.

Reactome is an open-source, open access, manually curated and peer-reviewed pathway database. Pathway annotations are authored by expert biologists, in collaboration with Reactome editorial staff and cross-referenced to many bioinformatics databases. These include NCBI Gene, Ensemble and UniProt databases, the UCSC and HapMap Genome Browsers, the KEGG Compound and ChEBI small molecule databases, PubMed, and Gene Ontology. The rationale behind Reactome is to convey the rich information in the visual representations of biological pathways fa-
Accurate Literature Mining

The U.S. Department of Homeland Security (DHS) Chemical Security Analysis Center (CSAC) identifies and assesses vulnerabilities and response to potential chemical threats to the U.S. and must consider thousands of chemicals when devoting limited resources to assess hazard and risk to human populations. Accurate human toxicity estimates are critical; inaccurate estimates lead to unreliable outcomes of predictive chemical terrorism assessments of risk, hazard, and countermeasure efficacy and safety. In contrast with existing Tox21 programs, DHS is concerned with: 1) an intentional release/attack and a one-time chemical exposure; 2) different chemicals of concern and chemical prioritization; 3) single chemicals and chemical combinations; 4) primarily acute toxicity effects, both lethal and non-lethal; 5) different exposure scenarios; and 6) rapid chemical identification and response to attacks. In addition, DHS has high interest in applications to countermeasure development. CSAC recently instigated a predictive toxicology program that leverages existing Tox21 programs to develop acute toxicity prediction models that combine in vitro toxicity testing with computational toxicology, applied specifically to DHS needs. Initial efforts are focused on investigating the objectives and current status of existing programs, forming collaborative relationships with participating organizations, and identifying shortfalls in existing programs in light of DHS needs. To this end, CSAC has conducted a market survey, collecting over 3,000 references and setting up a comprehensive structural framework for a searchable computational toxicology library. Databases containing information on DHS chemicals of interest have been identified. Once gaps in existing programs are characterized, CSAC will develop/review tools to address DHS-specific needs, test and validate developed models with representative chemicals from one selected toxicology (acetyl cholinesterase inhibitors), and potentially develop models to address other toxicodemes of interest.

2206 Quantitative Nanostructure Toxicity Relationship: Developing Predictive Cell Recognition Models for Gold Nanoparticles

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Drug delivery using nano materials (e.g. nanoparticles) is a promising method to achieve cell recognition. Folate receptors (FRs), which are overexpressed in many human cancer cells, have been used as ideal targets for the treatment of cancer and inflammatory diseases over several decades. In this study, we compiled a dataset consisting of 30 mono-ligand gold nanoparticles (GNPs) and 30 dual-ligand GNPs on cell recognition and uptake against four human cancer cell lines that express different levels of FR, one of which was used as a control cell line. Quantitative nanostructure toxicity relationship (QNTR) models were developed using this dataset. Specifically, we simulated the surface chemistry of GNPs by developing new weighted fragmental descriptors, since the accessibility of receptor-binding correlates to the distance from ligand-binding domain (LBD) to the gold core. Various modeling approaches (e.g. random forest, support vector machine and etc.) were applied to the resulting descriptor set using ten-fold cross-validation procedure. The modeling results clearly indicate the relationship between nanostructure (i.e. GNPs with different ligands) and cell recognition. The validated models can be used to design new GNPs with desired cell recognition and the developed QNTR approach can be used to build predictive models for other nanoactivity endpoints.
The new International Conference on Harmonisation (ICH) M7 guideline requires the use of both a statistics-based QSAR methodology and an expert rule-based methodology for the genotoxic hazard assessment of impurities. One of the major differences between these two methodologies is the nature of the alerts and how they are obtained. The objective of this work is to highlight similarities and differences between these two types of alerts and their effect on the prediction accuracy of the genotoxicity of impurities. We compared statistically-derived genotoxicity alerts and their equivalent rule-based alerts and present their effect on the sensitivity and specificity of predicting the toxicity of new test chemicals. The preliminary results found 88% sensitivity and 79% specificity for statistically derived alerts compared to 79% sensitivity and 61% specificity for the equivalent rule based alerts. Overall, we found that statistically-derived alerts performed better than rule-based alerts. However, we demonstrated in several cases that the two types of alerts are more complementary than redundant. We also show the impact of these two different types of alerts on: 1) the human expert review of the software test results, 2) understanding the mechanism of toxicity, 3) comparing across different endpoints or models, 4) validation of the (Q)SAR models and 5) adaptability when new experimental data become available.
In drug development, genetic toxicology studies are conducted using a battery of in vitro and in vivo tests to identify potential chemical–induced gene mutations and clastogenic damage, as described in the ICH S2 guideline. Varying numbers and types of studies are required for active ingredients, major metabolites, impurities, and degradants of drug substances. In some instances, (quantitative) structure-activity relationship (Q SAR) model predictions may be used to supplement or replace the use of experimental testing, in particular when empirical data are limited or lacking. In the current study, two commercially available (Q) SAR platforms were used to build models for predicting chromosomal aberrations in Chinese Hamster Lung (CHL) and Chinese Hamster Ovary (CHO) cell lines. The criteria set by OECD 473 were used to govern the data selected for model construction. Datasets were well-balanced, with 53% and 45% positives, respectively, and contained both re-evaluated legacy as well as new data to expand the chemical space of our previous models. Cross-validated (Q) SAR model performance of the CHL models built using 876 training compounds showed sensitivity of up to 79% and negative predictivity of up to 75%, an improvement over previous CHL models constructed from 749 compounds, which had cross-validated sensitivity up to 49% and negative predictivity up to 66%. For the CHO cell line, cross-validated performance of the new (Q) SAR models showed sensitivity up to 63% and negative predictivity up to 72% based on a training data set of 821 compounds, in contrast to the earlier models based on 688 compounds with sensitivity up to 47% and negative predictivity up to 70%. These two models more effectively predict in vitro chromosome aberration assay outcomes and cover a broader range of chemical structural attributes and functionality than earlier models, providing a state-of-the-art approach to genetic toxicity screening for components of drug products.

A three dimensional spectral-data activity relationship (3D-SDAR) approach utilizing fingerprints constructed from 13C and 15N NMR chemical shifts augmented with interatomic distances was used to model datasets of phospholipid (PLD) inducers and human Ether-a-go-go-Related Gene (hERG) blockers. A mapping technique based on the frequency tessellation (binning) of 8 ppm x 8 ppm x 1 Å for the C-C region, 8 ppm x 20 ppm x 1 Å for the C-N region and 20 ppm x 20 ppm x 1 Å for the N-N region and 3 latent variables produced a model which classified correctly 70% of the compounds in the external test set. A mapping technique based on the frequency of occurrence of highly weighted bins accumulated during multiple training/test set randomization cycles served to identify a three-center PLD toxicophore. This toxicophore was composed of two aromatic rings with a 4 Å to 5 Å distance between their centroids and a secondary or tertiary amino group (variably primary) at a distance of 3.5 Å to 7.5 Å to the centroid of one of the aromatic rings and 5.5 Å to 7 Å to the other. It was found present in the structures of many cationic amphiphilic drugs. Similarly treated dataset of 46 hERG+ and 115 hERG- blockers, resulted in a model with 80% classification accuracy. The 3D-QSDAR map revealed the existence of a toxicophore similar (although less geometrically restrained) to the one found in the structures of the PLD+ compounds. Thus, it was concluded that PLD and hERG may share a similar molecular mechanism. This support of this hypothesis is reported in Bioorg. Med. Chem. Lett. 2013, 23, 4587; BLAST searching amino-acid sequence of the pore domain of hERG to other known protein sequences from the UniProt database was used to identify potassium channel subfamily K protein members as potential PLD targets.
the Running Fisher's algorithm, the signatures were compared to -1500 biosets in an annotated human gene expression database to identify those that exhibited a significant positive correlation to the signature. Each signature identified an independent set of chemicals known to activate the TF. For example, the AhR signature identified biosets associated with known AhR-activating chemicals including TCDD, benzo[a]pyrene and quercetin (p < 1x10^-33), thus validating the method. Future work will focus on expanding the analysis to other TFs, allowing a comprehensive assessment of the chemical modulation of multiple human TFs in large, publicly available, genomic datasets. This abstract does not represent EPA policy.

2219 Data Integration and Visualization for Transparent Communication of the Category Read-Across Using ToxCast (Toxicological Priority Index) Tool: P-series Glycol Ethers Case Study

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The integration and communication of diverse information into a REACH read across application is often challenging given the wide variety of data types and sources. In this study we sought to utilize ToxPi software and graphical user interface to aggregate and visualize a diverse database of information that was assembled in support of a category read across submission under REACH. We chose a case study of P-series glycol ethers which are typically formed by combining propylene oxide (PO) with a C1-C4 primary alcohol (ex. methanol, ethanol, n-propanol, n-butanol) and a base catalyst to produce a mixture of mono-, di-, tri-, and higher propylene glycol ethers. The information available on these chemicals included physicochemical properties, environmental fate, mutagenicity/genotoxicity, as well as data from a variety of ecological and mammalian experimental model systems. The data was transformed into a ToxPi-compatible format, scaled, analyzed, and rendered multiple times to compose visualizations with alternate category grouping scenarios. Several data weighing schemes were also utilized to yield comparisons that may reflect various stakeholder priorities. To address the need for better analytical and communication approaches, the ToxPi-derived grouping and schemes can be applied according to different stakeholder criteria and the outputs can be adjusted in a transparent manner, along with the resulting visualizations, to communicate the results. We found overall that the glycol ethers group together within their structure-based category. ToxPi allows for data incorporation, differential weighing, relative ranking based on the input, and step retention to maximize working transparency. This is accomplished while encouraging a multidisciplinary framework, criteria and guidance to maximize flexibility of the application and further facilitate effective communication of complex data into relevant presentation.

2218 Integration into Big Data: First Steps to Support Reuse of Comprehensive Toxicity Model Modules

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Data surrounding the needs of human disease and toxicity modeling are largely siloed limiting the ability to extend and reuse modules across knowledge domains. Using an infrastructure that supports integration across knowledge domains (animal toxicology, high-throughput screening, genomics, proteomics, disease, exposure, product use, chemistry, etc.) increases the ability to evaluate, extend and expand models. For example, type II diabetes is a metabolic disorder caused and influenced by a combination of genetics, lifestyle and environment. In order to quantify the contribution of each factor and related confounders (e.g., diagnosis, screening, and treatment), the modeling framework relies on the ability to systematically access information across many knowledge domains to more accurately resolve the uncertainty resulting from the complexity within and across each factor. A first step to developing an integrated system was to develop an object model (i.e., a conceptual representation of each knowledge domain; ontologies) to resolve data redundancy and granularity issues from the complexity of the data. The advantage of an object model over siloed databases was the ability to conceptually link and merge previously disconnected datasets. The current object model enables the modular development of systems capable of providing an extensible framework for building a more comprehensive human disease model. This abstract does not necessarily reflect US EPA policy.
2221 An Integrative Approach for Predicting PAH Toxicity in Environmental Mixtures
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Our understanding of polycyclic aromatic hydrocarbon (PAH) mixture toxicity is limited by the fact that most PAHs are studied as individual chemicals. PAH exposure and risk are typically characterized by a small number of frequently monitored compounds based on equivalency to a reference. We propose that exposure and toxicity to PAHs in whole environmental mixtures can be addressed through integrative modeling of high-throughput chemical and biological data. We have developed a systematic approach for the management, integration and visualization of (1) quantitative PAH analysis of environmental samples collected from Portland Harbor Superfund site and Deepwater Horizon oil spill and (2) high-throughput toxicity screening of mixture extracts and individual PAHs in zebrafish embryos. Our model shows that the environmental samples collected 2009-2011 can be separated into 3 distinct groups, which distinguish both sampling location and date, based on their chemical PAH profiles. Zebrafish dose-response toxicity (26 developmental endpoints) for each mixture was summarized (BMD10, EC50, AUC, LEL) for integration with chemical data. When zebrafish BMD10s are evaluated within the context of the 3 chemical clusters identified, we find that mixtures causing the most toxicity in zebrafish are predominantly from the 2 clusters with the most PAHs detected at the highest concentrations. Overall, the total combined PAHs measured in each mixture extract significantly correlated (p<0.0001) with average BMD10 across all endpoints and specifically with caudal fin and combined PAHs measured in each mixture extract. These findings are consistent with previous studies showing that PAH exposure can cause dysfunction in the timing of onset of puberty.

In January 2014, a chemical mixture used for cleaning coal was accidentally released into the Elk River in West Virginia, resulting in contamination of the water supply for nearly 300,000 people. The mixture included 4-methylcyclohexanemethanol (MCHM), 1,4-cyclohexanedicarboximide (CHDM), 4-methoxycyclohexanemethanol (MMCHM), dimethyl 1,4-cyclohexanedicarboxylate (DMCHDC), and methyl 4-methylcyclohexanecarboxylate (MMCHC) a proprietary mixture of propylene glycol esters containing propylene glycol phenyl ether (PPH) and dipropylene glycol phenyl ether (DPPH). Limited toxicological data were available for these chemicals; therefore, a review of the available Tox21 high throughput screening (HTS) assay data was performed along with a structure activity relationship (SAR) analysis to predict potential toxicological effects. Four chemicals (MCHM, CHDM, DMCHDC, PPH) were evaluated in 27 stress response pathway and nuclear receptor assays. All four chemicals were inactive at concentrations up to ~100 μM. For SAR evaluations, 7 platforms were used (ADMET Predictor3M, CASE Ultra, Leadscope®, MetaDrugTM, SymmetrySM, Toxtree, VEGA). The results were organized based on the category of predicted endpoints and an integrated analysis was performed. Consistent with observed effects in West Virginia residents following the spill, the SAR analyses predicted that a number of the chemicals would be irritating to the skin, eyes, and lung. The chemicals also were predicted to produce effects in the liver and kidney, which is largely consistent with the limited, available toxicity data. A focused assessment of dose-response for effects on these and other target organs/systems will be performed as part of ongoing NTP toxicological studies.

2222 Converging Bioinformatics Evidence Implicates Endocrine-Disrupting Pesticide Mixture Exposure on Epigenetic Regulation of KISS1-GnRH Control on Precocious or Delayed Puberty
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The purpose of this research was to analyze epigenetic regulation of genes involved in dysfunction in the onset of puberty in response to pesticide mixture exposure. Kisspeptin (KISS1)-NRFI1-GPR54 signaling initiate gonadotropin-releasing hormone (GnRH)-gonadotropins (LH/FSH) secretion at puberty. Epigenetic regulation of KISS1 is involved in estrogen-mediated GnRH/gonadotropin surge. KISS1 via mitochondrial biogenesis provides significant excitatory neurotransmission and a concomitant decrease in inhibitory inputs to GnRH neurons for puberty to occur. To show that timing of puberty is under the control of transcriptional repression of Polycomb group (PcG) of silencers (e.g., CBX, RING, EED) in prepubertal hypothalimus. We applied Florida Department of Agriculture 2010 data on pesticide use and selected top 25 pesticides (herbicides, fungicides, insecticide and other chemicals) to analyze their epigenetic effects on the onset of puberty. Chemical Toxicogenomics Database (CTD) revealed ~25,000 interactions with ~1200 genes leading to complex diseases and precocious and/or delayed puberty. Results indicate that following pesticides, Mancozeb, Maneb, Endosulfan, Chlorpyrifos, Dimethoate, Thiram, Pendimethalin, H2O2, Atrazine, Paraoxan, Bifenthrin, Copper sulfate, Diazinon, Malathion may repress transcriptional silencing of the additional information now available we present a case study on the peroxisome proliferator-activated receptor gamma (PPARY). PPARY is a key modulator of adipocyte differentiation and has been implicated in a number of adverse health outcomes, including metabolic diseases, diabetes, atherosclerosis, and cancer. The ToxCast assay suite includes six assay endpoints specific to PPARY, with each varying sensitivity and specificity. Using only the hit calls across these six assays, the number of active chemicals in an assay range from 14 to 528 out of 1859 distinct chemicals. However, utilizing information derived from the updated analysis pipeline changes these results considerably. If we count only samples without analysis flags indicating potential data quality concerns, the number of active chemicals range from 12 to 382. Additionally, once we remove active with potency values within the range of cytoxicity, the number of active chemicals declines to a range of 5 to 88. After all filtering, we reduced the number of distinct chemicals active in at least one of the six PPARY assays from 671 to 157 (23.4% of the filtered list). The list of 157 includes known reference chemicals such as the pharmaceutical SSR-69071 and the tributyltin compounds. Through the case study with PPARY we highlight the inherent complexity of the ToxCast data and illustrate the necessity for external researchers to go beyond the summary potency values and utilize all available information. This abstract does not necessarily reflect Agency policy.

2223 HTS and SAR Analysis of Chemicals from the Elk River Spill

The USEPA ToxCast program has tested thousands of environmental chemicals in hundreds of assays to inform toxicity models and prioritization for further toxicity testing. Recently the ToxCast program updated its analysis pipeline, providing greater information to build more accurate models. To illustrate the importance of the additional information now available we present a case study on the peroxisome proliferator-activated receptor gamma (PPARY). PPARY is a key modulator of adipocyte differentiation and has been implicated in a number of adverse health outcomes, including metabolic diseases, diabetes, atherosclerosis, and cancer. The ToxCast assay suite includes six assay endpoints specific to PPARY, with each varying sensitivity and specificity. Using only the hit calls across these six assays, the number of active chemicals in an assay range from 14 to 528 out of 1859 distinct chemicals. However, utilizing information derived from the updated analysis pipeline changes these results considerably. If we count only samples without analysis flags indicating potential data quality concerns, the number of active chemicals range from 12 to 382. Additionally, once we remove active with potency values within the range of cytoxicity, the number of active chemicals declines to a range of 5 to 88. After all filtering, we reduced the number of distinct chemicals active in at least one of the six PPARY assays from 671 to 157 (23.4% of the filtered list). The list of 157 includes known reference chemicals such as the pharmaceutical SSR-69071 and the tributyltin compounds. Through the case study with PPARY we highlight the inherent complexity of the ToxCast data and illustrate the necessity for external researchers to go beyond the summary potency values and utilize all available information. This abstract does not necessarily reflect Agency policy.

2224 Making Sense of the New ToxCast Data: A Case Study with PPARY

The USEPA ToxCast program has tested thousands of environmental chemicals in hundreds of assays to inform toxicity models and prioritization for further toxicity testing. Recently the ToxCast program updated its analysis pipeline, providing greater information to build more accurate models. To illustrate the importance of the additional information now available we present a case study on the peroxisome proliferator-activated receptor gamma (PPARY). PPARY is a key modulator of adipocyte differentiation and has been implicated in a number of adverse health outcomes, including metabolic diseases, diabetes, atherosclerosis, and cancer. The ToxCast assay suite includes six assay endpoints specific to PPARY, each with varying sensitivity and specificity. Using only the hit calls across these six assays, the number of active chemicals in an assay range from 14 to 528 out of 1859 distinct chemicals. However, utilizing information derived from the updated analysis pipeline changes these results considerably. If we count only samples without analysis flags indicating potential data quality concerns, the number of active chemicals range from 12 to 382. Additionally, once we remove active with potency values within the range of cytoxicity, the number of active chemicals declines to a range of 5 to 88. After all filtering, we reduced the number of distinct chemicals active in at least one of the six PPARY assays from 671 to 157 (23.4% of the filtered list). The list of 157 includes known reference chemicals such as the pharmaceutical SSR-69071 and the tributyltin compounds. Through the case study with PPARY we highlight the inherent complexity of the ToxCast data and illustrate the necessity for external researchers to go beyond the summary potency values and utilize all available information. This abstract does not necessarily reflect Agency policy.

2225 Bioinformatic Analysis of Epigenome Response to Environmental Pollutants
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Toxic agents present in the environment could act directly on enzymes controlling epigenetic modifications or indirectly on signalling pathways causing long-term alterations in chromatin structure that may or may not be heritable. The aim of this study is to elucidate impact of arsenic compounds on nucleosome regulation and epigenetic modification associated with various human diseases, by applying sys-
tems biology and bioinformatics approach. We mapped comprehensive knowledge about biological pathways, including pathways involved in nucleosomial regulation, DNA damage and repair, reporting affected molecular processes and functions by manually annotating and processing information from the public domain and proprietary company datasets. We created a computational model of an epigenome regulation network, consisting of curated literature knowledge and high-throughput data related to protein-chemical, protein-gene, protein-protein interaction data. In this study we tested our model of arsenic health and safety issues, and proposed underlying molecular mechanisms, supported by current knowledge, confirming the role of epigenetic changes in key genes, such as tumor suppressors, as well as other epigenetic components that mediate arsenic toxic effects.

2226 Comparative Data-Mining Across Multiple Toxicity Databases Harmonized by eTOX Ontology to Test Hypotheses

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The eTOX database has been constructed from diverse preclinical toxicity reports from 13 pharmaceutical companies and continues to be expanded as it exchanges data with other domains. For example, over 100 marketed pharmaceuticals (>10% of the chemicals in the entire eTOX database) have been tested in Toxcast Phase II. The chemical space and associated toxicological findings have been also extended to include cosmetics, food additives, and industrial chemicals by importing databases from COSMO, ESS, and RepDose into eTOXsys (the user interface of eTOX) database. Based on the greater chemical and biological domains, two use cases were performed to identify and refine chemical categories. The first is based on the hypothesis that statogenic chemotypes (e.g., lipid vaculization) are similar even for compounds from quite different substance-use spaces (drugs, cosmetics, industrial chemicals). Such chemotypes enable building mechanistic categories for a particular toxicological finding. The second study focused on effects of C6-carboxylic acids and their analogs on specific hematology parameters. The database analysis was able to refute the hypothesis that specific chemical features tend to be associated with a decrease in erythrocytes count and hemoglobin content, increase in reticulocytes, thrombocytes, leukocytes, monocytes and neutrophils counts. It revealed a potential relationship between hematological effects and the pharmacological mode of action. Both data mining processes rely on well-controlled vocabulary of sites and effects. Results from both cases demonstrated that enriching the analysis with information from other substance domains, was critical to explore underlying mode-of-action pathways.

2227 Relating Adverse Events to Contraindications: A New Window into Drug Toxicity

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Contraindications (CIs) are risk factors that prevent a particular medicine from being prescribed to a specific category of patients due to increased risk of a harmful outcome. It is not always known what the underlying reasons are for the increased risk. This makes it difficult to identify CIs for a drug with high certainty. The situation is further complicated as many adverse events (AEs) are commonly reported for many drugs. It is pertinent to compare AEs vs CIs against a background signal so that medication is not withheld unnecessarily. From package inserts for 420 drugs, we processed clinical trial tables, (e.g., treatment vs. placebo), and harmonized AEs with the MedDRA vocabulary (http://www.meddra.org/). We narrowed the analysis to AEs associated to numerical values for AEs in both treatment and placebo (5595 pairs, representing 115 unique active pharmaceutical ingredients, APIs; and 645 unique AEs, respectively). By removing those that failed the Chi-squared test (right-tail < 0.1), 1236 API-AE pairs remained, representing 107 APIs and 315 AEs. For these 107 APIs, we further extracted 503 contraindications (CIs), as recorded on label, using a controlled vocabulary. We then investigated the relationship between AEs and CIs by counting the number of unique APIs found at the intersection between each AE and each CI. The 19,483 AE-CI relationships were normalized using the relative reporting ratio (RRR) against the backdrop of 164,112 absent values, presumed to not be significant in view of the Chi-squared test. Based on the RRR matrix, the top 30 APIs pairs, C: Abnormal Hepatic Function Tests; Dyspnea - Shortness of Breath; Nasal burning - Renal Disease; and Hostility - Depression, respectively. These are validated by clinical interpretation, e.g., Nasal Burning may occur in Nephrotic Syndrome. This work establishes a methodology that identifies significant CI and AE relationships from clinical information and takes RRR into account.

2228 Fish Connectivity Mapping: A Transcriptomics-Based Tool for Ecotoxicology


In spite of years of effort, the full potential of transcriptomics is yet to be realized for ecotoxicology both in terms of reliability and efficiency. Connectivity mapping (Cmap) is an approach originally proposed in biomedical research to establish linkages of chemical-chemical or chemical-disease states. They are made possible by statistical determination of the non-random distribution of query gene signatures on rank-ordered gene lists, which are generated from gene expression profiles (GEPs) associated with individual chemicals. The power of Cmap is a function of chemical coverage by rank-ordered gene lists. The Cmap concept should be equally applicable in ecotoxicology for exposure assessment, hazard identification, and mechanism of action determination. In the absence of dedicated resources to build a Cmap reference database, however, the development of fish Cmap will depend on a sequential integration of the massive amount of fish GEPs currently available in the public “omics” repositories such as the NCBI GEO. These GEPs are heterogeneous, representing data primarily from several major fish species of scientific and economic importance, multiple gene expression profiling platforms, and many different laboratories. This presentation will demonstrate a proof of concept study of fish Cmap where a large number of GEPs from zebrafish and fathead minnow were examined. A subset of them with significant responses to chemical treatments were selected to generate a reference database. A small group of gene signatures, genes showing differential expression between chemical-treated samples and controls, were then tested against this collection. A majority of these signatures were successfully connected to the GEPs associated with the intended chemical targets. To build on these promising results, future improvements of this tool will be made by expanding the coverage of chemicals in the fish Cmap database. This will be achieved first by cross mapping fathead minnow and zebrafish genomics so that the publicly available GEPs from both species can be utilized to generate a greater number of rank-ordered gene lists.

2229 Structure-Activity Model for Temporal Scaling Factors of Inhaled Hazardous Chemicals

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A critical component in setting short-term exposure limits for airborne concentrations of hazardous chemicals (as in Acute Exposure Guideline Levels or AELs) is the chemical’s temporal scaling factor (TSF, the n-value in the ten Berge equation, $C = x t = k$, where $C$ is the exposure-limit concentration, $t$ is exposure duration and $k$ is toxic load ). Using an empirically-derived TSF, a threshold concentration at one exposure duration can be extrapolated to other durations, although the necessary empirical data are available for only a few chemicals. Potentially, unknown TSFs can be extrapolated from available data using quantitative structure-activity relationship (QSAR) modeling. However, a QSAR model for TSFs has yet to be reported. To fill the data gap for hazardous chemicals lacking TSFs, a series of regression and categorical QSAR models were developed using 63 organic compounds with known empirical TSFs. The models relied on descriptors derived from the chemical’s 3D-structure related to the shape, topology, and electrostatic character. Quality of the models was contingent to size and diversity of the dataset. Use of these models in risk assessment may help in prioritization of chemical studies, rapid assessment in emergency situations, and complement insufficient empirical data in the development of public health guidelines.

2230 Assessment of the Predictive Use of the ECOSAR In Silico Modeling Program in Comparison to Experimental Data for the Fish Aquatic Toxicity Test Study Endpoint


Following the submission of high tonnage dossiers (>1000 t/a and 100-1000 t/a, respectively) for industrial chemicals to the European Chemicals Agency (ECHA) there is considerable data that could be used for the first time in a predictive manner. The aim of the review was to interrogate dossiers submitted and see, where experimental study data was submitted, if modelled data would have predicted the
actual results. The Ecological Structure Activity Relationships (ECOSAR) Class Program was selected as the modelling program because it is freely available and widely used. Eighty chemicals were studied and the general predictive accuracy agreed with a previously reported study but a bigger dataset is required to confirm this. It is possible that specific QSRs for aquatic toxicity could be developed and used to preferentially select chemicals, including crop protection products and their metabolites, for in vivo testing and so reduce animal testing.

2231 A Framework for Rapid Hazard Assessment of Chemicals Using Quantitative Adverse Outcome Pathways

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The increasing number of new and existing chemicals being developed, used, and released into the environment requires new approaches for early assessment of the potential hazards that these materials pose to both humans and the environment. Hence there is a great need to rapidly assess these chemicals. Here, we present a chemical hazard testing framework that utilizes a combination of chemical modeling, concentration-responsive effects assessment, in vitro to in vivo extrapolation, and a quantitative Adverse Outcome Pathway (AOP) approach to integrate data and derive a useful measure of chemical hazard. Quantitative Structure Activity Relationships (QSAR) are used initially to predict acute aquatic toxicity levels. The toxicity of a chemical is then examined through exposure of zebrafish embryos to 5 different concentrations of the chemical. Effects of the chemical are assessed morphologically and transcriptionally to identify potential toxicity pathways and target tissues. Concentration-dependent changes in gene expression are then used in a transcriptional pathway-based Benchmark Concentration approach to enable extrapolation of levels below which no effect is expected to mammals and adult fish using comparative genomics and reverse toxicokinetics. A novel quantitative Adverse Outcome Pathway (AOP) decision model using a numerical Weight of Evidence approach common to decision analysis is being used to integrate data from different sources (modeling, in vitro tests, ‘omics, zebrafish embryo tests) and provide a cumulative value for the hazard potential of a chemical. We demonstrate the utility of this concept with several chemicals. We expect that this framework will provide more accurate hazard level screening tools and enable the reduction and focusing of in vivo safety tests required for new chemical development resulting in significant savings in money and time.

2232 Chemical Structural Profiling of ToxCast II Compounds Predicted Lack of Reactivity in Nrf2, MTF2, and CREB3, Hallmarks for Nonspecific Cellular Stress


Toxicology testing is trending towards generation and utilization of large toxicity databases to prioritize chemicals based on prediction of hazard potential. This includes new in vitro high-throughput (HT) data generation and modeling, such as the US-EPA ToxCast Program. ToxCast makes HT data publicly available for scientists to develop predictive approaches for in vitro biological endpoints. ToxCast II tested > 1000 compounds in > 800 HT assays and it is important to discern those potentially specific for a given biological endpoint from those non-specifically active, such as a response to cellular stress. Several candidate HT assays have been identified as hallmarks for non-specific stress, including Nrf2 (nuclear factor erythroid 2-related factor 2), MTF2 (metal response element binding transcription factor 2) and CREB3 (cAMP responsive element binding protein 3), whose in vitro response might be used to attenuate non-specific responses in other assays. We developed an approach to chemically profile compounds using modeling tools embedded in the OECD Toolbox™, OASIS™, Pipeline Pilot™ and ToxTree™, to successively flag and filter chemicals with different functional groups such that remaining “unfiltered” compounds would have little if any reactivity. We applied this model to > 1800 compounds in ToxCast II with the hypothesis that unfiltered compounds would also be reactive in Nrf2, MTF2 and CREB3. The hypothesis was valid for the vast majority of compounds, except for several long chain fatty acids and vitamin-D that showed some reactivity in Nrf2, but are considered innocuous.

2233 Development of a Conceptual Module to Investigate Pharmacokinetic Influences When Evaluating Chemicals in Adverse Outcome Pathways


Adverse outcome pathways (AOPs) operate under the premise that known adverse outcomes may be linked via key events to an upstream molecular initiating event (MIE) and can be combined with high throughput in vivo methods to screen for chemicals able to trigger such an event. Absorption, distribution, metabolism, and excretion (ADME) can influence a compound’s ability to reach a molecular target in vivo, but are rarely considered. Our research attempts to address this issue through the development of a conceptual module incorporating both qualitative and quantitative aspects of ADME. The utility of our module was demonstrated using the AOP of acetylcholinesterase (AChE) inhibition. The physicochemical properties of AChE-inhibiting bioactive chemicals comprising 3% of the 1,059 compounds screened in the ToxCast program were analyzed in detail to assess their potential for exposure, absorption, and likelihood of a MIE. Seven compounds had a high probability of a MIE due to absorption potential and widespread use. Fourteen compounds had a low probability due to little absorption or exposure, and the remainder had an unknown probability due to limited exposures. Structural similarities of bioactive compounds were analyzed and compared against structures of non-reactive compounds using molecular fingerprint models to detect false negatives that might become biologically active when accounting for metabolism. Thirty-five non-reactive compounds exhibited a similarity threshold above 60% with their nearest bioactive neighbor. At least three of these compounds, such as chlorpyrifos and thiocarb, were parents of bioactive metabolites likely to elicit a MIE. Our results demonstrate the necessity for considering ADME properties in the screening of chemicals when utilizing an AOP framework. Disclaimer: This abstract has been cleared by the EPA but solely expresses the view of the authors.

2234 An In Silico Skin Absorption Model for Fragrance Materials

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Fragrance materials are widely used in cosmetics, fine fragrances, and other consumer products. At the Research Institute for Fragrance Materials (RIFM) we evaluate the safe use of fragrance ingredients. Dermal absorption is an important parameter in refining systemic exposure for topically applied fragrance materials. Currently, in RIFM’s safety assessment process, a 100% dermal absorption default value is applied for materials without experimental data. However, as discussed by Kros et al. at their in 2007 publication [1], “the assumption of 100% absorption is not scientifically supportable” and proposed an in silico methodology to assign a conservative skin absorption value based on the material’s maximum flux (Jmax). Jmax may be calculated by using QSAR models, that determine octanol/water partition coefficient (Kow), water solubility (S) and permeability coefficient (Kp). To apply Kros et al. methodology specifically for fragrance materials, each of these QSAR models was carefully evaluated and refined resulting in a detailed model workflow we refer to as SAM (Skin Absorption Model). SAM was developed using 105 materials with fragrance specific physicochemical characteristics and experimental Kp values by updating Potts and Guy’s proposed Kp model [2]. SAM was validated by using a set of 133 materials with fragrance specific physicochemical characteristics that had experimentally determined skin absorption data using human or pig skin in either in vitro or in vivo methods. All resulted in predicted values fitting Kros et al. three proposed skin absorption values based on Jmax ranges, i.e., 10%, 40%, 80%. Our findings agreed with a previously reported study but a bigger dataset is required to confirm this. It is possible that specific QSARs for aquatic toxicity could be developed and used to preferentially select chemicals, including crop protection products and their metabolites, for in vivo testing and so reduce animal testing.

2235 A Pilot Study of Clustering-Based Safety Assessment for Fragrance Ingredients

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With the development in science and technology and changes in the regulatory standards, there are growing needs to evaluate or re-evaluate large inventory of chemicals. Evaluating a set of similar chemicals together (i.e., clustering or grouping) is considered to be resource and time conservative. This practice is also encouraged by the regulatory agencies, such as ECHA and US EPA. ECHA and OECD have issued some guidance on chemical grouping and categorizing. The underlying
hypothesis is that chemicals with similar structures are expected to have similar toxicological profiles. However, chemical grouping, clustering, or categorizing usually depends on the chemists’ experience and may be subjective. As such, a transparent, objective, comprehensive, and reproducible method should be preferred and adopted. Chemical Assessment and Rating Engine (ChemARC) was developed by the U.S. EPA, Office of Pollution Prevention and Toxics (OPPT) to assist in the review and prioritization of large inventories of structurally diverse chemicals. In this study, 2664 fragrance ingredients were clustered using Chem/ACE. This resulted in 382 Clusters and 774 ingredients were considered as ‘orphans’ — i.e., chemicals that cannot be clustered when using a listwise parameter and 739 orphans were obtained. The clustering and not clustering of some material was questionable. To better the clustering outcome, we developed a set of restrictive criteria that resulted in less orphans and more appropriate clustering based on a toxicological end point. As a conclusion, clustering based only on chemical similarity is not enough to form a reliable cluster. Additional factors, such as structural alerts, metabolism and physicochemical properties, should be taken into consideration.

### 2238 Changes of DNA Methylation Patterns in Subjects Exposed to Polycyclic Aromatic Hydrocarbon

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Global hypomethylation, gene-specific methylation and genome instability are common events in tumorigenesis. To date, few studies have examined the aberrant DNA methylation patterns in coke oven workers, who are highly at risk for lung cancer by occupational exposure to polycyclic aromatic hydrocarbons (PAHs). We recruited 83 PAH-exposed workers and 62 unexposed controls, assessed exposure levels by urinary 1-hydroxypyrene, and measured genetic damage by comet assay, blomycin sensitivity and micronucleus assay. The lung cancer risk of coke oven emissions (COE) was estimated based on toxic equivalence factors. We used bisulfite-PCR pyrosequencing to quantify DNA methylation in long interspersed nuclear element-1 (LINE-1) and MGMT, Further, the methylation alteration was also investigated in COE-treated human bronchial epithelial (16HBE) cells. The estimated lifetime lung cancer risk for COE is 4.65 cases per person. We found lower levels of LINE-1 and MGMT methylation (with specific CpG site) in PAH-exposed workers. LINE-1, MGMT and its hot CpG site-specific methylation were negatively correlated to urinary 1-hydroxypyrene levels (r = -0.329, p < 0.001; r = -0.164, p = 0.049) and r = -0.176, p = 0.034, respectively. In addition, LINE-1 methylation was inversely associated with comet tail moment and micronucleus frequency, and a significant increase in micronucleus in low MGMT methylation group.

### 2239 HDAC Inhibition by Sodium Butyrate Reduces Insulin Resistance and Fat Deposition and Protects Beta Cell Dysfunction in Type 2 Diabetic Rat: Investigation on Chromatin-Derived Mechanisms

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Recent evidences highlighted the role of histone deacetylases (HDACs) in beta-cell development, differentiation, function, insulin signaling and resistance. HDACs can deacetylate the histone and various other transcription factors and regulatory proteins, which directly or indirectly affects glucose metabolism. We have also reported the protective role of sodium butyrate (NaB) on the beta-cell function and diabetic nephropathy in type-1 diabetic rats by HDAC inhibition and associated mechanisms. The current study was aimed to evaluate the protective mechanism of NaB on beta-cell dysfunction, fat deposition and insulin resistance as well as signaling in type-2 diabetic rat. Diabetes was developed in Sprague-Dawley rats by the combination of high-fat diet and STZ. NaB at the doses of 200 and 400 mg/kg twice daily as well as metformin (positive control) 150 mg/kg twice daily for 10 weeks were administered to rats by i.p. and oral route, respectively. Glucose and insulin tolerance tests were performed on fasted animals (6-8 h) to assess the glucose homeostasis, beta-cell function and insulin resistance. Dyslipidemia and glycaemia were evaluated by biochemical estimations, while fat accumulation and organ damage were assessed by histology. Protein expression and transcription factor of insulin signaling were evaluated by western blot and immunohistochemistry. NaB treatment significantly reduced the plasma glucose, HbA1c, insulin resistance, adiposity and fat deposition in BAT, WAT, liver and pancreas, which are comparable to metformin treatment. Further, NaB treatment restored the histological alterations, beta-cell dysfunctions and increased plasma insulin thus protects the beta-cells. Our findings provide the mechanistic insights on beneficial role of NaB in type-2 diabetes-associated beta-cell dysfunction and insulin resistance by HDAC inhibition and the modulation of insulin/AKT/FOXO1 signaling.

### 2237 In Silico Prediction of Physicochemical Properties of Environmental Chemicals in Combination of Molecular Fingerprints and Machine Learning Approaches

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According to OECD a chemical category is a group of chemicals whose physicochemical and human health and/or ecotoxicological properties are likely to be similar or follow a regular pattern. The building of categories has often been tried on the basis of conventional structure based approaches. In the present project we developed an approach by which toxicological and structural properties likewise contribute to the building of chemical categories for (sub)chronic toxicity. Two databases on repeated-dose toxicity (ReproTox and the “ELINCS” data base) served as data basis. The toxicological data are organized into organ toxicity split into subgroups according to phenotypic and mechanistic observations. For the definition of a category, the following characteristics were considered: organ investigated, effects, no effect potency in terms of no observed adverse effect level (NOAEL), organ specificity. A multi-label clustering by using predictive clustering trees (PCT) was established. Several decisions concerning structural features and chemical properties as well as the toxicological data had to be considered during development: - the selection of features and their SMARTS description - the non-use of PC parameters - imputation methods for missing values - the level of detail for a consistency of toxicological data versus data density in the matrix. All resulting category clusters were visualized and checked for plausibility. An important decision about a stop criterion for clustering was the use of toxicological variance data in combination with statistical significance. In the process of developing this approach we needed many incremental improvements; the final approach shows a set of useful and representative clusters now. This project was funded by BMBF.

### 2236 Development of Chemical Categories by Optimized Clustering Strategies

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In the present project we developed an approach by which toxicological and structural properties likewise contribute to the building of chemical categories for (sub)chronic toxicity. Two databases on repeated-dose toxicity (ReproTox and the “ELINCS” data base) served as data basis. The toxicological data are organized into organ toxicity split into subgroups according to phenotypic and mechanistic observations. For the definition of a category, the following characteristics were considered: organ investigated, effects, no effect potency in terms of no observed adverse effect level (NOAEL), organ specificity. A multi-label clustering by using predictive clustering trees (PCT) was established. Several decisions concerning structural features and chemical properties as well as the toxicological data had to be considered during development: - the selection of features and their SMARTS description - the non-use of PC parameters - imputation methods for missing values - the level of detail for a consistency of toxicological data versus data density in the matrix. All resulting category clusters were visualized and checked for plausibility. An important decision about a stop criterion for clustering was the use of toxicological variance data in combination with statistical significance. In the process of developing this approach we needed many incremental improvements; the final approach shows a set of useful and representative clusters now. This project was funded by BMBF.
2240 The Steroidal Saponin, Dioscins, Isolated from Wild Yam (Dioscorea villosa) Root Extract, Has the Potential to Modulate Human Breast Cancer Metastasis

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Previously, we have observed that wild yam root extract (WYRE) is able to activate GATA binding protein-3 (GATA3) gene in human breast cancer cells, MCF-7 and MDA-MB-231, targeting epigenome. The present study aims to find out bioactive molecules of WYRE which can modulate GATA3 gene functions and prevent metastasis in human breast cancer cell lines. We identified eleven saponins and one sapogenin from WYRE and found that four of them have the anticancer activity. In this study we have evaluated diosin (DS), a steroidal saponin which showed antiproteinase activity, preventing metastatic potential of cancer cells using MDA-MB-231 cells. Our data indicate that DS like WYRE is able to reduce cell viability and induce GATA3 mRNA expression in both MCF-7 and MDA-MB-231 cells in a concentration-dependent manner. The calculated IC50 is 3.85 \( \mu \)M for MCF-7 and 2.07 \( \mu \)M for MDA-MB-231 cells. Invasion analyses based on cell migration, invasion, and wound-healing assays indicate that DS is able to inhibit metastasis of MDA-MB-231 cells in vitro. In contrast to metastasis, GATA3 protein expression as evidenced by western blot and immunocytochemistry was enhanced by DS in MDA-MB-231 cells. To verify the effects of DS on metastasis at the molecular level we have evaluated the expression of four other genes which have significant influence on GATA3 expression as well as metastasis. The miRNAs of ZFPM2, a member of FOG family, and E-Cad which binds to the GATA motifs, were increased by DS (5.76 \( \mu \)M) while VIM, a downstream gene of GATA3, and MMP9 which is considered as a cellular marker for epithelial to mesenchymal transition (EMT), were decreased by DS. These findings indicate that DS has the potential to be used as a breast cancer preventive agent targeting metastasis.

2243 MicroRNAs and Their Potential Involvement in Arsenic-Induced Oxidative Stress

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Arsenic exposure is postulated to modify microRNA (miRNA) expression, leading to changes of gene expression and subsequent biologic outcomes, but studies relating the responses of miRNAs to arsenic exposure are lacking, especially with respect to in vivo studies. We utilized high-throughput sequencing technology and generated miRNA expression profiles of liver tissues from Sprague Dawley (SD) rats sub-chronically exposed to various concentrations of sodium arsenite. Unsupervised hierarchical clustering analysis of the miRNA expression profiles clustered the SD rats into different groups based on the arsenic exposure status, indicating a highly significant association between arsenic exposure and cluster membership (P-value of 0.0012). Multiple miRNAs expressions were altered by arsenic in an exposure concentration-dependent manner. Among the identified arsenic-responsive miRNAs, several are predicted to target Nf-22-regulated antioxidant genes, including glutamate-cysteine ligase (GCL) catalytic subunit (GCLC) and modifier subunit (GCLM) which are involved in glutathione (GSH) synthesis. Exposure to low concentrations of arsenic induced increased miRNA expression for Gclc and Gclm, while high concentrations significantly reduced their expression, which were correlated to changes in hepatic GCL activity and GSH level. Moreover, our data suggested that other mechanisms, e.g. miRNAs, rather than Nf-22-signaling pathway, could be involved in the regulation of mRNA expression of Gclc and Gclm post arsenic exposure in vivo. Together, our findings show that arsenic exposure disrupts the genome-wide expression of miRNAs in vivo, which could lead to the biological consequence, such as an altered balance of antioxidant defense and oxidative stress. (This work was supported by Zhejiang Provincial Natural Science Foundation, China (Y2110648 to H. W.), and by the NIEHS grant R21ES023239 and R01ES022629 to X.R. and P30ES007033 to T.J.K.).

2244 Aluminum Malolate Alters Cell Cycle Progression and Histone Modifications in Mouse Embryonic Stem Cells

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Metals induce histone modifications resulting in pathologies often seen in adulthood. Effects of aluminum malolate (AM) exposure was monitored by measuring cell viability, total histone protein (THP) production, H3K27 mono-methylation (H3K27me1) levels and cell cycle progression in mouse embryonic stem (mES) cells. AM (100–300 \( \mu \)M) decreased cell viability to 75% at 300 \( \mu \)M, whereas cell cycle was affected below IC25. However, AM induced cell cycle alterations at 24- and 48-h exposures, but not during recovery. In addition, flow cytometry data demonstrated a dose dependent G1/M suppression during 24- and 48-h exposures. At 48-h, the inability of mES cells to undergo mitosis in presence of AM was further highlighted by increased % cells appearing in the S-phase. Also, 24-2 h 200 \( \mu \)M AM exposure resulted in suppression of cell proliferation to a greater extent than THP
production, thus indicating the initiation of repair mechanisms in mES cells. AM exposure at 100 μM for 24-h did not exert significant effects on cell proliferation and THP production. However, the data suggests that cell proliferation in mES cells is more susceptible than chromatin stability, to AM exposure, as measured by THP production. To study the effects of AM on transcription we quantified H3K27me3 expression in both 3-h and 24-h AM exposure. H3K27me3 was detected at 200μM but not at 100μM. Moreover, H3K27me3 is elevated during 24-h AM exposure. Although the data suggests that cell proliferation in mES cells is more susceptible than chromatin stability, to AM exposure, as measured by THP production. To study the effects of AM on transcription we quantified H3K27me3 expression in both 3-h and 24-h AM exposure. H3K27me3 was detected at 200μM but not at 100μM. Moreover, H3K27me3 is elevated during 24-h AM exposure.

2245 Does the Epigenome Predispose for Susceptibility to DNA Damage?
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Although interindividual variation in responses towards chemical compounds, including carcinogens, is a well-known phenomenon, the causal factors for this variation are largely unknown. This complicates the issue of individual risk estimation. Interindividual variation in gene-methylation has been proposed as an additional factor that may explain the differences in responses to compound exposure within the human population. This study focuses on determining to what extent baseline methylation status of a gene influences the response of that gene towards compound exposure. Special emphasis has been given to the evaluation of the DNA-damage response genes and on interindividual variation. Primary human hepatocytes of several donors were exposed to aflatoxin B1 (AFB) or benzo[a]pyrene (BaP). The response to DNA-damage was evaluated by H2A-staining, these results were used to divide the study-population into two groups per compound; a low and a high responder group based on the benchmark dose for H2A induction. For each of the sub-groups, genes that are unique in their expression responses were evaluated with respect to their baseline methylation status and baseline gene expression levels. Although clear differences between high and low responders to DNA-damage could be defined, baseline methylation status only seems to have marginal impact on induced gene expression levels. Interindividual variation in baseline gene expression levels does not seem to be related to the variation over individuals in baseline methylation levels. In turn, variation in baseline methylation was not reflected in compound-induced gene expression responses. Thus, the overall conclusion is that the interindividual variation in baseline methylation status explains to only a limited degree the interindividual differences in susceptibility to carcinogen exposure of groups/individuals. Investigations of further (epi)genetic factors are required for a better understanding of regulatory mechanisms underlying interindividual variation in genotoxic responses.

2246 Reversal Treatment Results in a miRNA-Regulatory Network in Renal Cancer Cells and Suppresses Cancer Growth and Metastasis by Therapeutically Targeting miR-181d, miR-21, miR-10a, miR-17, BCL-2, CDC25a, MCL-1, and STAT3
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Renal cell carcinoma is the most common type of kidney cancer in adults. The cancer has a very high risk of metastasis, most often to the lungs and other organs. In about one-third of patients, the cancer has already metastasized at the time of diagnosis, and the patients have a poor prognosis. Resveratrol, a natural product stilbenoid, has been shown to suppress malignant proliferation, angiogenesis and metastasis. In the current report, we investigated the potential antitumor effects of resveratrol in vitro and in vivo on murine renal carcinoma cell line (Renca). We found that resveratrol suppressed the growth, migration, and invasion of Renca cells. Resveratrol also caused apoptosis of Renca cells. The metastasis of Renca cells and the tumor angiogenesis were inhibited in vivo after resveratrol treatment. To understand the underlying mechanisms, we performed simultaneous examination of miRNAs, mRNAs, and proteins in these cells retreated with resveratrol. With functionally interfering manipulation using siRNA against expression of miR-181d, -21, -17, and -10a, as well as BCL-2, MCL-1, CDC25a, and STAT3, we identified a microRNA-regulated signaling network in Renca cells. This network signaling phenocopied JAK2/STAT3 pathway and was elicited by tumor-secreted IL-6. These findings provide insights into the role of microRNAs in renal carcinogenesis and can facilitate the identification of novel therapeutic targets, as well as the development of more effective renal carcinoma treatment strategies. This work was supported in part by National Institutes of Health grants P01AT003961, R01AT006888, R01ES019313, R01MH094755, and P20GM103641 to PN and MN.

2247 Persistence of Genomic Effects following Early-Life Exposure to a Nongenotoxic Carcinogen in Mice
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Early life environmental exposures have been associated with a variety of adverse health outcomes later in life. Previously we reported that postnatal exposure to dichloroacetic acid (DCA), a byproduct of drinking water chlorination, increased liver tumor incidence in mice 80 weeks after prior exposure. In this study we evaluated genomic changes underlying this carry-over effect. Male B6C3F1 mice 28 d of age were given the following treatments in drinking water for up to 93 wk: deionized water (dH2O; control); 3.5 g/l DCA continuously; or 3.5 g/l DCA for 4, 10, 26, or 52 wk followed by control dH2O. Endpoints included liver tumor incidence, cell proliferation, global mRNA and microRNA profiles, and RT-qPCR validation of gene expression measured during eight time points. Liver tumor incidence was increased in all DCA treatment groups, but no persistence in liver cytotoxicity or cell proliferation was observed. Distinct patterns of persistence were observed for differentially expressed mRNAs and microRNAs. Target microRNAs related to cell metabolism that were highly induced (e.g. AcoT1, Cyp4a14) or repressed (e.g. Nqo1) by DCA returned to control levels typically within 5 weeks of stopping DCA (9-fold over/under control, significant by one-way ANOVA with post hoc test, multiple comparison corrected p<0.05). In contrast, early life DCA exposure led to long-term suppression of age-related increases in microRNAs specific to a chromosome X cluster potentially involved in cellular differentiation. Transient DCA treatment induced clear carcinogenic effects later in life in the absence of sustained cytotoxicity and increased cell proliferation. Transcriptional changes over time showed different patterns of persistence that may elucidate epigenetic mechanisms for non-genotoxic carcinogenesis. This abstract does not necessarily reflect the policy of the US EPA.

2248 Dioxin (TCDD) Induces Epigenetic Transgenerational Inheritance through Alterations in DNA Methylation, Histone Modifications, and microRNA Profile
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Dioxin (TCDD), is a potent AhR ligand, and a global environmental contaminant. While the toxicity of TCDD in adult vertebrates has been well characterized, the transgenerational effects remain to be elucidated. In the current study, we investigated the effect of TCDD in parent (P), F1, and F2 generations by examining epigenetic modifications of genomic DNA and microRNA (miR) profile. To this end, pregnant mice were injected with TCDD on GD 14. The thymi of mothers (P), F1, and F2 generations of mice were harvested and high-throughput miR arrays for miR profile; McDIP sequencing for global promoter methylation, and CHIP DNA sequencing for histone methylation were performed. We observed more than 160 miRs that were common to and dysregulated greater than 1.5 fold in thymocytes of all three generations of mice post TCDD exposure, when compared to controls. Also, miR arrays data post-TCDD exposure demonstrated changes in miR profile affecting expression of important genes which are known to regulate signaling, toxicity, apoptosis, thymic atrophy, cancer, immunosuppression, and other physiological pathways. Methylation arrays data showed significant differences in methylation status of many genes including AhR, CYP1A1, IFN-γ, IL-4, FoxP3, and IL-17. The data obtained from CHIP DNA sequencing for histone modification revealed TCDD-mediated changes in all the three generations. Together, our studies suggest that TCDD triggers long-term and transgenerational effects by alterations in miR profile, methylation in the promoter region of various genes, as well as histone modifications that together regulate gene expression. Our findings support the concept of fetal basis of adult disease following environmental pollutants (Supported in part by NIH grants P01AT003961, R01AT006888, R01ES019313, R01MH094755, and P20GM103641 as well as by Veterans Affairs Merit Award BX001357).
2249  MicroRNA hsa-miR-128-3p Reduced CYP2C9 Expression in Human Liver Cells  

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Published studies have identified genetic variants, somatic mutations, and changes in gene expression profiles that are associated with hepatocellular carcinoma (HCC), particularly involving genes that encode drug metabolizing enzymes (DMEs). CYP2C9, one of the most abundant and important DMEs, is involved in the metabolism of many carcinogens and drugs and is down-regulated in HCC. To investigate the molecular mechanisms that control CYP2C9 expression, we applied integrative approaches including in silico, in vitro, and in vivo analysis to elucidate the role of microRNA hsa-miR-128-3p in the regulation of CYP2C9 expression and translation. RNA electrophoresis mobility shift assays demonstrated a direct interaction between hsa-miR-128-3p and its cognate target, the CYP2C9 transcript. Furthermore, the expression of a luciferase reporter gene containing the 3'UTR of CYP2C9 and the endogenous expression of CYP2C9 were suppressed by transfection of hsa-miR-128-3p. Importantly, chemically-induced up- or down-regulation of hsa-miR-128-3p correlated inversely with the expression of CYP2C9. Finally, an association analysis revealed that the expression of hsa-miR-128-3p is significantly inversely correlated with the expression of CYP2C9 in HCC tumor tissues. Altogether, the study partially elucidated the mechanism of CYP2C9 regulation by hsa-miR-128-3p, and their inverse association in HCC. 

2250  DNA Methylation Profiles in Liver and Kidney during the Rat Life Span Show Age, Sex, and Tissue Differences  

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Liver and kidney are two major organs responsible for drug metabolism, disposition, and excretion. Recent studies indicate that epigenetic modifications, such as methylation of cytosine, are critical in regulating gene and miRNA expression, cellular differentiation, and sex-chromosome inactivation, and have been shown to be involved in human diseases including cancer. Epigenetics may play an important role in liver and kidney development and disease, but comprehensive genomewide profiling is lacking on the DNA methylation landscape at different life stages. In this study, liver and kidney tissues from Fischer 344 rats at 2, 5, 6, 15, 21, 78, and 104 wk of age of both sexes were examined for sex- and age-specific DNA methylation at 372,645 sites that are associated with 29,751 methylation regions using methylated DNA immunoprecipitation and Roche methylation microarrays. Biweight mean centering normalization was applied before downstream analysis. One-way ANOVA was used to identify differences between groups at FDR<0.001. Principal component analysis indicated that there were greater sex differences than age differences in terms of methylation patterns in both tissues. Within each sex, the results also showed methylation changes occurred from early age through old age. In contrast, the differences between older age (78 and 104wk) animals were much smaller in male compared to female animals, in which there were greater differences between middle-aged (15 and 21wk) and older animals in both tissues. There were 7,017 and 6,926 CpG regions methylated in liver and kidney, respectively, in at least one age and sex. Among them, 931 and 840 methylated regions were unique to liver and kidney, respectively. A total of 6,086 regions were methylated in both tissues, but the methylation often occurred at different ages or sexes. These age-, sex-, and tissue-related differences in DNA methylation may provide insights in susceptibility to liver and kidney disease, adverse drug events and their progression.

2251  Epigenetic Markers of B Cell Differentiating Transcription Factor Genes in Mice Exposed to Dust from Nellis Dunes Recreational Area, Clark County, Nevada  

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Many cellular processes are managed by epigenetic mechanisms including normal cell tissue function, maintenance and regulation of chromatin organization, cellular differentiation, and the regulation of gene expression. Environmental exposures can interfere with these normal mechanisms leading to altered gene expression and potential pathogenesis. More connections between environmental exposures and disease pathogenesis are being linked to epigenetic processes and may elucidate mechanisms of toxicity on a molecular level, beyond what traditional genomic approaches can achieve. Our objective is to understand a possible mode of action for suppression of antigen specific IgM antibody production observed in mice exposed to dust from Nellis Dunes Recreation Area (NDRA), Clark County, Nevada. In our current study, we examined methylation patterns in key promoter regions of genes encoding transcription factors known to be critical in B-cell to plasma cell differentiation. DNA was extracted from splenic tissue preserved in RNAlater® from B6C3F1 mice exposed to mineral dust from four areas of the NDRA; each with a different compositions and characterization. The DNA was bisulfite converted and amplified using methylation specific primers and genotyping HDI1 and PAI5 transcription factor genes regulating the B-cell to plasma cell differentiation pathway. Exposing B6C3F1 mice to 100 mg/kg dust from NDRA did not have effects on the methylation of CpG islands located in promoter regions of PRDM1 or PAI5 as compared to controls. Future studies will evaluate other genes involved in the B-cell to plasma cell differentiation pathway and antibody production.

2252  DDT Exposure Reduces DNA Global Methylation in Susceptible MTHFR C677T Pregnant Mexican Women  

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DNA-methylation is an epigenetic biomarker related to gene expression which may be altered by genetic and environmental factors. We aimed to test the hypothesis that DNA methylation is associated to p,p’DDE (DDT) and p,p’DDT (DDT) exposure and that methylethylenedihydrofolate reductase variant allele MTHFR 677 C>T genotype (CC, CT, TT) modify these associations in a sample of 197 pregnant women. Global DNA methylation was determined in blood cells by the LUMinometric Methylation Assay (LUMA). DDE and DDT serum levels were determined by ECD-GLC. Genotypes of interest were evaluated by PCR. In addition, dietary folate intake was estimated by a validated food frequency questionnaire. The association between DDE, DDT and global methylation was estimated by linear regression models. Global DNA methylation was not significantly associated with folate consumption estimates or with DDE or DDT serum levels. However, the association between global DNA methylation and DDT was negatively modified in MTHFR 677 TT carriers. Global DNA methylation was significantly and negatively associated with DDE (β= -1.8) and DDT (β= -0.6) only among MTHFR 677 TT carriers. These results suggest that DDT exposure reduces global methylation levels in carriers of the MTHFR 677 TT adverse genotype. Further studies are needed to explore the underlying mechanisms and biological relevance of these interactions. Supported by CONACyT, Mexico (31034-M; 41708; 13915), and the Mount Sinai International Training and Research in Environmental and Occupational Health (ITREOH) of the Fogarty International Center (D43 TW006460).

2253  DNA Methylation Modifies Urine Biomarker Levels in 1, 6-Hexamethylene Disocyanate Exposed Workers: A Pilot Study  

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DNA methylation may mediate inter-individual responses to chemical exposure and, thus, modify biomarker levels of exposure and effects. We analyzed inter-individual differences in inhalation and skin exposure to 1,6-hexamethylene diisocyanate (HDI) and urine biomarker 1,6-hexamethylene diamine (HDA) levels in 20 automotive spray-painters. Genome-wide 5-methyl cytosine (5mC) DNA methylation was assessed in each individual’s peripheral blood mononuclear cells (PBMC) DNA using the Illumina 450K CpG array. Mediation analysis using linear regression models adjusted for age, ethnicity, and smoking was conducted to identify and assess the association between HDI exposure, CpG methylation, and urine HDA biomarker levels. We did not identify any CpGs common to HDI exposure and biomarker level suggesting that CpG methylation is a mediator that only explains the phenotype. Functional significance of genic- and intergenic-CpG methylation status was tested using protein-protein or protein-DNA interactions and gene-ontology enrichment to infer networks. Combined, the results suggest that methylation has the potential to affect HDI mass transport, permeation, and HDI metabolism. We demonstrate the potential use of PBMC.
methylation along with quantitative exposure and biomarker data to guide further investigation into the mediators of occupational exposure and biomarkers and its role in risk assessment.

2254  Furan Induces Long-Term Persistent Epigenetic Alterations in the Livers of Fisher 344 Rats

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The rodent liver carcinogen furan is present in a broad range of food products. The mechanisms leading to the furan-induced liver tumor development are still unclear. Previously, we reported dose- and time-dependent epigenetic changes in the liver of rats treated with furan. The goal of the present study was to investigate the possible role of epigenetic events in furan-induced liver carcinogenesis by examining the extended evolution of epigenetic alterations in the liver of rats after stopping the exposure to furan. Male Fisher 344 rats were treated by gavage 5 days per week with 8 mg furan/kg body weight (bw)/day for 90 days. After the last treatment, rats were divided randomly into four groups; one group of rats was sacrificed 24 hours after last treatment, while other groups were maintained without further furan treatment for 90, 180, and 360 days. Treatment with furan for 90 days resulted in alterations of histone lysine methylation and acetylation, oxidative damage to DNA, and changes in the gene expression. The majority of these furan-induced molecular changes were restored after the furan treatment cessation. In contrast, acetylation of histone H3 lysine 9, H3 lysine 27, and H3 lysine 56 was decreased in a time-dependent manner following furan treatment. This sustained histone H3 lysine deacetylation was accompanied with a formation of heterochromatin and decrease in gene expression. Furan treatment caused also a gene-specific reduction in the level of histone H3K9 acetylation, which correlated with reduced expression of these genes. The results of the present study indicate that furan-induced adverse effects may be mechanistically related to sustained changes in histone lysine acetylation that compromise the ability of cells to maintain and control properly the expression of genetic information.

2255  Methylation Profiles in Chronic Smokers and Moist Snuff Consumers


Alternations in gene methylation and other epigenetic changes regulate normal development as well as drive disease progression. Chronic cigarette smoking results in hyper- or hypo methylation which could contribute to smoking-related diseases. For example, methylation status of genes involved in xenobiotic metabolism and growth regulation is altered in smokers (SMK). Yet there is limited information on the global methylation changes in smokers. Further, it is unclear whether consumers of non-combustible tobacco, such as moist snuff, also exhibit perturbations in their methylome. Here, we present global methylation changes in the buccal cells collected from smokers and moist snuff consumers (MSC) in a biomarker discovery study. Generally healthy adult male study subjects were recruited into SMK, MSC and Non-Tobacco Consumers (NTC) cohorts (40 subjects/ cohort). The subjects fasted overnight from food and tobacco and buccal cells were collected. Global methylation profiling was performed on Illumina 450K methylation array using the buccal cell DNA. Approximately 1250 gene loci were found to be differentially methylated in tobacco consumers (SMK and MSC) relative to NTC. Hypermethylation of the gene loci was the more frequent mode of differential gene methylation than hypomethylation. Overall, the SMK cohort exhibited largest qualitative and quantitative changes relative to MSC. Hierarchical clustering of top 20 significant gene loci suggested that MSC and NTC cocluster. Approximately half of the total number of gene loci were classified as Combustible Tobacco-related Signatures/changes, and a third of the changes, termed Tobacco Related Signatures/changes, that were commonly detected in the tobacco consumers. Consistent with other published work, AHRR was hypermethylated in smokers, while F2RL3 was hypomethylated. Initial bioinformatic analyses indicated that SMK, not MSC, exhibit activated AHR pathway and perturbations in vitamin metabolism. In summary, we describe global gene methylation changes in buccal cells of long-term tobacco consumers.
Epigenetic regulation of gene expression plays pivotal roles in the orchestration of in vivo cytokine-driven T cell polarization and may determine the vigor, quality and/or longevity of such responses in vivo. T cell polarization in vivo is stimu-
lated by chemical contact allergens that result in type 1/type 17 responses, or by chemo-
icallary regulatory factors that induce preferential type 2 responses. BALB/c strain mice were exposed topically to dinitrochlorobenzene (DNCB; contact aller-
gen), trimellitic anhydride (TMA; respiratory allergen) or to vehicle control for 13 days and the CD4+ draining lymph node cell (LNC) populations isolated. Naïve CD4+ LNC were activated and cultured with interleukin (IL)-12 or IL-4 for 6 days to produce in vitro polarized Thelper (Th1) or Th2 populations, respectively. Follow-
ning polarization, changes in DNA methylation and mRNA expression were measured by methylated DNA immunoprecipitation and qPCR. Several differen-
tially methylated regions that were identified previously in a genome-wide study of allergen-activated DNA methylation in unfractored LNC populations were investigated. Intrinsic regions of dap12, a signal transducer, and nuc (a newly dis-
covered gene from within the rag locus) exhibited differential DNA methylation between Th1 (generated in vivo by DNCB treatment and in vitro) and equivalent Th2 (from in vivo TMA-activated tissue and those polarized in vitro) populations. These changes correlated with functional differences in gene expression in cytokine-
polarized Th1 and Th2 cells. These data demonstrate that exposure to chemical aller-
gens results in characteristic DNA methylation patterns indicative of epigen-
etic regulation of divergent T cell populations in vivo that are paralleled in Th1/
Th2 LNC generated by cytokines in vitro. Furthermore, these patterns of DNA methylation may contribute to functional changes in mRNA expression that may underpin divergent T cell responses.

Substantial research has established an evidence base in model systems that shows environmental factors, including chemicals, can cause adverse effects to both ex-
posed organisms and their progeny; although usually at exposure levels higher than
those normally encountered. The mechanisms underlying this multigenerational toxicity are not well understood, but a number of studies have shown a role for heritable epigenetic modifications, including DNA methylation and small RNA-
mediated changes. Both of these types of epigenetic mechanisms play vital roles during normal early embryo and germ cell development and are thought to be
affected by environment, including environmental chemical exposure. It is therefore
possible that environmental chemical induced changes during these epigenetically
vulnerable windows enable the establishment and transmission altered phenotypes
across multiple generations. To test the hypothesis that environmental chemicals can
affect the erasure and re-establishment of epigenetic marks during gamete for-
mation, an in vitro germ cell test system was developed. Using this system effect(s)
of different environmental chemicals on the differentiation of primordial germ
cells from embryonic stem cells was tested and linked to chemically-induced global
developmental windows to epigenetic perturbation, although the relevance to
environmental exposure levels needs to be established.

Epigenetic changes, such as DNA methylation, are reversible events that are trans-
missible both mitotically and meiotically, but do not involve modification of DNA
sequences. Changes in DNA methylation patterns have been shown to be induced by
cigarette smoke. For instance, a large scale analysis from white blood cells has
suggested DNA methylation as a possible biomarker of Chronic Obstructive Pulmonary Disease (COPD). Recent studies indicate that DNA methylation also plays a role in COPD. The C57BL/6 mouse model is a useful model for cigarette
smoking-induced COPD and provides valuable insights into emphysema initiation
and progression. We have used this model to analyze the DNA methylation pat-
terns in the lungs of mice that develop emphysema upon smoke exposure and to
investigate the impact of smoking cessation or switching to a prototypic modified
risk tobacco product (pMRTP) on lung methylation. We conducted whole genome bisulfite sequencing on lung DNA samples collected after smoke exposure for 7
months from a reference cigarette (3R4F), as well as following smoking cessation
and switching to a pMRTP after 2 months. The lungs of mice continuously ex-
posed to 3R4F presented a larger increase in the amount of hypermethylated CpG
sites over time than after either smoking cessation or switching to a pMRTP. The
results indicated that smoking cessation or switching to a pMRTP reduces the
3R4F induced repressive changes in the mouse lung DNA methylation pattern.

Introduction. Human post-mortem evidence indicates that frontal cortex neuronal
cells have distinct DNA methylation signatures compared to non-neurons. Also,
the methylation landscape of neurons varies depending on neuronal activity, based
on in vivo research in adult mice. This evidence suggests that the methylome may
be plastic, and particularly sensitive to environmental conditions such as lead (Pb)
exposure. Studies in rodents and monkeys exposed to Pb in the first few weeks
and first year of life, respectively, suggest that gene expression in the adult brain is
associated with early life exposures. Epigenetic epidemiology research in the brain
examines whole tissue containing mixed cell types, but cell-type specific epigenetic
analyses may be more informative. Methods. We have conducted a novel study of
neuron-specific epigenetic signatures associated with Pb exposure in an isogenic
mouse model. Utilizing post-mortem frontal cortex tissues from 10-month old
mice exposed perinatally to Pb and controls, we optimized a Fluorescence Assisted
Cell Sorting (FACS) assay to separate neuronal nuclei from non-neuronal nuclei
in 3 pooled control samples (total n=9 mice) and 6 pooled Pb exposed samples
(total n=19 mice). The offspring were exposed via the maternal drinking water to
0 ppm, 2.1 ppm, or 32 ppm of Pb two weeks before mating, throughout gesta-
tion, and three weeks after birth. Using NimbleGen Promoter Tiling Arrays, we
probed DNA methylation levels in neuron-specific cells at a genome-wide level.
Results and Conclusions. Using a bioinformatics bumphunting method and fami-
ly-wise error rate cutoff of 0.3, we report 5 novel exposure-dependent differentially
methylated regions associated with the Hmna3, Skin3, and Hmna genes. Hmna3
(histamine N-methyltransferase) is interesting due to its association with regulation
of neurotransmitter levels. This work is a step towards identifying epigenetic signa-
tures that may be used as biomarkers of exposure and disease.

Our objective was to determine the impact of transplacental exposure of polycyclic aromatic hydrocarbons (PAHs) on the epigenome of fetal and adult lung tissues in
offspring exposed to either dibenzo[a]anthracene (DBA) or benzo[a]pyrene (BaP;
BaP) during gestation. Methylation of the promoter regions of Cdkn2a, Rarb, Dapk1, Mgmt and Cadh13 genes and expression of these target genes and of DNA methyltransferase enzymes 1, 3a and 3b in neonate lungs was
assessed by bisulfite sequencing and quantitative RT-PCR, respectively. Expression of Cdkn2a mRNA was twofold higher in neonate lung of DBC exposed mice compared to controls, while no significant differences in expression in other target genes were observed. Genome wide methylation of lung tumors in the adult offspring was determined using the NimbleGen DNA Methylation array. We employed laser capture microdissection to determine the timing of methylation dependent tumor suppressor gene silencing during progression of preneoplastic lesions to atypical adenomatous hyperplasia (AAH) and lung adenocarcinomas. Analysis of methylation for the Cdkn2a promoter revealed a localized region of moderate methylation associated with CpG sites 27 to 30 in all samples evaluated. Within the Mgmt gene promoter region, progression of methylation was observed from 25 week normal and AAH samples to 45 week adenocarcinoma samples. The other target genes of interest showed no apparent differences in methylation. Lung tumor incidence in 45-week old mice initiated with BaP was 30%, much lower than that of the DBC-exposed offspring at 100%. The spontaneous lung tumor rate was 9% in control offspring at 45-weeks. Although we did not observe profound differences in methylation for these target genes, it is possible that other tumor suppressor genes are subject to hypermethylation in this lung cancer model. In future studies, we plan to perform more comprehensive analysis of the complete CpG island region of other target genes identified from analysis of the genome wide methylation data set for lung tumor tissues.

### 2263 Epigenome-Wide Effects of Perinatal Bisphenol A Exposure in Adult Mice with Hepatic Tumors

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Developmental exposure to the endocrine-active compound bisphenol A (BPA) has been linked to epigenetic and potential carcinoenic effects in rodent prostate and mammary glands. We have previously reported a dose-dependent increase in hepatic tumors in 10-month mice perinatally exposed to one of three doses of BPA (50 ng, 50 μg, or 50 mg BPA/kg chow). These tumors represent early-onset disease and lack classical sexual dimorphism in incidence, suggesting a distinct disease etiology. Here, we investigate epigenome-wide DNA methylation profiles at gene promoters associated with BPA exposure and disease via methylcytosine enrichment microarrays (β-ratio of enriched to input signal) in 10-month mice with and without liver tumors (n=4 male and n=4 female offspring exposed to 50 ng or 50 μg BPA/kg chow; n=4 male and n=4 female offspring exposed to 50 mg BPA/kg chow with tumors; n=3 male and n=3 female control diet offspring without tumors). We report four novel exposure-dependent differentially methylated regions in 5' gene promoters of Lctm1 (tumor vs. non-tumor; β̂=0.641, p=7.1E-6), Shtm2 (low BPA vs. high BPA; β̂=3.162, p=2.2E-7), Foxp2 (low BPA vs. control; β̂=0.383, p=4.6E-6), and Ifit4 (high BPA vs. control; β̂=3.181, p=2.2E-5) in mice. These genes also display functional links to human disease in the literature. LCE1 (Late Cornified Envelope Group 1) genes are downstream targets of tumor suppressor p53 and may carry novel driver mutations in breast cancer.
2266 Nickel Induces Epigenetic Dysregulation through Repressive Chromatin Domain Disruption

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Nickel (Ni) compounds are environmental pollutants prevalent in the atmosphere due to the extensive consumption of Ni products and combustion of fossil fuels. A multitude of human health risks is associated with exposure to Ni compounds, including lung and nasal cancers, cardiovascular diseases as well as allergic dermatitis. Although Ni is a proven carcinogen, its mutagenic potential is very low and the molecular basis of Ni-induced carcinogenicity is not fully understood. Emerging evidence suggests that Ni, as well as several other environmental pollutants induce development of cancer and other diseases through dysregulation of chromatin modifications. In this study, using ChIP-Seq, we comprehensively analyzed Ni-induced alterations to several histone modifications in the immortalized non-cancerous human lung epithelial, BEAS-2B cells. Our results indicate that Ni exposure triggers disruption of repressive chromatin domains marked by the silencing histone modification H3K9me2, resulting in its spreading. Spreading of H3K9me2 is associated with gene silencing. Interestingly, we find loss of DNA binding of the insulator protein CTCF at the Ni-disrupted H3K9me2 domain boundaries. This suggests that Ni interferes with the insulation mechanism that delimits the active and the repressive regions of the genome. Since epigenetic alterations are believed to be important for Ni-induced cancer, our findings of H3K9me2 domain disruption by Ni will have profound implications in understanding the process of carcinogenesis. Supported by NIH, NIEHS grants R01ES023174 and P30ES000260.

2267 Early Epigenetic Modulation of Nrf2 and Lipogenic Genes by PNPP Exposure of Bisphenol A Is Associated with Hepatic Steatosis in Female Mice

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Bisphenol A (BPA) is chemical used in manufacturing of polycarbonate and epoxy resins. Studies demonstrate a potential health concern with exposure to low BPA levels through direct or indirect mechanisms via in utero and lactational exposure. In humans, urinary BPA levels have been positively associated with general and abdominal obesity, with increased adverse liver effects described in children. Rodent studies have demonstrated increased steatosis on BPA exposure. Nuclear Factor E2-Related Factor 2 (Nrf2) has been recently shown to be a possible contributor to hepatic lipid accumulation. We hypothesized that epigenetic changes occur before BPA-induced steatosis, possibility via modulation of Nrf2 expression. Pregnant CD-1 mice were administered 25 or 250 μg BPA/kg/day via osmotic pump, and after weaning on PND 20, the resulting daughters were further exposed to BPA via drinking water until PND 35. Tissues were collected at PND 32 (Week 5) and 39 weeks of age and mRNA and protein expression profiling was performed. Liver tissues were then used for follow up studies to determine transcription factor binding and DNA methylation via methylated DNA immunoprecipitation. Both 25 and 250 μg BPA/kg/day BPA increased lipid deposition in livers of young and adult female offspring, along with increased fatty acid synthase, acyl-Co-A carboxylase, steryl-Co-A desaturase, peroxisome proliferator activated receptor-gamma, and sterol regulatory element binding protein 1c (Srebp1c) mRNA and protein expression, with induction of Nrf2 protein expression at W39. At W5, BPA exposure increased Nrf2 binding to a putative ARE consensus sequence in the Srebp1c promoter and hypomethylation of the Nrf2 and Srebp1c promoters was observed in livers from offspring that were developmentally exposed to BPA. Overall, the work herein presents new findings that developmental BPA exposure induces fat accumulation via hypomethylation of key lipogenic genes and promotes Nrf2 binding to the Srebp1c promoter.

2268 Effect of Prenatal Environmental Tobacco Smoke Exposure on Promoter Methylation and Asthma

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Environmental tobacco smoke (ETS), which can lead to poor respiratory health among susceptible children, remains a major public health concern. Although it is widely recognized that prenatal exposure to ETS may play a significant role in allergic disease in offspring, the mechanism driving development of asthma due to prenatal ETS exposure is not fully understood. In order to examine the effect of in utero ETS exposure on asthma susceptibility via the proposed mechanism of epigenetic programming, a house dust mite (HDM) induced-asthma murine model was used. Pregnant female C57BL6 mice were exposed to ETS (1.0mg/m3, 6hours/day, 7days/week) or filtered air through gestation and after the dams had given birth, 6 week old offspring were further exposed to HDM or PBS intranasally to the nose. At 6 weeks of age, offspring were sacrificed and lungs were collected for DNA and protein extraction. DNA was then used to identify alterations in coat color distribution. Compared to the controls, Mediterranean HFD offspring displayed a bimodal distribution distinct from the unimodal control distribution. To examine the effect of ETS exposure on asthma susceptibility in the proposed mechanism of epigenetic programming, a house dust mite (HDM) induced-asthma murine model was used. Pregnant female C57BL6 mice were exposed to ETS (1.0mg/m3, 6hours/day, 7days/week) or filtered air through gestation and after the dams had given birth, 6 week old offspring were further exposed to HDM or PBS intranasally to stimulate allergen-induced airway inflammation. Promoter methylation of Il-4, Il-13, Ifn-γ and Foxp3 in lung tissue from offspring was measured by pyrosequencing assay and BALF cell differential counts and cytokine production were measured for allergic response to HDM challenge. Prenatal exposure to ETS induced a severe increase in the immune response when challenged with HDM. We also found Ifn-γ to be hypermethylated in the HDM group and further hypermethylated after prenatal ETS exposure. In addition, Il-4 was hypomethylated in the HDM group and further hypomethylated after ETS exposure. Obtained results suggest that prenatal ETS exposure increases the susceptibility to an allergic and asthmatic response when exposed to allergen, with promoter methylation as a proposed mechanism.

2269 High-Fat Diet Modifies the Effect of Perinatal Bisphenol A Exposure on the Epigenome

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Epidemiological and animal data indicate that the risk of developing adult-onset diseases such as obesity is influenced by persistent adaptations to perinatal environmental conditions. One such environmental exposure is bisphenol A (BPA), a chemical monomer used in production of polycarbonate plastics and epoxy-resins. Few studies, however, have investigated perinatal BPA exposure in combination with nutritional modifiers such as diet. To investigate the effects of high fat diet (HFD) and BPA on developmental programming, isogenic female mice with 95% identity to C57BL/6J were randomized to 1 of 6 diets 2 weeks prior to mating with Agouti viable yellow (Avy) males: 1) Control Chow, 2) Mediterranean HFD, 3) Western HFD, 4) Control + 50 μg BPA/kg diet, 5) Mediterranean diet + 50 μg BPA/kg diet, or 6) Western diet + 50 μg BPA/kg diet. Dams remained on their respective diet throughout gestation and lactation, and at PND 22, Avy offspring (n=40-64/group) were visually rated for coat color status, which serves as a visual proxy for DNA methylation at the Avy locus. A chi-squared goodness of fit test was used to identify alterations in coat color distribution. Compared to the controls, Mediterranean HFD offspring displayed a marginally significant shift towards the yellow hypomethylated coat color (p=0.07). Conversely, Western HFD offspring displayed a bimodal distribution distinct from the unimodal control distribution (p=0.07), and BPA offspring shifted slightly towards yellow, but did not reach significance (p=0.37). Compared to Mediterranean HFD, the distribution of offspring exposed to Mediterranean HFD + BPA was shifted towards the brown hypermethylated coat color (p=0.02), but Western HFD compared to Western HFD + BPA offspring did not differ (p=0.20). Quantitative analysis of DNA methylation at the Avy locus and other genes (Calh1AP, Igf2, and H19) is ongoing. The present results serve as preliminary evidence that HFD diet modifies the effect of BPA on the epigenome, indicating attention should be paid to diet composition in environmental epigenetics studies.
In early pregnancy, the conceptus is highly sensitive to reactive oxygen species (ROS). Maternal spiral artery remodeling and trophoblast invasion serve as the beginning of placentation development and protect the fetus against ROS. Improper invasion and remodeling can result in preeclampsia. Preeclampsia is associated with low birth weight, preterm labor, SGA, IUGR, and altered placental gene expression as a result of increased ROS. We previously demonstrated altered expression profiles of apoptosis-related miRNAs in cytotrophoblasts exposed to ROS which was modulated by the natural product CAPE. Here we test the hypothesis that CAPE modulates ROS-induced changes in miRNAs and apoptotic proteins is not only dependent on its antioxidant properties. To test this hypothesis, we compared CAPE to the known antioxidant NAC on antioxidant parameters, apoptosis-related miRNA and apoptosis protein targets. Villous 3A trophoblast cell lines were mock exposed, exposed to H2O2, CAPE, or NAC alone or exposed in combination to H2O2-CAPE or H2O2-NAC for 4h. We measured LDH as viability marker, SOD, GSH and TBARS as antioxidant capacity markers, apoptotic miRNA levels and Bax and Bcl-2 protein levels, and caspase-3 activity. Our data indicate that CAPE is more effective compared to NAC in preventing LDH leakage and preserving the Bax/Bcl-2 ratio. We measured increased GSH and SOD levels. In comparison to NAC, CAPE resulted in a significant increase in Nrf2 followed by epigenetic up-regulation of MT. Consequently, histone acetylation at MT1 promoter leads to the open chromatin structure which provides binding site for Nrf2, a transcription factor, to regulate MT expression. Hence, histone acetylation and HDAC inhibition could up-regulate MT expression at MT promoter. Second, histone acetylation at MT promoter leads to the open chromatin structure which provides binding site for Nrf2, a transcription factor, to facilitate MT expression. To test this hypothesis, mouse model of type 2 diabetes was induced by high-fat diet and STZ injection. Diabetic mice were then treated with or without SFN for 4 months. Diabetic mice exhibited renal dysfunction, fibrosis, inflammation, and oxidative damage along with increased HADC activity. These effects were significantly alleviated by SFN treatment along with Nrf2 and MT upregulation. Mechanically, SFN significantly reduced the HADC2 expression and HDAC2 binding at AT1 promoter, leading to the enhanced histone acetylation at AT1 promoter in diabetic kidney. Consequently, histone acetylation at AT1 promoter leads to the open chromatin structure which provides binding site for Nrf2 to facilitate MT expression. These results suggest that SFN-mediated increase in Nrf2 followed by epigenetic up-regulation of MT may play a critical role in its renal protection from diabetes.

MicroRNAs (miRNAs) are small —15-22 nucleotide—noncoding RNAs that play pivotal role in regulation of gene expression; miRNAs bind via complementary base-pairing to target transcripts to repress translation or promote mRNA degradation. Mounting evidence indicates that miRNAs are frequently overexpressed or downregulated in various types of cancer. We have previously extracted the miRNAs commonly up or downregulated in the liver of rats treated with several different classes of non-genotoxic hepatocarcinogens, including a key immunoinhibitory molecule in tumor, Cd276, by using a toxicogenomic database named TG-GATEs. In the present study we hypothesized that miRNA play pivotal roles in regulation of gene expression in non-genotoxic hepatocarcinogenesis and genome-wide miRNA expression analysis can aid the understanding the detailed mechanisms involved in the regulation of miRNAs in non-genotoxic hepatocarcinogenesis. Genome-wide miRNA expression data were obtained in the liver of rats repeatedly treated with thioacetamide at 3 different dosages for up to 28-day. Following statistical filtering, 16 miRNAs were extracted as differentially expressed miRNAs and their target transcripts were predicted by TargetScan and miRanda database. Integrative analysis of mRNA and miRNA profiles successfully identified several miRNA-mRNA pairs with inverse correlation in their expression changes. Among them, Cd276 mRNA expression was inversely correlated with miR-29a levels. This is suggestive of miR-29a repression increases Cd276 mRNA expression. Furthermore, network analysis on microRNA-mRNA pairs demonstrated novel key biological events possibly involved in the regulation of miRNA expression in hepatocarcinogenesis. This integrated analysis can help to discover coordinated expression signatures of miRNAs and their target mRNAs in hepatocarcinogenesis.
Introduction: Past oral administration of powdered senna to rats has resulted in pigment deposits in renal tubules. Renal tubule brownish pigment was seen in mice and rats dosed with emodin (an anthraquinone metabolite of sennosides) and with anthraquinone in NTP studies. Since some species of Cassia (Senna) cause skeletal muscle degeneration and necrosis in domestic animals, we wanted to see if the renal pigment in rodent studies was myoglobin or some other pigment(s). Methods: Slides from archived paraffin blocks from 2 rats and 2 mice with kidney pigment from the emodin and anthraquinone chronic studies were stained with Schmorl’s, PAS, Hall’s Bile, Prussian Blue (PB) stains and myoglobin by immunohistochemistry (IHC). Results: The IHC reaction for myoglobin resulted in positive staining of small intracellular granules of brown pigment within the proximal renal tubule epithelial cells in rat kidneys treated with emodin and anthraquinone. The same reaction did not stain any of the pigment as myoglobin in the mouse kidney sections. The Schmorl’s reaction was variably positive in rats. However, when positive, the reaction was associated with larger droplets and not the small distinct granules. The PB reaction was variably positive in emodin rats and mice and anthraquinone rats. Anthraquinone mice were strongly positive for the iron stain. The PAS reaction and Hall’s bile stain were noncontributory. Conclusion: In rats treated with emodin or anthraquinone a portion of the renal cortical tubule pigment may be myoglobin. Lipofuscin was also a component of some of the brownish pigmentation, but was associated with larger droplets, not smaller distinct granules. This “wear and tear” pigment is likely related to the renal pathology induced by anthraquinone in rats and emodin treated rats and mice. PAS and Hall’s Bile stains were negative. In mice the only stain that was strongly positive was the PB (iron) correlating with hemosiderin (not myoglobin) related to an induced anemia by anthraquinone in mice.
affected by F more seriously. We used the renal tubulointerstitial fibrosis model of rats induced by unilateral ureteral obstruction (UUO). We examined whether or not F exposure deteriorates renal tubulointerstitial fibrosis in rats with UUO. In addition, the mechanisms responsible for the aggravation of renal tubulointerstitial fibrosis caused by fluoride exposure in the rats were examined by the pathological observation. Left ureter was ligated and sham-operation was done in 6-week-old male rats weighing 230-250 g. F was administered to rats with UUO at 0, 75 and 150 ppm and sham-operated rats at 0 and 150 ppm in drinking water for 2 weeks. After 2 weeks, HE, PAS and Masson trichrome staining were done for the kidneys, and they were subsequently examined by immunohistochemical staining using anti-ED-1 antibody and by double immunostaining with anti-ED-1 and TGF-β1 antibodies. Fibrosis area stained with Masson trichrome was examined by imaging analysis and ED-1 positive cell number was determined by counting the number of the cells within the renal cortex. Fibrosis area and ED-1 positive cell number were significantly increased in the 150 ppm obstructed kidney group when compared to the 0 ppm obstructed kidney group. The double positive cell using anti-ED-1 and TGF-β1 antibodies were also observed in the renal cortex of the 150 ppm obstructed kidney group. These observations indicate that the aggravation of renal tubulointerstitial fibrosis caused by fluoride exposure may be due at least in part to infiltrated macrophages via increased production of TGF-β1 driving fibrosis.

2280 Canine Renal Proximal Tubule Cells As an In Vitro Model for Toxicity Assessment of Pet Food Ingredients
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Comprehensive in vitro toxicity testing of pet food ingredients was conducted in a previously validated canine proximal tubules cell (CPTC) in vitro model to assess the safety and comparative toxic potential of denatonium benzoate (DB), green tea catechin extract (GTE), sodium copper chlorophillin (SCC), tetrahydroisohumulone (HEX) and eucalyptol (EUC); commercial ingredients and their purified components. The model to assess in vitro previously validated canine proximal tubules cell (CPTC) model may serve as a valuable in vitro screening tool for assessing and comparing safety of diverse feed additives in a defined humane alternative model system. (This research was supported by Mars, Inc.)

2281 Diglycolic Acid, the Toxic Metabolite of Diethylene Glycol, Inhibits Renal Mitochondrial Respiration
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Diethylene glycol (DEG) ingestion has repeatedly led to numerous mass poisonings characterized by acute kidney injury, hepatotoxicity and peripheral neuropathy. To better understand the mechanism of toxicity and to advance therapy for DEG poisoning, recent animal and cellular studies have shown that diglycolic acid (DGA) is the primary metabolite that accumulates and produces toxicity in the kidney proximal tubule. Although necrosis is the dominant feature, the specific mechanism for the toxic effects is not fully understood. Studies in cultured cells indicated that DGA decreases the mitochondrial membrane potential, oxygen consumption and the NADH/NAD+ ratio, suggesting that it induces mitochondrial dysfunction. To localize this mitochondrial inhibition, rat kidney mitochondria were isolated by differential centrifugation and then exposed to toxicologically-relevant concentrations of DGA (0 - 100 mM). Mitochondrial respiration (States 3 and 4) was determined using either succinate or glutamate/malate as substrate and electron transport chain (ETC) complex activities were measured at mitochondrial complexes I-IV via spectrophotometric analysis. DGA selectively inhibited Complex II, but had no effect on the other ETC complexes. Also, DGA decreased State 3 respiration with either succinate or glutamate/malate as energizing substrate, but did not affect State 4 respiration or the ADP/O ratio. Glutamate/ malate respiration was inhibited by very low DGA (6 mM), while succinate respiration was reduced only at 100 mM. These results suggest that DGA has two anti-mitochondrial effects - directly inhibiting succinate dehydrogenase, due to a structural similarity to succinate, and indirectly reducing Complex 1 respiration (glutamate/malate), by decreasing the supply of reducing equivalents (NADH). These results could explain how DGA inhibits oxidative phosphorylation in kidney cells, thereby leading to necrotic cell death and eventually to the acute kidney injury displayed by DEG.

2282 Effects of Polyphenols of Coccs nucifera Husk Fiber on Selected Kidney Function Indices in Rat

Polyphenols have been found to be mainly responsible for the antimarial activity of Coccs nucifera husk fibre. The husk fibre polyphenols were therefore evaluated for their effects on selected kidney function indices in Swiss albino mice. Fifty mice were randomly grouped into five each containing ten mice. The mice in the control group were orally administered 5% DMSO solution (the solvent used in dissolving the polyphenols) while the other four groups were orally administered 31.25, 62.5, 125 and 250 mg/kg body weight of the polyphenols respectively for seven days. The serum urea, creatinine and uric acid concentrations were determined. The serum levels of sodium, potassium, chloride and calcium ions were also determined. The activities of alkaline phosphatase (ALP), glutamate dehydrogenase (GDH) Na+,K+ ATPase, Ca2+, Mg2+ ATPase and gamma-glutamyltransferase (GGT) in the kidney were determined. The results showed that the polyphenols significantly reduced (p<0.05) serum urea concentration at 250 mg/kg body weight and serum creatinine concentration at all doses compared to controls. The polyphenols caused no significant change (p>0.05) in serum uric acid concentration at all doses compared to control. There was a significant increase (p<0.05) in serum level of sodium ion at doses of 31.25, 125 and 250 mg/kg body weight whereas the significant increase (p<0.05) in potassium and chloride ions was observed at 62.5 and 250 mg/kg body weight compared to control. The polyphenols caused no significant change (p>0.05) in the activities of ALP, GGT and GDH compared to controls. The polyphenols significantly (p<0.05) reduced Na+, K+ ATPase activity in the kidney at all doses compared to control. The polyphenols significantly (p<0.05) reduced kidney Ca2+, Mg2+ ATPase activity at 125mg/kg body weight compared to control. The results of this study suggest that the polyphenols of Coccs nucifera husk fibre may adversely affect osmoregulatory functions of the kidney.

2283 Exploring the Role of Oxidative Stress in 3, 5-Dichloroaniline (3, 5-DCA)-Induced Nephrotoxicity In Vitro
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Chlorinated anilines are important chemical intermediates in the production of many agricultural products. These chlorinated anilines have been shown to be nephrotoxics both in vivo and in vitro. Of the chlorinated anilines previously tested, 3,5-dichloroaniline (3,5-DCA) proved to be the most potent nephrotoxicant. These studies showed significant increase in lactate dehydrogenase (LDH) release following exposure to 1.0 mM 3,5-DCA for 90 minutes with isolated renal cortical cells (IRCC) obtained from male Fischer 344 rats as a model. Previous studies also showed that antioxidants significantly attenuated 3,5-DCA induced oxidative stress in 3,5-DCA induced nephrotoxicity. The current study was designed to explore the role of oxidative stress in 3,5-DCA induced nephrotoxicity. IRCCs (4 x 10^6) were treated with DMSO or 3,5-DCA (0.5 or 1.0 mM) for 60 or 90 minutes. Following exposure to 3,5-DCA, aliquots were taken for LDH release assays, protein carbonyl detection using Oxyblot kits from Millipore, and glutathione determination assays. Results showed a significant increase in protein carbonyl levels only after exposure to 1.0 mM 3,5-DCA for 90 minutes. Also seen was a shift in the ratio of oxidized/reduced glutathione, suggesting the presence of oxidative stress, but only at 1.0 mM 3,5-DCA for 60 minutes. Both these events occur after there was a significant increase in LDH release. This data suggests that while oxidative stress occurs following exposure to 3,5-DCA, it is not an early initiator of toxicity.
markers of nephrotoxicity. The concentrations of Fe were higher than WHO MCL of 1.5 mg/L in 50.6% of drinking water samples, showing a positive and significant correlation between water (0.1-5.12 mg/L) and urine (0.34-22.7 μg/mL) Fe concentrations, suggesting that the main source of exposure is the water supply network. In the multiple linear regression analysis, the urinary Fe concentrations were positively associated with the urinary excretion of Cystatin-C (β = 0.026, 95%CI 0.016-0.036), Clusterin (β = 0.072, 95%CI 0.015-0.13), KIM-1 (β = 0.059, 95%CI 0.023-0.095) and Osteopontin (β = 0.06, 95%CI 0.023-0.098), and the R-squared adjusted values were between 0.16 to 0.32, confirming the kidney damage process activation. In conclusion, these results show an association between the Fe exposure and the excretion of urinary kidney injury biomarkers, supporting the nephrotoxic role of the chronic Fe exposure in drinking water. Prospective studies are needed to establish kidney damage risk by Fe exposure. Funded by Conacyt (grant 180847).

2287 Imaging Techniques Supporting Human Risk Assessment of Drug-Induced Crystal Nephropathy in Preclinical Studies
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Drug or metabolite precipitation in tubules of the kidney and associated nephropathy is usually an exposure related finding. Knowing the nature of the precipitates can be important for human risk assessment. We recently observed kidney findings suggestive of drug-induced crystal nephropathy with multiple molecules with varying chemical structures and pharmacological activities which led to further investigations. Kidney samples from toxicity studies of different durations in rat, mouse or monkey were evaluated. Undisturbed and freeze-dried kidneys were examined microscopically for the presence of crystals. Raman and Infrared microspectroscopy were performed on crystals detected. For one compound, for which the exact nature of the crystals could not be determined LC-MS on crystal rich areas, SEM/EDX and MALDI-imaging were performed. Nephropathy was observed in treated rodents or monkeys and was characterized by presence of crystals in tubules in addition to dilation, tubular basophilia and inflammation. For some compounds crystals were identified to be parent compound or metabolite by Raman and Infrared microspectroscopy. However, for other nephropathies, crystals were only shown to be similar but not identical to the compound by these techniques. Subsequent LC-MS for one of these compounds and presence of the glucuronide of the major metabolite (M1) in the tissue. By MALDI imaging the presence of M1 glucuronide in crystals was confirmed but also some parent was detected. Sodium was the only cation present in higher concentrations in SEM/EDX suggesting precipitation of an M1 glucuronide salt. Conclusion: The additional investigations confirmed precipitation of compound and/or metabolite in affected kidneys. MALDI imaging was crucial in the case the other techniques did not provide conclusive data. Applying imaging techniques can support human risk assessment in case of drug-induced crystal nephropathy in preclinical studies.

2288 Hepcidin: A Novel Treatment for Heme-Mediated Kidney Injury?
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Introduction: Multiple clinical observational studies demonstrated that increased urinary hepcidin levels are associated with reduced risk of developing acute kidney injury (AKI) due to hemolysis and hypoxia in cardiac surgery patients. This study aimed to get more insight in renal hepcidin handling and its potential protective effects against heme-mediated AKI. Methods: C57Bl/6 mice were treated with i) a single i.p. dose of 10 μg human hepcidin-25 (hhep25) to study renal handling of systemic hepcidin, ii) a single i.v. dose of 5 mg hemoglobin (Hb) to induce AKI, and iii) Hb combined with hhep25 to evaluate the protective effects of hhep25 on Hb-mediated kidney injury. Results: Systemically administered hhep25 was cleared rapidly from plasma and excreted in the urine. Hhep25 was biologically active in mice as observed by a negative feedback on mouse endogenous hepcidin plasma levels (60% reduction, p<0.05) and hepatic mRNA expression (10-fold decrease, p<0.05). Urine hhep25 was increased 20-fold in maligen deficient mice compared to control (p<0.05) and immunofluorescence staining showed that hepcidin was present in tubules expressing maligen, but not in maligen-deficient tubules. Administration of hhep25 simultaneously or 4h after Hb injection significantly attenuated the Hb-induced rise in urinary NGAL (p<0.05) and KIM1 (p<0.05) levels, and renal IL6 (p<0.05) and NGAL (p<0.05) mRNA expression. Interestingly combined administration of Hb and hhep25 resulted in an increase in renal hamp1 mRNA expression (15-fold, p<0.05) and reduction in renal mRNA expression of
HO-1, DMT1, H-ferritin and L-ferritin (all p<0.05), possibly reflecting the pathway by which hepcidin exerts its protective effects. Conclusion: Systemic hepcidin is filtered to the urine and partly reabsorbed via megalin in the proximal tubules. Moreover, our data suggest that both systemically delivered hepcidin and locally produced hepcidin are involved in renal protection against heme-induced AKI.

2289 Acute Renal Toxicity to Diglycolic Acid: Preliminary Findings of an In Vitro-to-In Vivo Comparison

Division of Toxicology, Food and Drug Administration, Laurel, MD. Sponsor: L. Yourick.

Diglycolic acid (DGA) is an impurity produced during the synthesis of carboxymethyl starches, which are used as fillers/viscosity stabilizers/tablet binders in food and drugs. DGA exposure is known to induce acute renal cortical tubular degeneration and proximal tubular necrosis. To assess the ability of our in vitro test system to accurately predict in vivo renal toxicity, cell and mitochondrial viability and several FDA-qualified biomarkers of acute kidney injury were measured in cultured human kidney proximal tubule cells exposed to increasing doses of up to 100 mM DGA for 24 hours. Additionally urine samples were collected after 2, 4, 8, 16, and 22 days of exposure from an ongoing in vivo DGA 28 day repeat oral dose toxicity study (see Keltner et al., SOT 2014) and the same biomarkers of acute kidney injury were measured. A non-monotonic dose response relationship was observed in vitro and levels of KIM-1 and NGAL in culture supernatants treated with the 10 mM dose were significantly elevated. In contrast, a non-monotonic trend was not observed in our urine analyses. Instead, we found urinary levels of KIM-1, but not NGAL, exclusively elevated in the high dose DGA treatment group (300 mg/kg bw compared to control) after 2 days of daily DGA exposure. The levels of these urinary biomarkers in all other treatment groups, which received 100 mg/kg doses or less, were similar to control urine samples. After 22 consecutive days of DGA treatment at the 100 mg/kg dose and below, KIM-1 levels remained low in the urine of DGA-treated and control animals. The analysis of the urine samples obtained from the in vivo study suggest the NOEL for DGA is 100 mg/kg bw.

2290 High-Concentration SGLT2 Inhibitor-Mediated Change in Renal Glucose Metabolism
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Pharmaceutical inhibition of sodium-glucose co-transporter 2 (SGLT2) as treatment for Type II diabetes mellitus is one major approach pursued over the last years. SGLT2 is exclusively expressed in the epithelium of the proximal tubuli and responsible for the bulk of renal glucose reabsorption. By SGLT2 inhibition, high capacity renal glucose reabsorption is inhibited thus inducing glucosuria and reducing hyperglycemia. Preclinical and clinical evaluations of SGLT2 inhibitors are sufficiently robust as to consider the use of these pharmaceuticals as safe for human use. However, using the specific inhibition of glucose uptake via SGLT2 inhibitors and well-defined serum-free culture medium the question was addressed whether restricted glucose supply could alter the energy metabolism in human renal tubular epithelial cells. Treatment with 10 or 20 μM canagliflozin (C) but not dapagliflozin (D) for 24 h significantly raised cellular lactate production by 29 % and 34 % respectively while glucose consumption was increased by 20 % and 21%. 10 or 20 μM C mildly but significantly increased the conversion rate from glucose to lactate from 1.61 to 1.73 and 1.79 respectively, suggesting increased glycolysis and decreased oxidative phosphorylation since ATP levels remained unchanged. Consistent with the latter, activation of AMPKα by C (5-fold increase in phosphorylation) and an increase in mitochondrial membrane potential was observed. AMPKα activates phosphofructokinase in the glycolytic pathway, thus suggesting that high in vitro concentrations of C triggered alterations in cellular glucose metabolism that may be attributed to metabolic stress. Since increased and altered glucose metabolism were not observed with D, the effects described are not likely to originate from specific SGLT2 inhibition. Future investigations will elucidate the mechanism(s) underlying the metabolic switch from oxidative phosphorylation to glycolysis in tubular epithelial cells induced by C using D as control.

2291 Unravelling the Phenomenon of Propionivore-Induced DAAO Accumulation in Rat Kidney: A Direct Approach
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Propionite (PV) (Apophega Arzneimittel GmbH, Germany) is a common and well-tolerated pharmaceutical for bladder control diseases and urinary incontinence. Subchronic rat studies demonstrated PV-induced renal intra-nuclear and cytosolic protein accumulation, which was later identified as D-amino acid oxidase (DAAO). Similar observations were reported in rat exposed to other pharmaceuticals, e.g. a norepinephrine serotonin reuptake inhibitor (Pfizer, USA). To elucidate whether the mechanism underlying DAAO droplet formation is of human relevance, the direct interaction of PV and its main metabolite Propionivore-N-oxide (PVO) with DAAO was first investigated. Recombinant rat and porcine DAAO activity was investigated using two different activity assays, PV, PVO and known DAAO substrates and inhibitors. The same conditions were also applied to human and rat DAAO expressed in HEK293 cells. Moreover, spectroscopic binding studies (differential spectra; Near-UV circular dichroism) were performed by titrating recombinant human and rat DAAO with PV and PVO. Interestingly, PV and PVO did not affect DAAO functionality, since they do not act either as substrates or as inhibitors. DAAO binding studies with PV and PVO supported the enzyme activity studies: no direct binding or induced conformational changes were observed. These results indicated that PV and PVO do not interact with mammalian DAAO’s altering their activity and conformation/oligomerization state. Thus, the observed PV-induced DAAO accumulation in rat kidney must arise from mechanisms that still need to be elucidated.

2292 Unravelling the Mechanism of Propionivore-Induced DAAO Accumulation in Rat Kidney: The Role of Proteasomal Degradation
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Propionite (PV), a frequently prescribed pharmaceutical for treatment of overactive bladder and incontinence provokes massive protein accumulation in the cytosol and nucleus of renal proximal tubule epithelial cells in rats, whereby the severity of accumulation is higher in males. Previously, the accumulated protein was identified as D-amino acid oxidase (DAAO), a long-lived, peroxisomal flavoenzyme. Contrary to studies in rats, no DAAO accumulation was observed in mice or dogs, therefore suggesting the presence of a species-specific and sex-susceptible phenomenon. Notably, neither PV nor its main metabolite Propionivore-N-oxide (PVO) influences DAAO mRNA level in vivo or in vitro indicating that DAAO accumulation occurs rather at protein level, for example by impairment or alteration of DAAO degradation or trafficking. Direct interaction studies of PV and PVO with human, porcine and rat DAAO demonstrated no effect on DAAO activity and conformation state (see Heusner et al. Abstract ID 2112220) suggesting that for example inhibited proteasomal processing of DAAO may be responsible for the observed DAAO accumulation. Indeed, using proteasomal inhibitors like MG132, epoxomicin and bortezomib in HEK293 cells stably transfected with EFPP-tagged DAAO, we detected similar accumulation of DAAO as observed in vivo. Proteasomal inhibition induced the accumulation of human and rat DAAO as juxtanuclear and nuclear inclusions confirming the presumed role of the proteasome in cellular degradation of DAAO. Consequently, the direct influence of PV and PVO on proteasomal degradation activity was assessed using a human, murine and rat constitutive proteasome activity assay.

2293 Identification of Epigenetic Modifications of CDK Inhibitors Induced by Nephrotoxicants Using Targeted DNA Methylation and Next-Generation Sequencing
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Bromate (BrO3-) is a drinking water disinfection byproduct formed from bromide during the ozonation process. Our laboratory previously showed that BrO3—induced nephrotoxicity in vitro and in vivo correlates to increased expression of the cyclin-dependent kinase (CDK) inhibitor p21. We hypothesized that BrO3-induced p21 expression via epigenetic mechanisms. This is supported by our recent published study using methylation specific PCR demonstrating decreased methylation and next-generation sequencing.
EMT is postulated to be a pathway leading to fibrosis due to nephrotoxics such as cyclosporine A and cadmium. Inhibition of EMT has been shown to prevent kidney fibrosis in several studies. EMT involves the switch of epithelial cells into a mesenchymal phenotype via loss of epithelial markers such as E-cadherin and acquisition of mesenchymal markers such as α-smooth muscle actin (α-SMA), vimentin and extracellular matrix proteins such as collagen, resulting in increased toxicity in in vitro models. Transforming growth factor β1 (TGF-β1) can trigger fibrogenic injury and has been used as an inducer of EMT. TGF-β1 causes activation of Akt through phosphorylation by P38. Activated Akt phosphorylates and deactivates glycogen synthase kinase 3 β (GSK-3 β) which is a repressor of EMT and leads to degradation of transcription factors β-catenin and Snail. Our previous work showed that the flavonoid chrysin inhibits motility in LLC-PK1 cells. This study was performed to test the hypothesis that chrysin inhibits EMT induced by TGF-β1 in LLC-PK1 cells. Cells were treated with chrysin, TGF-β1 or TGF-β1 + chrysin for 48 hrs. Upon treatment with TGF-β1 alone, expression of collagen increased by 10 time points before and after Cp administration (pre, post, 8, 12, 24, 48, 72, 96, 120 and 144h). All three miRs (measured by qRT-PCR and normalized to miR-489) were significantly (p<0.01) higher in urines from APAP or Cp treated patients with AKI as compared to patients without AKI. ROC-AUC analysis revealed that expression of miR-200c (AUC: 0.61 and 0.40, respectively), miR-203 (AUC: 0.65 and 0.40, respectively) and miR-21 (AUC: 0.52 and 0.40, respectively) were able to discriminate AKI patients. These results suggest that F-prooxidant properties could be related with mir-144 induction expression, it’s concomitant inhibition of NR2F2 and the increase of TGF-β1 expression and activity. Funded by CONACYT (Grant 152416).

2295 Renal Expression of TGF-β1 and Inflammation-Related miRNAs in a Rat Model after Subchronic Exposure to Fluoride

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Fluoride (F) is an endemic water pollutant across the world. The WHO recommends a concentration of ≤1.5 mg F/L in drinking water. However, many regions worldwide present higher levels of F in water. F effects have been described mainly in bone and teeth, nevertheless, other organs have been indicated as targets of F toxicity. F excretion occurs mostly in urine and for this reason, kidneys are commonly exposed to high concentrations of F, thereby inducing deleterious effects like epithelial cell degeneration and interstitial nephritis. These effects are evidenced by the induction of several biomarkers of proximal tubular damage (NAG, GGT and KIM-1). The aim of this study was to establish if F-exposure cause alterations in cytokines profile and inflammation-related microRNAs in rat kidney. Recently weaned male Wistar rats were exposed to 15 and 50 mg F/L in drinking water for 40 days. Urine samples were obtained every 10 days for F determination as an exposure biomarker. At the end of the exposure, both kidneys were extracted and frozen, and afterwards protein and total RNA were obtained from renal cortex. A panel of inflammatory cytokines was evaluated using Luminex xMAP technology. Quantitative RT-PCR was performed for the analysis of a panel of inflammation-related microRNAs. The results showed an increment in TGF-β1 (p<0.05) as well as a decrement on TNF-α (p<0.05) in renal cortex in the group exposed to 50 mg F/L. The microRNAs panel presented more than a two-fold increment in the levels of mir-144-3p in both F-exposed groups. mir-144-3p is involved in the inhibition of NR2F2 expression, an antioxidant-related element. Moreover, mir-144 has been also related with an increase in TGF-β1 activity and expression. These observations suggest that strong oxidant properties could be related with mir-144 induction expression, it’s concomitant inhibition of NR2F2 and the increase of TGF-β1 expression and activity. Funded by CONACYT (Grant 152416).
Fluoride (F) is an inorganic ion that is usually found as salts and can be released into groundwater and contaminated drinking water. Several studies have shown that exposure to F can cause kidney damage through mechanisms of cellular toxicity (e.g. damage in the mitochondria, induction of ROS and apoptosis). However, after suffering damage from exposure to a toxic, kidney cells can trigger survival mechanisms such as autophagy. But, at present it is not known if fluoride is able to induce or to alter autophagy in renal cells. It was therefore proposed: (1) assess autophagy in NRK-52E cells exposed to sodium fluoride (NaF) for 24 h; (2) assess the autophagic response in cells deprived of nutrients for 6 h previously exposed to NaF for 18 h. Autophagy was determined using Monodansylcadaverine (MDC) and Light Chain-3 (LC3). Autophagy model was induced by nutrient deprivation and was characterized showing an effect time-dependent manner (P<0.001) in NRK-52E cells. When autophagy was determined in cells exposed to NaF for 24 hours, through LC3 marker, we observed a decrease in the levels of LC3-II as a result of high lysosomal activity. When we inhibited lysosomal degradation, at concentrations of 2 and 4 mM of NaF, observed an increase in the proportion LC3-II/I was observed. Which would indicate a decrease in the induc-treated cells in the range of 0 to 2 mM of NaF; a dose-dependent decrease of the indicates an induction of autophagy. Finally, we determined that in pre-fluoride toxicity (Eg damage in the mitochondria, induction of ROS and apoptosis). However, after exposure to F, kidney cells can trigger survival mechanisms such as autophagy. But, at present it is not known if fluoride is able to induce or alter autophagy in renal cells. It was therefore proposed: (1) assess autophagy in NRK-52E cells exposed to sodium fluoride (NaF) for 24 h; (2) assess autophagic response in cells deprived of nutrients for 6 h previously exposed to NaF for 18 h. Autophagy was determined using Monodansylcadaverine (MDC) and Light Chain-3 (LC3). Autophagy model was induced by nutrient deprivation and was characterized showing an effect time-dependent manner (P<0.001) in NRK-52E cells. When autophagy was determined in cells exposed to NaF for 24 hours, through LC3 marker, we observed a decrease in the levels of LC3-II as a result of high lysosomal activity. When we inhibited lysosomal degradation, at concentrations of 2 and 4 mM of NaF, observed an increase in the proportion LC3-II/I was observed. Which would indicate a decrease in the induction of autophagy. We conclude that fluoride can induce autophagy and is able to decrease the autophagic response in the pre-fluorode-treated cells. Funded by CONACyT (grant 152416).

Effect of Cadmium Exposure during Gestation over HIF-1 in Rat Fetal Kidney

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Cadmium (Cd) has been linked to the development of nephropathies and hyper-tension in the progeny of dams exposed during gestation. Although the underlying mechanism has not been elucidated yet, the Hypoxia-Inducible Factor 1 (HIF-1) might be involved since it controls gene expression in hypoxic conditions, such as those found in early developmental stages, and its activity in various cell lines is modified by Cd; however, the effect of an in utero Cd-exposure over HIF-1 and its target genes has not been studied yet. Pregnant Wistar rats were exposed by inhalation to isotonic saline solution (CT group) or CdCl2 solution (Cd group, Delivered Dose-2.2 mg Cd/kg/day) during gestational days (GD) 8-20. Another group was administered with losartan (LOS group, gavage, 100 mg/kg/day) the same GD to modify the expression and/or activity of HIF-1 in fetal kidneys. On GD21, fetal kidneys, and maternal livers, lungs, placenta and kidneys were ob-tained. Cd content in maternal organs was assessed and, as expected, the levels in the Cd group were higher than in the CT and LOS groups (P<0.05) showing the biodisponibility of the metal. Cd and losartan treatments caused low fetal weight (P<0.001) without changing kidneys relative weight. Also, the mRNA expression of HIF-1α, PHD2 and VEGF (target gene of HIF-1) was evaluated in fetal kidneys by qRT-PCR. Cd exposure decreased VEGF expression (55%, P<0.01) without altering HIF-1α or PHD2 expression. However, in the LOS group only HIF-1α was modified. Finally, the ability of HIF-1α to bind to its response element was eval-uated with a Luminescence-based assay (Procarta® FF-Plex kit) that showed a decreased activity of HIF-1α in fetal kidneys of dams exposed to Cd (37%, non-significant). Losartan treatment did not alter HIF-1α activity. These results suggest that Cd might affect kidney development by reducing VEGF expression. This alteration is partially explained by a decrease of HIF-1α activity, but other factors might be involved and need to be studied. Funded by CONACyT (grant 152416).

Resveratrol attenuates cisplatin cytotoxicity in HK-2 cells: Effects on TnFaLpha and Oxidative Stress

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Cisplatin is a chemotherapeutic agent used in treatment of various types of cancer. Treatment with cisplatin is associated with nephrotoxicity. The purpose of this study was to first evaluate cisplatin cytotoxicity using a human proximal tubular kidney cell line (HK-2) and further investigate whether resveratrol (RES) would reduce renal cytotoxicity. HK-2 cells were pretreated for 1 h with 0-20 μM RES (vehicle control 10% DMSO). Renal cells were next exposed to cisplatin at a final concentration of 0-60 μM for 24 or 48 h. Viability was assessed using MTT release. Cells pretreated with 10 μM RES were protected from cisplatin cytotoxicity at 24 and 48 h. Due to high cytotoxicity after 48 h exposure to cisplatin, additional studies examined only 24 h exposure to cisplatin. Cisplatin caused a concentration dependent decline in MTT viability after 24 h exposure to 0, 3.25, 7.5 and 15 μM cisplatin. RES pretreatment for 1 h with 10 or 15 μM RES increased HK-2 viability relative to cells exposed only to RES Vehicle (DMSO). Additional studies examined whether RES stimulated cell growth as part of the mechanism for cellular protection. Additional studies examined Caspase cleavage and protein carbonyla-tion following cisplatin exposure 24 h after cisplatin exposure in cells pretreated with DMSO or RES. Western blot using 20 μg protein loading showed an increase in protein carbonylation with 24 h cisplatin exposure which was reversed by RES. In summary, RES protects human proximal tubular epithelial cells from cisplatin cytotoxicity using concentrations similar to clinical levels. (Supported by NIH Grants INBRE 5P20RR016477-09S4; 5P20RR016477 and 8P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence).

SOMC2 Mediates Kidney Fibrosis via Activating Fibroblasts

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Fibrosis is the common final outcome of progressive chronic kidney disease (CKD), characterized by excessive deposition of extracellular matrix (ECM) materials by activated myofibroblasts. Using temporal RNA sequencing, we identified and confirmed SPARC related modular calcium binding 2 (SOMC2) to be amongst the highest upregulated genes in folic acid (FA)-induced progressive kidney fibrosis in

ERK1/2 Suppresses PGC-1α and Mitochondrial Biogenesis in Endotoxin and I/R-Induced AKI

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Acute kidney injury (AKI) is characterized by rapid loss of renal function and leads to substantial morbidity and mortality. Causes of AKI include ischemia/reperfusion (I/R), sepsis and drug toxicity. Our laboratory demonstrated that following I/R and endotoxin-induced AKI there is a persistent depletion of peroxisome pro-liferator-activated receptor gamma coactivator-1α (PGC-1α), a master regulator of MB, and mitochondrial proteins. The goal of this study was to determine the role of extracellular-regulated kinases 1/2 (ERK1/2) in the mitochondrial changes observed in mice subjected to bilateral I/R injury or exposed to endotoxin. In the I/R model, phospho-ERK1/2 to total ERK1/2 ratio increased 4-fold at 1, 3, and 24h post I/R. Phospho-ERK1/2 to total ERK1/2 ratio increased 3-fold at 1h after endotoxin exposure and remained elevated over 24h. Increased ERK1/2 activation was associated with decrease in mRNA levels of PGC-1α, NRF1, TFAM, and COX1. Renal dysfunction was observed, as indicated by BUN and KIM1 at 3h and 24h. The MEK1/2 inhibitor trametinib administered IP in mice 60min prior to I/R or endotoxin exposure inhibited ERK1/2 phosphorylation by 50% after 24 h after I/R and by 90% at 3h after endotoxin exposure. Trametinib treatment in both AKI models attenuated suppression of PGC-1α and renal dysfunction as measured by BUN and KIM1. Unexpectedly, trametinib administered to control mice increased mRNA of PGC-1α, NDUF51, and NRF1 at 4h. To study the role of ERK1/2 on mitochondrial homeostasis, rabbit renal proximal tubule cells (RPTC) were treated with 100nM trametinib. An increase in PGC-1α mRNA was noted at 1, 4, and 24h, with a 2.7 fold increase at 4h post-trametinib. NDUF51, NRF1, and TFAM were also increased at 4h and 24h after trametinib treatment of RPTC. These results demonstrate that ERK1/2 regulates renal PGC-1α, MB, and mitochondrial homeostasis under control conditions and that ERK1/2 activation following renal injury suppresses PGC-1α transcription and MB, and contributes to both mitochondrial and renal dysfunction.
mice over time (250 mg/kg ip, n=6/time point). SMOC2 increase was confirmed in another mechanistically distinct model of kidney fibrosis induced by unilateral ureteral obstruction (UUO) where SMOC2 mRNA increased 20 fold increase and its protein increased 40 fold over controls at day 7 following surgery. SMOC2 was also significantly increased and localized to the basolateral membrane of the proximal tubular epithelial cells in human kidney biopsy specimens from patients with kidney fibrosis. Urinary levels of SMOC2 increased 7.5 fold in patients with CKD as compared to patients without any evidence of kidney disease suggesting its biomarker potentials for detection of kidney fibrosis. SMOC2 overexpressing transgenic mice exhibited increased kidney wild type as compared to wild type in both models of kidney fibrosis (FA administration or UUO). Markers of fibrosis such as collagen, α-smooth muscle actin, fibronectin (by RT-PCR and immunoblotting) as well as histological indicator Sirius red were ~ 2-4 fold higher in the SMOC2 transgenic mice as compared to wildtype at 7 day. Mechanistically, overexpression of SMOC2 (or addition of 10 ng/ml recombinant SMOC2 protein) to fibroblasts resulted in activation (α-smooth muscle actin, F-Actin, and Collagen); proliferation (~150-200% increase measured by MTT and EdU) and also ~125-175% increase in migration (measured by scratch and tranwell assays). Thus, we conclude that SMOC2 plays a critical role in progression of kidney fibrosis and may be a potential biomarker as well as therapeutic target for kidney fibrosis.

2303 Altered slc6a20b Sequence in Chemokine Receptor D6 Knockout Mice May Be Related to Protection from Diabetic Nephropathy

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Renal inflammation is a contributor to diabetic nephropathy (DN). The scavenger receptor D6 binds and promotes degradation of inflammatory CC chemokines thus reducing chemokine levels and inflammation. Therefore, potentially D6 reduces DN. For studying the effect of D6 on DN, D6 knockout (KO) mice, originally created on the 129/sv background were crossed ten times to the FVB strain before crossing with diabetic OVE mice in our laboratory. We hypothesized more severe DN due to increased renal inflammation. Contrary to our expectations urine albuminuria, renal fibrosis and inflammation were greatly reduced in D6-KO diabetic mice. To explore possible mechanisms Affymetrix gene array was performed on kidney RNA. A cluster of 4 genes located within 1.5 Mb of the D6 gene showed strikingly downregulated in kidneys of D6-KO mice when compared to D6 wild type mice. Of the 4 genes, slc6a20b was most reduced and reduced expression was completely limited to exons4. Sequence of exons in FVB and D6-KO mice showed multiple nucleotides mutations. Three mutations located on the segment hybridized with array probe resulted in a poor hybridization but did not change protein coding. The other mutation produced a non-conservative codon change of slc6a20b protein from threonine to isoleucine which may alter posttranslational modifications. The gene mutation of slc6a20b in D6 knockout mice might be a potential biomarker for renal fibrosis.

2304 The Hedgehog-Gli1 Pathway Promotes Renal Fibrosis

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Chronic alcoholism induces hepatotoxicity; yet it is acute kidney injury that is an early predictor of mortality in patients with alcoholic hepatitis. We showed ethanol induces oxidative metabolism in kidney via CYP2E1, and causes renal inflammatory injury and development of fibrosis. The mechanism of ethanol-induced renal fibrosis remains unclear. We hypothesized that activation of Hedgehog signaling contributes to renal fibrosis after chronic ethanol ingestion. In this study we find that mice fed a Lieber-DeCarli liquid ethanol diet for 25 days had increased levels of renal dysfunctional markers such as blood urea nitrogen and serum creatinine along with increased infiltrating neutrophils and tubular apoptotic cells in kidney. Immunohistochemistry and mRNA levels revealed chronic ethanol ingestion in mice induces fibrotic markers such as α-smooth muscle actin, collagen I and IV in kidney. Similarly, the probiotic mediators Soni and Indian Hedgehog increased in kidney after chronic ethanol exposure. This resulted in urinary Sonic Hedgehog in mice fed with ethanol diet relative to pair-fed control. We also find increased urinary Sonic Hedgehog in hospitalized patients with alcoholic hepatitis. β-Galactosidase staining of LacZ, knock-in reporter mutant mice for Gli1 (Gli1tm2Alji), clearly shows chronic ethanol feeding induces Sonic Hedgehog signaling in kidney. These results suggest that activation of Hedgehog-Gli1 pathway promotes ethanol-induced renal fibrosis, and urinary Sonic Hedgehog could be a potential biomarker for renal fibrosis.

2305 Effects of Pregnancy and Renal Insufficiency on the Disposition of Mercuric Ions

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Pregnant women with chronic kidney disease (CKD) represent a significant population of adults in the United States. Normal pregnancy is associated with increases in renal plasma flow (RPF) and glomerular filtration rate (GFR). In pregnant women with CKD, the normal increase in RPF and GFR is attenuated, and RPF and GFR decrease as CKD worsens. These reductions could lead to an increased body burden of toxicants or other xenobiotics to which the mother is exposed. The fetus of a pregnant woman suffering from CKD may be more susceptible to maternal exposure to a toxicant. A toxicant of particular concern is methylmercury because of its prevalence in the environment and its known reproductive toxicity. Pregnant women with CKD may be less able to excrete mercuric ions and thus the burden of mercury in fetuses from these mothers may be greater than that of fetuses from healthy mothers. In the current study, we utilized 50% and 75% nephrectomized female Wistar rats as models of reduced renal mass. Three weeks after surgery, rats were mated and pregnant females were exposed to a non-nephrotoxic dose of methylmercury (either chronically or acutely). The disposition and toxicity of mercury was subsequently assessed in dams and pups. Significant changes in the disposition of mercury were observed between pregnant and non-pregnant rats as well as between sham and nephrectomized rats. The burden of mercury in fetal and placental tissues was also altered by nephrectomy. These data suggest that pregnancy and CKD can have significant effects on the handling and disposition of toxicants.

2306 Fenofibrate Uregulates FGF21 and Nrf2 Function to Prevent Diabetic Nephropathy

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Lipid lowering medicine Fenofibrate (FF) is a peroxisome proliferator-activated receptor α (PPARα) agonist and shows beneficial effects on diabetic complications, such as the amelioration of diabetic nephropathy, along with anti-oxidative effect. However the underlying mechanisms had not been understood well. This study was designed to investigate FF protective effects on diabetic nephropathy and its underlying mechanisms. Diabetes was induced with a single intraperitoneal injection of streptozotocin in male C57BL/6J mice. Diabetic and age-matched non-diabetic control mice were treated with or without FF at 100 mg/kg by gavage every other day for 3 and 6 months. Diabetes induced progressively renal oxidative damage, inflammation response, apoptotic cell death, lipid and collagen accumulation, and dysfunction, accompanying with significant decreases in Akt and GSK-3β phosphorylation. All these diabetic effects were significantly prevented by FF treatment. We also found significant increases in renal fibroblast growth factor 21 (FGF21) expression and nuclear factor erythroid 2-related factor (Nrf2) expression and function in FF-treated mice. Moreover, the renal protection in diabetes and up-regulation of renal Nrf2 expression were abolished or diminished in FGF21 gene deletion (FGF21-null) diabetic mice compared to the wild-type (WT) diabetic mice, suggesting that the FGF21-mediated Nrf2 expression and function is required for the renal protection in diabetes by FF. Further mechanistic study showed that FF treatment stimulated the phosphorylation of Akt and GSK-3β and decreased the nuclear accumulation of Fyn (a Nrf2 negative regulator), along with increased expressions of Nrf2 downstream antioxidants at mRNA levels. These results suggest that FGF21 is required for FF to prevent diabetic nephropathy, which may be mediated by up-regulating Nrf2 function via Akt/GSK-3β-mediated reduction of Fyn’s nuclear accumulation.
2307 Hepaticocyte-Targeted Deletion of Aryl Hydrocarbon Receptor Increases Hepatic Energy Storage and Alters Energy Homeostasis


The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor with an established role in mediating TCDD toxicity. In mice, global AHR deletion has revealed its involvement in numerous biological pathways. More recent evidence indicates the AHR also plays a role in metabolic homeostasis. For example, we previously demonstrated that AHR attenuates de novo cholesterol/fatty acid synthesis in the liver. However, the homeostatic role of AHR in the absence of exogenous ligand remains largely unknown. Here, we examined the effects of hepaticocyte-targeted AHR deletion on energy homeostasis using a conditional AHR knockout mouse model Cre-AllAhr-Fx/Fx. When maintained on a defined diet, Cre-AllAhr-Fx/Fx mice exhibit no difference in body composition and display similar serum chemistry compared to the parental strain (Ahr-Fx/Fx). However, NMR analysis reveals a significant difference in the relative abundance of several liver metabolites between these two species. In particular, Cre-AllAhr-Fx/Fx mice exhibit a 26% decrease in p-hydroxybutyrate, a 13% decrease in lactate, and a 30% decrease in acetate levels in comparison to Ahr-Fx/Fx mice. Histological analysis of liver sections from these mice further shows that glycogen and lipid storage is increased in Cre-AllAhr-Fx/Fx mice. Specifically, glycogen stores are more than 3-fold higher, while triglyceride content is 50% higher than in Ahr-Fx/Fx mice. We also examined the effects of long-term, high-fat diet feeding in these two strains of mice. Cre-AllAhr-Fx/Fx mice demonstrate a level of resistance to the increases in liver and body weight that are associated with increased dietary fat. In summary, our data demonstrates that AHR plays a role in energy homeostasis in the absence of exogenous ligands. We hypothesize that AHR activation by ligands derived from the diet and/or those produced from the gut microbiota may therefore play an integral role in maintaining energy homeostasis. This project is supported by NIH grants ES004869 and ES019964.

2308 Characterization of Human and Mouse PXR- and CAR-Mediated Transcriptional Activation by Bisphenol A and Its Related Compounds

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Pregnane X receptor (PXR) and constitutive androstane receptor (CAR), which are members of the nuclear receptor superfamily, are involved in the gene regulation of xenobiotic metabolism, and it is known that there are species differences between humans and rodents on their transcriptional activation induced by ligands. Bisphenols are a group of chemicals structurally similar to bisphenol A (BPA) in current use as the primary raw material in the production of polycarbonate and epoxy resins. In this study, using cell-based transactivation assays, we characterized the agonistic and/or antagonistic activities of BPA and its related compounds against human and mouse PXR or CAR. In the PXR assays, nine of the 19 compounds showed hPXR agonistic activity in a dose-dependent manner, and 1,1’-bis(4-hydroxyphenyl)cyclohexane (BPCH) was the most potent hPXR activator among them. However, none of test compounds showed any mPXR agonistic activity. In the CAR assays, eleven of the 19 compounds showed inhibitory effects on hCAR-mediated basal activation, and namely acted as hCAR inverse agonists. In particular, the hCAR inverse agonistic activities of BPA, bisphenol B (BPB), bisphenol AF, BPCH, and bisphenol A-dimethyl were more potent than those of other compounds. On the other hand, in the mCAR assay, only three compounds showed weak CAR inverse agonistic activity with the most potent activity of 3,3’,5,5’-tetramethyl bisphenol A. Taken together, these results suggest that BPA and its related compounds have species differences on PXR and CAR activation. This also provides the first evidence that several BPA derivatives, including BPA, BPB and BPCH, have pleiotropic activity via PXR and CAR in humans.

2309 The In Vitro and In Vivo Response to PPARα Activation in Rats

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In vivo, sustained PPARα activation leads to tumors in rats, but not humans. To better understand this mechanism, we cultured primary rat hepatocytes and exposed them to the PPARα-selective agonist GW7647 over a range of 3 concentrations (0.001–10μM) and assessed gene expression via microarray after 5 intervals of exposure (2–72h). Additionally, we performed an in vivo study in which male rats were exposed to GW7647 via oral gavage for 4 days across three doses. Analytical chemistry confirmed that GW7647 serum concentrations of treated animals are dose dependent and complementary to the concentrations used for our in vitro experiments. We observed a dose-dependent increase in liver weight in treated animals, but serum ALT levels were only slightly increased, indicating the absence of overt hepatotoxicity. Cell division was increased in the livers of treated animals. Lower doses of GW7647 that did not cause significant increases in proliferation in vivo produced gene expression profiles that were similar to those observed at in vitro concentrations of 1μM, showing upregulation of β-oxidation enzymes and other lipid metabolism machinery. High in vivo exposures, however, led to activation of additional biological functions, including genes associated with mitosis and cell-cycle checkpoint control. Immunohistochemical staining suggests that induction of peroxisome-specific markers in response to PPARα activation is primarily a centriolobular phenomenon. We used laser capture microdissection to separate perportal, midzonal, and centrilobular tissues from GW7647-treated rats and are using microarrays to determine regional differences in the response to PPARα activation. Purified rat hepatocytes do not proliferate in response to PPARα activation, supporting the model that hepatic proliferation requires mitogenic signaling from non-parenchymal cells. We are using this information to design better in vitro testing strategies for nuclear-receptor-mediated proliferation.

2310 Genome-Wide Analysis of Aryl Hydrocarbon Receptor (Ahr)- and AhR Repressor (AhRR)-Bound Regions in Dioxin-Treated MCF7 Cells

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The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor that mediates the toxic effects of various environmental contaminants, including 2,3,7,8-tetrachlorodibenzo-p-dioxin (dioxin; TCDD). After binding ligand, the AHR translocates to the nucleus and heterodimerizes with AHR nuclear translocator (ARNT). The activated complex binds to aryl hydrocarbon response elements (AHREs; 5’-GCGTG-3’) located in the regulatory regions of its target genes. The AHR repressor (AhRR) is an AHR target gene and functions as a ligand-induced repressor of AHR. However, its mechanism of repression is controversial and very little is known about AHRR signaling that is independent of AHR and vice versa. To gain insight into the unique signaling differences between AHR and AHRR, and to unravel the mechanism of AHR-dependent repression of AHR, we determined the DNA binding profiles of AHR and AHRR in MCF7 human breast cancer cells treated with 10 nM TCDD for 24h. A 24h treatment was needed to induce AHR protein expression in MCF7 cells. Using chromatin-immunoprecipitation sequencing (ChIP-Seq), we identified 6850 AHR-bound regions and 7405 AHRR-bound regions with 1494 regions bound by both proteins. These regions corresponded to 3079 AHR-bound genes, 3643 AHRR-bound genes and 1625 genes common to both. AHRR-bound regions mapped significantly closer to the transcription start sites than did AHR-bound sites, suggesting the AHRR preferentially binds to promoter regions. De novo DNA binding motif analysis of the top 300 regions bound by AHR or by AHRR identified a sequence that was identical to the AHRE sequence. Within the genes that were uniquely bound by AHRR, we identified and confirmed AHRR binding to XRCGC6 and MAP2K7. Both genes are potential tumor suppressors, suggesting that they might mediate the tumour suppressive action of AHRR. In summary, we identified shared but also unique genomic regions bound by AHR and AHRR, supporting the notion that AHRR may regulate distinct genes and signaling pathways compared with AHR.
Role of the Aryl Hydrocarbon Receptor in the Development and Function of Breast Cancer Stem Cells

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Self-renewing, chemoresistant cancer stem cells (CSCs) are believed to contribute significantly to cancer metastasis and patient relapse. Therefore, the identification of signaling pathways regulating CSC development and function is an important step towards understanding why patients relapse and development of novel therapeutics, which specifically target CSCs. Recent studies indicate a role for the aryl hydrocarbon receptor (AHR), an environmental chemical receptor implicated in cancer, in tissue-specific stem cell self-renewal. These observations inspired the hypothesis that the AHR plays a role in CSC development. To test this hypothesis, AHR activity in aggressive, Hs578T triple negative and SUM149 inflammatory breast cancer cells were modulated with environmental or putative endogenous AHR ligands, shRNA, or AHR-specific inhibitors, and phenotypic, genomic, and functional CSC characteristics evaluated. Data presented demonstrate that: 1) ALDHHigh cells express elevated levels of Ahr and the AHR-driven gene, Cyp1b1. 2) AHR knockdown reduces ALDH activity. 3) AHR hyper-activation with ligands, including 2,3,7,8-tetrachlorodibenzo(p)dioxin, significantly increases ALDH activity and expression of genes critical to self-renewal, pluripotency, and metastasis. 4) The AHR interacts directly with Sox2, a master regulator of self-renewal, and this interaction is increased with exogenous AHR ligands. 5) Tumorsphere formation is inhibited by AHR knockdown. 6) AHR inhibition blocks the rapid migration of ALDHHigh cells and reduces ALDHHigh cell chemoresistance. 7) AHR knockdown inhibits tumor growth and reduces tumor Aldh1a1, Sox2, and Cyp1b1 expression in an orthotopic xenograft model. These data demonstrate that the AHR plays an important role in CSC development and imply that environmental AHR ligands may exacerbate cancer by driving CSC production. These data also suggest AHR targeting for treatment of aggressive, triple negative breast cancers.

Nuclear Receptor 4A1 (NR4A1) As a Drug Target for Breast Cancer Chemotherapy

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The orphan nuclear receptor 4A1 (NR4A1) is overexpressed in mammary tumors and breast cancer cell lines. The functional activity of this receptor was investigated by treating with RNAi (siNR4A1) and by treatment with NR4A1 antagonists. Upon treatment with NR4A1 antagonists or siNR4A1 transfection, effects on cell proliferation and apoptosis were determined in MCF-7, SKBR3 and MDA-MB-231 cells. Effects on NR4A1-regulated genes/pathways were determined by western blots of cell lysates and tumor growth inhibition by NR4A1 antagonists was investigated in athymic nude mice bearing MDA-MB-231 cells as xenografts. Transfection of the three breast cancer cells with siNR4A1 decreased cell proliferation and induced apoptosis. Decreased expression of Sp-regulated genes (survivin, bel-2, and EGFR), inhibition of mTOR signaling in MCF-7 cells (wild-type p53), and activation of oxidative and endoplasmic reticulum stress through downregulation of thioredoxin domain-containing 5 (TXNDC5) and isocitrate dehydrogenase 1 (IDH1), resulting in ER oxidative stress. Increased oxidative stress also increased sestrin2, which in turn activated AMPK to inhibit mTOR signaling. Thus, NR4A1 antagonists block NR4A1-regulated pro-oncogenic pathways in RMS, and represent a potential clinical approach for this disease.

Nuclear Receptor 4A1 (NR4A1) is a Drug Target for Treating Rhabdomyosarcoma

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Rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma primarily observed in children and adolescents and accounts for 5% of all pediatric cancers and 50% of soft tissue sarcomas in children. Some RMS patients can be successfully treated with surgery and cytotoxic drug combinations; however there are several long-term adverse health effects of these drugs. Therefore, the development of relatively non-toxic, RMS mechanism-based agents is essential. Data mining of RMS patient array data indicates orphan receptor, NR4A1, expressed in the tumors as well as detection in RMS cell lines, including RD and RH30 cells, which represent embryonal and alveolar RMS tumors, respectively. The function of this receptor in RMS and RH30 cells was investigated by RNAi (siNR4A1), which shows a significant decrease in cell growth and induction of apoptosis as evidenced by increased Annexin V staining. Recent studies in this laboratory have shown that among a series of 1,1-bis(3’-indolyl)-1-(p-substituted phenyl) methane (C-DIM) analogs, the p-hydroxyphenyl (DIM-C-pPhOH) and p-carboxymethylphenyl (DIM-C-pPhCO2Me) analogs bind to the ligand binding domain of NR4A1 and exhibit antagonist activity. Treatment of RD and RH30 cells with DIM-C-pPhOH and DIM-C-pPhCO2Me inhibited cell growth and induced apoptosis; these effects were accompanied by decreased expression of several specificity protein (Sp1)-regulated genes, including EGFR, c-Myc, Bel-2, and cyclin D1. These results are consistent with previous studies showing NR4A1-Sp1-mediated gene regulation. NR4A1 antagonists also decreased expression of thioredoxin domain-containing 5 (TXNDC5) and isocitrate dehydrogenase 1 (IDH1), resulting in ER oxidative stress. Increased oxidative stress also increased sestrin2, which in turn activated AMPK to inhibit mTOR signaling. Thus, NR4A1 antagonists block NR4A1-regulated pro-oncogenic pathways in RMS, and represent a potential clinical approach for this disease.

Functional Differences among Polymorphic TCDD-Inducible Poly-ADP-ribose Polymerase (TIPARP) Variants

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TCDD-inducible poly-ADP-ribose polymerase (TIPARP) is an aryl hydrocarbon receptor (AHR) target gene and a member of the poly-ADP-ribose (ADPR) polymerase (PARP) family that transfers ADPr onto target proteins. The majority of PARPs transfer mono-ADPr (MAR), and not poly-ADPr (PAR), to their substrates. ADP-ribosylation is involved in several biological processes, such as immune cell function, the regulation of transcription, and DNA repair. We have previously shown that TIPARP MARYlates both itself and core histones, and as part of a negative feedback loop it represses AHR activity by increasing its proteolytic degradation. Based on these findings, we support the view that TIPARP is a key component and regulator of AHR signaling pathways and that any changes in TIPARP activity will impact AHR function or sensitivity to TCDD toxicities that are mediated by AHR. A number of single nucleotide polymorphisms (SNPs) in the human TIPARP gene have been identified; however, the impact of these SNPs on TIPARP activity and its ability to repress AHR function has not been investigated. In this study we determined the effect of 21 TIPARP SNPs, most of them located within known functional domains of TIPARP including 6 in its catalytic domain, on TIPARP activity and its ability to repress AHR function. Using an ADP-ribosylation assay, we observed that some of the TIPARP variants displayed reduced or increased catalytic activity compared with wild-type. Small, but significant differences, in the ability of different polymorphic TIPARP variants to repress AHR-dependent CYP1A1-regulated reported gene activity were also observed. For example, F405, located in the nuclear localization signal, increased the ability of TIPARP to repress AHR, while M558T, located in the catalytic domain, decreased its ability to repress AHR. Collectively, our data suggest that differences in TIPARP activity and variation in TIPARP function in humans and among other species may influence their sensitivity to environmental contaminants, including TCDD.
The orphan nuclear receptor NR4A1 exhibits pro-oncogenic activity in solid tumors and the main objectives of this study were to (a) determine expression of NR4A1 in renal cell adenocarcinoma (RCC) ACHN and 786-O cell lines and tumors and the main objectives of this study were to (a) determine expression of NR4A1 in renal cell adenocarcinoma (RCC) ACHN and 786-O cells were transfected with siNR4A1 (RNAi), and effects on cell proliferation and apoptosis were determined. The antineoplastic activities of 1.1-bis(3-indolyl)-1-(p-substituted phenyl)methane (C-DIM) NR4A1 antagonists on RCC cell growth, apoptosis and related pathways/gene expression were also determined by western blots and compared to effects of siNR4A1. In vitro effects were investigated in a mouse xenograft model. Transfection of ACHN and 786-O cells with siNR4A1 resulted in a 40-60% decreases in cell proliferation and induction of apoptosis. Moreover, siNR4A1 in RCC cells decreased bcl-2, survivin and EGFR expression, inhibited mTOR signaling, induced oxidative and endoplasmic reticulum stress, and decreases TXNDC5 and IDH1. C-DIM compounds including the p-hydroxyphenyl(DIM-C-pPhO) and p-carboxymethyl(DIM-C-pPhCO2Me) analogs that are NR4A1 antagonists that bind NR4A1, also inhibited growth of cells and tumors and induced apoptosis in RCC cells. The functional and genomic effects of the NR4A1 antagonists were comparable to those observed after NR4A1 knockdown, indicating that NR4A1 antagonists target multiple growth promoting and pro-survival pathways in RCC cells and tumors including their activity as mTOR inhibitors, thus representing a novel chemotherapeutic approach for treating RCC.

The gut microbiota impacts persistent organic pollutant-associated inflammation via anaryl hydrocarbon receptor-dependent mechanism

2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD), a potent aryl hydrocarbon receptor (AhR) ligand, is among the most environmentally toxic compounds and its impact on the gut microbiome and host metabolism relatively unknown. In this study, wild type, Ahr-null and gnotobiotic (germ free) male C57Bl/6j mice were treated with and without TCDD for five days at 24 μg/kg body weight. Metagenomics, 1H nuclear magnetic resonance (NMR) metabolomics, and biochemical assays were used. Weighted UniFrac principal coordinate analyses of 165 rRNA gene sequencing results indicated that TCDF exposure induced a remarkable change in the overall gut microbiome population whereas no significant changes were observed between vehicle- and TCDD-treated Ahr-null mice. Firmicutes and Bacteroidetes exhibited significant changes with a significant reduction of the Firmicutes/Bacteroidetes ratio after TCDD exposure. Interestingly, we found significant elevation of inflammatory factors in serum including C3-CSF, Entaxin, IP-10, MCP-1, IL-6 and IL-12 in TCDD-treated gnotobiotic mice compared to vehicle-treated gnotobiotic mice whereas no significant differences were observed between vehicle- and TCDD-treated conventional mice. Further, 1H NMR-based metabolomics results showed that TCDF led to hepatic lipogenesis and inhibition of gluconeogenesis and glycogenolysis in wild type mice and these changes were AhR-dependent. Further, significant elevation of hepatic glutathione, glucose and glycogen but reduction of lactate and amino acids was observed in TCDD-treated gnotobiotic mice suggesting oxidative stress and inhibition of glycolysis induced by TCDD. These findings provide new evidence that exposure to persistent organic pollutants strongly impacts the gut microbiome, host inflammation, and the metabolome.
The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor that mediates toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and structurally related compounds. AHR also functions in endogenous processes, including cardiovascular, hepatic, and immune system development. Humans have a single AHR gene. In contrast, the African clawed frog (X. laevis) expresses AHR1α and AHR1β, paralogous proteins resulting from a genome duplication ~40 million years ago. X. laevis AHR1s exhibit 86% amino acid identity and different expression patterns in vivo. We hypothesize that X. laevis AHR1s exhibit distinct transcriptional regulatory functions in xenobiotic toxicity or development. As one test of this hypothesis, we used transcription activator-like effector nucleases (TALENs) to edit the genome of the frog cell line XLK-WG, generating strains lacking functional AHR1α or AHR1β. Both AHR1s were required for maximal induction of the prototypical target gene Cytochrome P450 1A6 (CYP1A6). Compared to wild type cells exposed to 100 nM TCDD, CYP1A6 mRNA induction was reduced 81% in AHR1α cells and 87% in cells lacking functional AHR1β. The large effect of the AHR1β mutation is surprising, since expression of AHR1α mRNA and protein is at least 3-fold greater than AHR1β in wild-type cells. In the AHR1β mutant, the dramatic effect from the modest reduction in overall AHR content suggests that XLK-WG cells largely lack “spare” AHRs for this response. These mutant cell lines will enable the use of RNA-seq to characterize the role of each AHR paralog in regulation of TCDD-responsive genes on the genomic scale. Dissection of the functions of the two X. laevis AHR paralogs will ultimately contribute to the understanding of the pleiotropic roles of the single human AHR. [NIH R15 ES011130]

### 2320 Interactions between Thyroid Hormone and Dioxin Signaling in the Frog Xenopus laevis

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Dioxin-like compounds are environmental contaminants that elicit toxic effects in vertebrates, including developmental defects, endocrine disruption, and death. Toxicity results from binding to the aryl hydrocarbon receptor (AHR) and subsequent alterations in gene expression. Exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD or dioxin) has been associated with Graves Disease and hyperthyroidism in adults. However, the mechanism of dioxin effects on TH function during development is less well understood. Tadpole metamorphosis is a developmental process driven by TH. We used tadpoles of the African clawed frog (Xenopus laevis) and an X. laevis cell line (XLK-WG) as models to examine effects of dioxin exposure on TH function at the molecular, cellular, and organismal levels. Our results suggest the possibility of a functional interaction between the thyroid hormone receptor (TR) and AHR signaling pathways. In cells, expression of Cytochrome P450 1A6 (CYP1A6) a well-characterized AHR target gene, was induced at least 300 fold by 100 nM TCDD. The primary TH target gene, Kruppel-Like Factor 9 (KLF9) was induced 5-10 fold by 50 nM TH and approximately 2 fold by dioxin, which increased target gene induction following co-exposure of XLK-WG cells to TH and TCDD occurred in the absence of serum in culture media. Therefore, this phenomenon was not due to lack of interaction with serum-binding proteins and resulting changes in bioavailability of these compounds. Additionally, target gene induction was sensitive to TR- or AHR-specific antagonists (1-850 and SR1, respectively), demonstrating that transcriptional effects of dioxin and TH co-exposure depend on TR and AHR agonism. These results suggest that the thyroid hormone and dioxin signaling pathways may interact to enhance upregulation of genes induced by either TH or TCDD alone. Preliminary results in zebrafish cultures suggest that TCDD may hasten tail resorption during metamorphosis. [NIH R15 ES011130]
immediately after dosing in addition to regular feed. Chocolate consumption was measured at pre-designed intervals and compared with the control group that was treated with the vehicle (sunflower oil + 2.5%/6% DMSO). As hypothesized, all of the AHR activators induced practically total aeration to novel chocolate. However, the duration of the aeration varied: FCZC – 24 hours, β-naphthoflavone – 47 hours and benzo[a]pyrene – 71 hours. In contrast, there was no statistically significant difference in chocolate consumption between the control and the AHR antagonist or TPD groups; all consumed –6 g of chocolate by 24 hours after exposure. In individual rats, fading of the aeration did not consistently coincide with subsidence of the hepatic enzyme induction. These findings indicate that avoidance of novel foodstuffs is a behavioral change in rats triggered not only by TCDD but also by other AHR activators, with its duration apparently depending on the half-life of the agonist. However, they do not support a tight link between the aeration and the hepatic enzyme induction responses.

The adverse effects of dioxins and related compounds (DRCs) are mediated largely by its binding to the aryl hydrocarbon receptor (AHR). Our previous study reported that the red seabream (Pagrus major) has two AHR isoforms denoted as AHR1 and AHR2 (Yamauchi et al. 2006). Moreover, we found that AHR2 mRNA level was elevated by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) exposure in the early life stage of red seabream embryos, while AHR1 mRNA level was not altered. In this study, to investigate the regulatory mechanism of these AHR transcripts, we cloned each 5′-flanking regions of AHR1 and AHR2 genes. Both the 5′-flanking regions of AHR1 and AHR2 genes contained 3 potential XRE-like sites (XRELs). We measured the transactivation potency of the 5′-flanking regions of AHR1 and AHR2 genes in AHR1/2-driven reporter gene assay. Only AHR2 XRELs-containing reporter plasmid showed a clear TCDD dose-dependent transactivation by AHR1 and AHR2 that were transiently expressed in COS-7. This result suggests that AHR2 XREs have a functional to mediate AHR2/mediated transactivation, supporting in vivo induction of AHR2 mRNA levels by TCDD exposure. TCDD-Ec50 values for the AHR2 XREL-driven transactivation by AHR1 (1.3nM) and AHR2 (1.4nM) were higher than in vivo TCDD-Ec50 (0.30 nM) for cytochrome P450 1A (CYP1A) mRNA induction, but were closer to in vivo TCDD-Ec50 (1.5 nM) for AHR2 mRNA induction. Mutations in XRELs of AHR2 promoter led to decreased luciferase induction. Electrophoretic mobility shift assay showed that each of 3 XREL in AHR2 gene has a potency to bind TCDD-activated AHR1 and AHR2. This suggests that TCDD-activated AHR1/2 up-regulate the AHR2 mRNA levels and this auto-induced AHR2 may amplify the signal transduction of its downstream targets including CYP1A in the red seabream.

Esosomes are naturally occurring small membrane enclosed nanovesicles generated and released constitutively by various cell types, and are more frequently released by tumor cells. The normal functions of exosomes include transporting proteins, nucleic acids and may facilitate communication between cells within the local microenvironment. Recently, several reports postulated the involvement of exosomes in tumor cell metastases including melanoma. Metabolic glutamate receptor 1 (GRM1), a neuronal receptor, when ectopically expressed in melanocytes, is sufficient to induce melanocyte transformation in vitro and spontaneous malignant melanoma development in vivo in a transgenic mouse model. GRM1-transformed melanocytes exhibited abundant level of exosomes compared to normal melanocytes as measured by electron microscopy, CD-63, a protein marker of exosomes, and Nanosigart. Similar results were observed in GRM1-expressing human melanoma cells. Modulation of GRM1 expression levels by genetic (via silencing RNA) or pharmacological (inhibitor of GRM1-mediated glutamate signaling) means led to a reduction in cell growth in vitro and tumor progression in vivo. Furthermore, concurrent reduction in exosomal production/secretion was observed, while an increase in GRM1 expression by exogenous GRM1 cDNA resulted in parallel enhancement in exosomal production/secretion. Possible involvement of exosomes in promoting the metastatic phenotypes detected in our melanoma prone transgenic mice provided opportunities to explore this notion directly. Whether GRM1 regulates exosome production directly, via its signaling cascade, or by another route, is currently being investigated.

Esosomes naturally occurring small membrane enclosed nanovesicles generated and released constitutively by various cell types, and are more frequently released by tumor cells. The normal functions of exosomes include transporting proteins, nucleic acids and may facilitate communication between cells within the local microenvironment. Recently, several reports postulated the involvement of exosomes in tumor cell metastases including melanoma. Metabolic glutamate receptor 1 (GRM1), a neuronal receptor, when ectopically expressed in melanocytes, is sufficient to induce melanocyte transformation in vitro and spontaneous malignant melanoma development in vivo in a transgenic mouse model. GRM1-transformed melanocytes exhibited abundant level of exosomes compared to normal melanocytes as measured by electron microscopy, CD-63, a protein marker of exosomes, and Nanosigart. Similar results were observed in GRM1-expressing human melanoma cells. Modulation of GRM1 expression levels by genetic (via silencing RNA) or pharmacological (inhibitor of GRM1-mediated glutamate signaling) means led to a reduction in cell growth in vitro and tumor progression in vivo. Furthermore, concurrent reduction in exosomal production/secretion was observed, while an increase in GRM1 expression by exogenous GRM1 cDNA resulted in parallel enhancement in exosomal production/secretion. Possible involvement of exosomes in promoting the metastatic phenotypes detected in our melanoma prone transgenic mice provided opportunities to explore this notion directly. Whether GRM1 regulates exosome production directly, via its signaling cascade, or by another route, is currently being investigated.

The aryl hydrocarbon receptor (AhR) is a ligand-dependent bHLH-PAS containing transcriptional factor that mediates toxic and biological effects of structurally diverse chemicals, including 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). The AhR PASB domain is involved in multiple steps of AhR activation including ligand binding, hsp90 binding and dimerization with ARNT. Functional activities of the AhR were systematically compared across a panel of the AhRs containing PASB mutations, with the goal of generating functional sequence maps for various steps of the mechanism of AhR activation. Ligand binding and ligand-dependent DNA binding (in the presence of ARNT) were analyzed with the in vitro synthesized AhR using hydroxypatite binding and gel retardation assays, respectively, while hsp90 binding was analyzed by co-immunoprecipitation of AhRs from transiently transfected COS-1 cells. Effects on AhR/ARNT dimer formation were estimated through outlitter analysis of ligand-binding/DNA binding plots. Ligand-selectivity of AhR mutants was also evaluated using a representative panel of 12 structurally diverse AhR ligands. Based on mutational analysis, multiple PASB residues were identified that affected ligand binding, ligand selective AhR activation, hsp90 binding and ARNT dimerization. PASB mutations that affected TCDD binding affinity all belonged to the TCDD fingerprint residues proposed by the PASB homology model. Several residues were identified as involved in hsp90 binding and these residues significantly overlapped with those involved in ligand-selective AhR activation, suggesting a role for hsp90 binding in the latter mechanism. Lastly, a single PASB residue was shown to affect AhR dimerization with ARNT (Asp371), and this residue did not overlap with any other functional PASB maps suggesting that the PASB-dependent mechanism affecting AhR/ARNT dimerization was distinct from other PASB-mediated functional steps in AhR activation. (NIEHS ES007685 & ES004609).

The aryl hydrocarbon receptor (AhR) is a ligand-dependent bHLH-PAS containing transcriptional factor that mediates toxic and biological effects of structurally diverse chemicals, including 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). The AhR PASB domain is involved in multiple steps of AhR activation including ligand binding, hsp90 binding and dimerization with ARNT. Functional activities of the AhR were systematically compared across a panel of the AhRs containing PASB mutations, with the goal of generating functional sequence maps for various steps of the mechanism of AhR activation. Ligand binding and ligand-dependent DNA binding (in the presence of ARNT) were analyzed with the in vitro synthesized AhR using hydroxypatite binding and gel retardation assays, respectively, while hsp90 binding was analyzed by co-immunoprecipitation of AhRs from transiently transfected COS-1 cells. Effects on AhR/ARNT dimer formation were estimated through outlitter analysis of ligand-binding/DNA binding plots. Ligand-selectivity of AhR mutants was also evaluated using a representative panel of 12 structurally diverse AhR ligands. Based on mutational analysis, multiple PASB residues were identified that affected ligand binding, ligand selective AhR activation, hsp90 binding and ARNT dimerization. PASB mutations that affected TCDD binding affinity all belonged to the TCDD fingerprint residues proposed by the PASB homology model. Several residues were identified as involved in hsp90 binding and these residues significantly overlapped with those involved in ligand-selective AhR activation, suggesting a role for hsp90 binding in the latter mechanism. Lastly, a single PASB residue was shown to affect AhR dimerization with ARNT (Asp371), and this residue did not overlap with any other functional PASB maps suggesting that the PASB-dependent mechanism affecting AhR/ARNT dimerization was distinct from other PASB-mediated functional steps in AhR activation. (NIEHS ES007685 & ES004609).
The constitutive androstane receptor (CAR) is a key nuclear receptor that transcriptionally regulates diverse biochemical and physiological processes, including bile acid and xenobiotic metabolism, as well as lipid and energy homeostasis. Many of these CAR-regulated functional activities appear conserved between rodent and humans. However, CAR activation in mice, by direct ligands or indirect activators such as phenobarbital (PB), is causally linked to the development of hepatocarcinogenesis, while these processes do not appear to occur in humans. These observations suggest species-specific genomic and regulatory interactions for CAR. In this study, we used an adenosine delivery system to humanize liver hepatocytes in CAR knockout mice with human CAR. The human CAR constructs were fused with a YFP tag, allowing their visualization and also chromatin immunoprecipitation (ChIP) of nuclear CAR. We performed deep sequencing of the ChIP’d samples on an Illumina HiSeq platform. Thousands of CAR genomic targets were identified, including regulatory regions of genes that encode processes related to all three phases of biotransformation, and an expanded array of CAR gene targets related to energy and cholesterol metabolism. For example, we identified several strong binding sites for human CAR within the 15kb upstream region of mouse cytochrome P450 4A1 (Cyp4a1) and mCyp4a1 activation of the CYP4A xenobiotic response element module (XREM), and direct repeat (DRx53) DNA response element reporter at equal levels when co-expressed with the heterodimer partners, hRXRα, hRXRβ and mRXRα. Lower activation was observed with hRXRγ. With the DRx43 DNA response element reporter, hCAR1 and 2 exhibited increased activity in co-transfection with hRXRα, whereas equal levels of activity were achieved with hRXRα, hRXRβ, and mRXRα. hCAR3 activation of the DRx43 reporter was similar across all of the RXRs. mCAR activated 2B6XREM, DRx43, and DRx53 reporters at equal levels when co-expressed with hRXRα and mRXRα. Interestingly, mCAR with mRXRα activated the 3A4XREM reporter at higher levels than when co-expressed with hRXRα. The results of this study demonstrate that hCAR and mCAR differentially interact with DNA response elements, and suggest that hCAR and mCAR may have distinct genomic targets.
by co-expressing a GAP-tagged p100 or Cherry-tagged RelB in combination with the ARNT isoforms in HEK 293T cells. Remarkably, we found that both ARNT isoforms are necessary to promote p100, rather than p52, nuclear translocation and increase nuclear levels of RelB. Moreover, in a similar experiment using ARNT isoform 1 and 3 mutants lacking nuclear localization signals, GAP-tagged p100 accumulates in the cytoplasm, rather than in the nucleus. Thus, we show that ARNT regulates NF-κB signaling by regulation of p100-RelB nuclear localization, implicating ARNT as a general nuclear transporter.

2333 Constitutive Androstane Receptor/Pregnane X Receptor-Dependent CYP450 Inhibition and Metabolism of Antifungal Itraconazole

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Itraconazole is an antifungal agent that inhibits of the fungal cytochrome P450 (CYP450) enzyme 14-α-t-methylsterol. Itraconazole is metabolized by and inhibits both CYP3A activity and expression in human and rodent systems. Previously, we showed that itraconazole represses the constitutive androstane receptor’s (CAR) transcriptional activity, which may contribute to CYP3A repression. However, the role of pregnane X receptor (PXR) in this process is unclear. Here, we evaluated the effect of itraconazole on CAR/PXR activation and regulation. Itraconazole treatment robustly repressed both hCAR and hPXR-mediated CYP2B6/3A4 induction in human primary hepatocytes (HPHs). P450-Glo™ assays confirmed that itraconazole inhibited CITCO or rifampicin-induced CYP450 activity in HPHs. Moreover, itraconazole activated nuclear translocation of hCAR in HPHs, although mammalian two-hybrid assays demonstrated that itraconazole disrupted the interaction of hCAR with the nuclear co-activator, SRC1. Additionally, cell-based reporter assays showed that itraconazole caused a dose-dependent inhibition of rifampicin-modulated hPXR activation, which was previously observed for hCAR and mCAR. Similar to wild-type mouse hepatocytes, chemically-activated CYP2B10/3A11 induction from either CAR-knockout or PXR-knockout mice was decreased by itraconazole treatments, and effect that was absent in CAR/PXR double knockout mice. Pharmacokinetic analyses revealed that the average serum concentration vs. time curves of itraconazole after oral administration in CAR/PXR double knockout mice were reduced. This data suggests that itraconazole are both CAR and PXR dependent. The co-antagonist activities of itraconazole are both CAR and PXR are important for considering potential drug-drug interactions with the use of this agent.

2334 Hormetic Mechanism-Receptor/Cell Signaling Pathways

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Hormesis is a general adaptive response characterized by a biphasic dose response that is identified by specific dose-response features including a modest stimulation amplitude at a critical dose (the hormetic window) and a subsequent down modulation of the hormetic response at the zero equivalent point generally < 5 fold, and a stimulatory range generally > 20 fold. This review evaluates receptor and cell signaling pathway mediated mechanisms in relation to the hormetic dose response. For this assessment, three a priori criteria were required: 1) a reproducible biphasic dose/concentration response was observed; 2) the dose/concentration-response stimulation was hypothesized to be mediated by a specific receptor or cell signaling pathway; and 3) the hypothesized receptor or cell signaling pathway was tested using a specific antagonist. More than 100 agents from a wide range of chemical classes with their approximately 400 dose responses demonstrated hormesis mediated via a specific receptor or cell signaling pathway. Different mechanistic approaches emerged: 1) the same receptor/cell signaling pathway mediated both the stimulatory and the inhibitory response, 2) one receptor/cell signaling pathway mediated the stimulatory response while another mediated the inhibitory response, and 3) the stimulatory response was mediated by a receptor/cell signaling pathway, but the mechanism for the inhibitory response was unknown. The data suggest a measure of biological plasticity due to the similarity of the quantitative features of the dose response, regardless of the numerous model types (both in vitro and in vivo). Inducing agents, endpoints measured, or receptors and/or cell signaling pathways evaluated; however, none of the data indicate why there is a modest stimulatory response nor the molecular trigger for the turn in the response.

2335 Aryl Hydrocarbon Receptor +/- Mice Are Protected from High-Fat Diet-Induced Disruption of Metabolic Rhythms

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The Aryl Hydrocarbon Receptor (AhR) is an integral player in the crosstalk between circadian rhythms and metabolism. AhR shares structural homology to clock genes, containing both PAS domains and basic helix-loop helix structural motifs, allowing interaction with components of the primary circadian feedback loop. We have previously demonstrated that activation of AhR alters circadian rhythmicity, primarily through inhibition of CLOCK-BMAL1-mediated regulation of Per1 in peripheral tissues. We have also reported that AhR +/- mice are protected from diet-induced metabolic syndrome exhibiting enhanced insulin sensitivity and glucose tolerance. High fat diet consumption disrupts the synchronized circadian timing system resulting in harmful loss or gain of transcriptional oscillations. To determine the role of AhR in diet-induced disruption of rhythms, C57BL/6 AhR +/- and AhR +/- mice were fed a high fat diet (60% fat) for 15 weeks followed by sample collection every 4 hours. High fat diet feeding altered the rhythmic strength of serum glucose and the circadian and metabolic transcription, including nuclear receptors Rev-erb, PPARz and PPARy. However, AhR deficiency provided protection against diet-induced transcriptional oscillation changes, restoring glucose and gene rhythm strength comparable to levels needed to maintain circadian homeostasis. Protection of AhR +/- mice against harmful rhythmic disruption further our understanding of the intrinsic role AhR plays in both circadian rhythms and metabolism and provides a potential therapeutic target for diseases characterized by rhythmic desynchonry.

2336 Identification of Human Constitutive Androstane Receptor (hCAR) Modulators

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CAR is a well-known nuclear receptor which plays an important role in every phase of drug metabolism. Due to its regulation of drug metabolizing enzymes and transporters, altering CAR activation can result in drug-drug interactions which may lead to toxicity or changes in therapeutic efficacy. It has also been reported recently that this nuclear receptor is involved in energy metabolism and cancer therapy. Therefore, it is essential to gather information on how drugs will affect the modulation of CAR. We screened approximately 2800 clinically approved and investigational drugs from the National Institutes of Health Chemical Genomics Center Pharmaceutica Collection (NCPC) for their capacity to activate or deactivate CAR using a double stable hCAR/CYP2B6 HepG2 cell line. Each compound was tested at eight different concentrations in a quantitative high throughput screening format. After the primary screening, 115 agonists and 154 antagonists were identified. The selected drugs were confirmed in human primary hepatocytes by determining the induction or inhibition of CYP2B6 mRNA expression. Ten agonists and 11 antagonists were further evaluated in the more biologically relevant assay using human primary hepatocytes. Four agonists were shown to induce the mRNA expression of CYP2B6 significantly, one of which, celebrex, appears to have increased the expression to a greater extent than the known CAR activator CITCO. Eight of the 11 antagonists significantly inhibited the mRNA expression of CYP2B6 as well. These results demonstrate the ability of many drugs to modulate CAR activity and subsequently regulate drug metabolizing enzymes and transporters, highlighting the importance of characterizing the impact drugs have on CAR.

2337 Ligand Activation of PPARβ/δ Attenuates Inflammation in Both Primary and KC13-2 Kupffer Cells

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 Peroxisome proliferator-activated receptor β/δ (PPARβ/δ) can inhibit pro-inflammatory activities in the liver. Since activated Kupffer cells can modulate hepatic inflammation, the role of PPARβ/δ in modulating Kupffer cell activities was examined. Kupffer cells were isolated from wild-type, Pparβ/δ-null or Pparβ/δ-null mice expressing a DNA binding mutant form of PPARβ/δ in the Kupffer cells (Pparβ/δ DBM). Ligand activation of PPARβ/δ in primary Kupffer cells caused an increase in expression of the PPARβ/δ target gene, adipocyte differentiation-related protein (Adip). In wild-type but not in Pparβ/δ-null or Pparβ/δ DBM Kupffer cells. Ligand activation of PPARβ/δ attenuated lipopolysaccharide (LPS)-induced expression
of the pro-inflammatory gene, tumor necrosis factor-alpha (Tnfsf), interleukin-6 (Il-6) and interleukin-1p (Il-1p) mRNA in Kupffer cell cultures from wild-type and Pparb/- DDB mice but not in Kupffer cells from Pparb/-null mice. Ligand activation of LPS treated immortalized KC3-2 Kupffer cells reflected the changes in mRNA expression of the induced AHR target gene, Tnfsf, mRNA expression observed in primary Kupffer cells. This attenuation of inflammatory cytokine expression with ligand activation of PPARb/δ was not observed in primary hepatocytes of both wild-type and Pparb/-null mice. Combined, results from these studies suggest that activation of PPARb/δ inhibits hepatic inflammation via transrepression of pro-inflammatory signaling in Kupffer cells but not hepatocytes. Since this effect can be found with a DNA binding mutant form of PPARb/δ, this suggests that the attenuation of pro-inflammatory signaling could be due to direct protein-protein interaction of PPARb/δ with other inflammatory signaling molecules such as NF-kB. (Supported by CA140369)

2338 The Nuclear Receptor Constitutive Androstane Receptor (CAR) Is Essential for Toxaphene-Induced Liver Tumorigenic Response in Mice
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Chronic exposure to toxaphene results in increased liver tumors in B6C3F1 mice. Available data suggest that liver tumor induction is through a non-DNA reactive mode of action (MoA), similar to that of phenobarbital. Several short-term animal studies were performed to examine the potential MoA. In prior studies in B6C3F1 mice, toxaphene produced dose dependent increases in 1) liver weight; 2) hepatocyte DNA synthesis as measured by BrdU incorporation; and 3) mRNA expression levels of both nuclear receptors CAR and AhR. The present study used transgenic mice to determine whether CAR or AhR is the primary mediator of the toxaphene liver effects. CAR and AhR knockout mice (CAR-/- and AhR-/-) and wild-type C57BL/6 mice were treated with toxaphene; phenobarbital was a control for CAR activation. We confirmed that CAR regulated genes Cyp3a11 and Cyp2b10 were induced in C57BL/6 mice by toxaphene at 320 ppm and by phenobarbital following 14 or 28 d of treatment. In the CAR-/- mice, Cyp3a11 and Cyp2b10 were not induced at 14 d of toxaphene exposure and strongly attenuated at 28 d. Cyp2b10 expression was not induced in the CAR-/- mice following treatment with phenobarbital. Similarly, induction of PROD by toxaphene and phenobarbital was abolished in the CAR-/- mice after 14 and 28 d. EROD activity in the CAR-/- mice treated with toxaphene or phenobarbital was higher compared with control, but was remarkably lower in comparison with that of the C57BL/6 mice exposed to toxaphene. Notably, the induction of CAR- and AhR-responsive genes, as well as PROD and EROD in the AhR-/- mice was similar to the C57BL/6 mice exposed to toxaphene. Together, these results show that toxaphene induced effects in mouse liver can be attributed mainly to CAR activation; the contribution of AhR in these processes is low but measurable, and may be secondary to or reflect cross-talk with CAR activation. This work was supported in part by internal funding of the Klaunig Lab and by Hercules Inc. a wholly-owned subsidiary of Ashland Inc.

2339 The Role of Nuclear Receptors Constitutive Androstane Receptor and Pregnane X Receptor in Dieldrin-Induced Liver Tumors in Mouse
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Dieldrin, an organochlorine pesticide, has been shown to induce liver tumors selectively in mice. Available evidence has suggested that dieldrin induces liver tumors in mice through a non-genotoxic mechanism acting on tumor promotion stage. The current study was performed to examine the role of nuclear receptor activation as a possible mode of action (MoA) for dieldrin inducing mouse liver tumors. In the initial study, male C57BL/6 mice were treated with dieldrin 10 ppm in diet for 7, 14 or 28 days. Phenobarbital (PB) was used as a positive control for CAR (constitutive androstane receptor) activation. A significant increase in hepatocyte DNA synthesis measured by BrdU incorporation was observed in mice treated with dieldrin for 14 and 28 days as compared with the untreated controls. The liver CAR/PXR responsive genes (Cyp2b10 and Cyp3a11) and enzyme (PROD) activity were induced significantly in the mice treated with dieldrin for 14 and 28 days. The AhR genes (Cyp1a1 and Cyp1a2) were also slightly induced in liver following 28-day treatment of dieldrin. The liver EROD activity (repressors AhR activation) was increased significantly after 7-, 14-, and 28-day treatment of dieldrin. PPARα target genes (Aox1, Cyp4a10) and enzyme activities (ACO) were not increased after dieldrin treatment up to 28 days. In addition, treatment with dieldrin produced significant elevation in the hepatic MDA (malondialdehyde), a biomarker of oxidative damage to lipids. Based on these findings, we propose that dieldrin induced liver tumors in mice is through nuclear receptor CAR/PXR-mediated mode of action. The oxidative stress and damage may also play a role in the MOA of dieldrin-induced hepatocarcinogenesis. Studies using CAR, PXR, CAR/PXR knockout mice are in progress to confirm the involvement and relative contribution of CAR and PXR in dieldrin induced liver carcinogenesis in the mouse. This work supported in part by internal funds in the Klaunig laboratory.

2340 LXRα Antagonist, SPA088 Attenuates T0901317-Induced Nonalcoholic Fatty Liver
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LXR is a member of nuclear receptor superfamily, it regulates various biological events such as de novo lipogenesis, cholesterol metabolism and inflammation. Recently, some papers suggest that LXR double knock-out mice are completely rescued from fatty liver when metabolic syndrome is induced. In this study, we discovered the LXRα selective antagonist to use as therapeutics for fatty liver disease. Meso-Dihydrougauaretic acid (MDGA) inhibits LXRα selectively in liver leading to decrease hepatic lipogenesis in our recent study. So, we have developed SPA088, MDGA derivative and evaluated the effects of SPA088 on LXRα activation and its ability to attenuate fatty liver in mice. SPA088 activates recruitment of NCoR, the corepressor of LXRα and decreased the luciferase activity in LXRα-, SRE-Luc-transfected cells. SPA088 decreased lipid accumulation and the gene expression of lipogenesis, including SREBP, FAS, and SCD-1 which are LXRα target genes. Based on the in vitro results, we examined the inhibitory effect of SPA088 on lipogenesis in T0901317-induced steatosis model in vivo. SPA088 significantly alleviated fatty liver induced by T0901317. SPA088 increased the interaction of NCoR with LXRα and down-regulated the target gene expressions associated with lipogenesis in T0901317-treated mice. The expression of genes related to reverse cholesterol transport was not decreased. These results demonstrate that SPA088 has the potential of therapeutic for nonalcoholic fatty liver disease mediated by selective inhibition of LXRα in the liver in mice.

2341 Deletion of TIPARP Increases Sensitivity to Dioxin-Induced Hepatosteatosis and Lethality
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The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor that mediates the toxic effects of the environmental contaminant dioxin (2,3,7,8-tetrachlorodibenzo-p-dioxin; TCDD). Dioxin causes a diverse range of toxic responses, including hepatic damage and lethal wasting syndrome; however, the mechanisms of dioxin-induced toxicity are still unknown. We have shown that TCDD-inducible poly(ADP-ribose) polymerase (TIPARP), an ADP-ribosyltransferase and AHR repressor, is part of a negative feedback loop that inhibits AHR activity by increasing its proteolytic degradation. In this study, we show that the loss of TIPARP increases sensitivity to dioxin-induced toxicity and lethality. Male Tiparp-/- mice treated with a single injection of 100 or 10 µg/kg dioxin display an accelerated lethal wasting syndrome with no Tiparp-/- mice surviving beyond day 5 or 8, respectively. All Tiparp+/+ mice survived at least 30 days post treatment. For the 100 µg/kg treatment, Tiparp-/- mice exhibit dramatic weight loss, increases in liver steatosis and hepatotoxicity. Dioxin-treated Tiparp-/- mice have markedly reduced glucose levels, but phosphoenolpyruvate carboxykinase mRNAs were similar between Tiparp+/- and Tiparp-/- mice. At the molecular level, TIPARP selectively ADP-ribosylates AHR, but not AHR nuclear translocator (ARNT) nor AHR interacting protein (AIP). The TIPARP-dependent repression of AHR is reversed by the macrodomain containing mono-ADP-ribosylase Macrod1, but not Macrod2. Overall, these novel findings provide evidence that AHR activity is dynamically regulated by TIPARP, Macrod1, and ADP-ribosylation, and show that TIPARP, a well-known AHR target gene, is protective against dioxin-induced toxicity and lethality.
2342 Preliminary Characterization of a Suite of Nuclear Receptor Knockout Rats

The nuclear pregren X receptor (PPAR), constitutive androstane receptor (CAR) and the aryl hydrocarbon receptor (AHR), are transcription factors involved in the regulation of drug metabolizing enzymes and transporters in response to xenobiotics and other pollutants. Null and humanized mouse models for these receptors have been generated, characterized (for review see ref 1), and been a very important part of the toxicity evaluation during the drug development process. Because of their larger size, rats are a preferred ADME/Tox model system over mice. We created PXR, CAR and AHR knockout rats in Sprague Dawley (SD) background using ZFN technology (2). In this study, we treated 8-week old wild-type and null male SD rats with known rodent activators of PXR, pregnenolone-16α-carbonitrile (PCN), CAR, 1,4-Bis-[2-(3,5-dichlorophenylxylo)]benzene, 3,3',5,5'-tetra-chloro-1,4-bis(pyridyloxy)benzene (TCPOBOP), and AHR, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). RNA was extracted from liver tissue of treated animals and analyzed via qRT-PCR (SYBR-arraies and Taqman assays). P450 treated null-PXR, TCPOBOP treated null-CAR, and TCDD treated null-AHR rats lost the activation of the Cyp3a4 family (Cyp3a18, 3a9, 3a23/3a1, 3a2), Cyp2b2 and Cyp1a (Cyp1a1, 1a2) respectively, as compared to treated wild-type rats. These models alone should be useful for studying metabolism of xenobiotic compounds and hepatotoxicity. In addition, these models are also critical components for the humanization of the cytochrome P450 pathways in the SD rat. 1. Scheer N, Wolff CR, (2013) Xenobiotic receptor humanized mice and their utility Drug Metabolism Review 45(1): 110-121. 2. Geurts et al., (2009) Knockout rats via embryo micro-injection of zinc-finger nucleases. Science 325(5939):433.

2343 Biokinetics and Effects of Barium Sulfate Nanoparticles
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Nanoparticulate barium sulfate has wide use in the polymer and paint industries and a great potential for future novel applications. We performed a comprehensive biokinetic study of radio labeled 131BaSO4 NPs administered via multiple routes and examined acute pulmonary responses to intratracheally (IT) instilled BaSO4 in rats. We followed the tissue distribution of 131BaSO4 over 28 days after IT instillation, and over 7 days after gavage and intravenous (IV) injection of 131BaSO4. We measured multiple parameters in bronchoalveolar lavage (BAL) fluid in separate groups of rats 24 hours after instillation of BaSO4. We found that IT-instilled BaSO4 caused dose-dependent increases in BAL parameters of injury and inflammation. The clearance half-life of instilled 131BaSO4 (0.28 ± 0.004 mg/ lung) was approximately 9.6 days. At 28 days post-IT, translocated 131Ba from the lungs was predominantly detected in the bones (29% dose), far more than in all tissues combined (7%). Nevertheless, the concentration of the gavage dose was excrated in the feces: only 0.15% was detected in tissues at day 7. IV-instilled 131BaSO4 were mostly localized in the liver, spleen, lungs and bone at 2 hours. 131Ba was redistributed from the liver to bone over 7 days. Fecal excretion was the dominant elimination pathway for all routes of exposure. In summary, instilled BaSO4 caused dose-de- pending inflammatory and inflammatory lung injury of 131BaSO4. Lung clearance of 131BaSO4 was decreased and resulted in higher tissue retention than when ingested. Since the bioavailability of barium from ingestion is very low, no significant contribution from ingestion of BaSO4 should occur during whole-body inhalation studies in rats. Injected BaSO4 localized predominantly in reticuloendothelial organs but redistributed to the bone over time. BaSO4 exhibited lower toxicity and biopersistence in the lung than other poorly soluble NPs such as CeO2 and TiO2.

2344 Identification of the Appropriate Dose Metric for Pulmonary Inflammation of Silver Nanoparticles in an Inhalation Toxicity Study
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A number of studies have shown that induction of pulmonary toxicity by nanoparticles of the same chemical composition depends on particle size, which is likely in part due to differences in lung deposition. Particle size mostly determines whether nanoparticles reach the alveoli, where they might induce toxicity. For the risk assessment of nanomaterials, there is need for a suitable dose metric that accounts for differences in effects between different sized nanoparticles of the same chemical composition. The aim of the present study is to determine the most suitable dose metric to describe the effects of silver nanoparticles after short-term inhalation. Rats were exposed to different concentrations (ranging from 61 to 1105 μg silver /m3 air) of 18 nm, 34 nm, 60 nm and 160 nm silver particles for 4 consec- tive days and sacrificed at 24 hours and 7 days after exposure. We observed an exposure concentration dependent increase in pulmonary toxicity parameters like cells counts and pro-inflammatory cytokines in the bronchoalveolar lavage fluid. All results were analysed using the measured exposure concentrations in air, the measured internal dose in the lung and the estimated alveolar dose. In addition, we analysed the results based on mass, particle number and particle surface area. Our study indicates that using the particle surface area as a dose metric in the al- veoli, the dose-response effects of the different silver particle sizes overlap for most pulmonary toxicity parameters. We conclude that the alveolar dose expressed as mass is the most suitable dose metric to describe the toxicity of silver nanoparticles after inhalation.

2345 Effects of Amorphous Silica Coating on Cerium Oxide (CeO2) Nanoparticle-Induced Pulmonary Responses
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Recently cerium compounds have been used in a variety of consumer products including diesel fuel additives for increasing fuel combustion efficiency and decrease diesel soot emissions. However, CeO2 has been detected in the engine exhaust, which raises a health concern. Since the potential pulmonary toxicity of cerium nanoparticles (NPs) may be due to the release of CeO2 from by intratracheal (IT) instillation induces sustained pulmonary inflammation and lung fibrosis. The present study examines pulmonary responses to CeO2, or CeO2 coated with a nanofiber layer of amorphous SiO2 (aSiO2/CeO2), generated from the Harvard Versatile Engineered Nanomaterial Generating System. Male Sprague Dawley rats were exposed to various doses of CeO2 or aSiO2/CeO2, by a single IT and sacrificed at various times post exposure. The first acellular bronchoalveolar lavage fluid (BALF) was collected from all exposed animals and analyzed. CeO2 (0.15 mg/kg), but not aSiO2/CeO2, induced cytotoxicity, inflammatory cytokines, matrix metalloproteinases (MMP)-9, and tissue inhibitor of MMP in BALF at 1 day post exposure. In contrast, morphological analysis indicated that collagen fibers were increased in CeO2 or aSiO2/CeO2, at 3.5 mg/kg, but not at 0.15mg/kg, at 28 days post-exposure. CeO2 and aSiO2/CeO2 particles were evident in particle-exposed lungs up to 28 days post exposure. Energy dispersive x-ray spectroscopy (EDX) analysis indicated Ce and O in all particle-exposed lungs up to 28 days post exposure, whereas Si was only detected in aSiO2/CeO2-exposed lungs up to 3 days after exposure. The qualitative data from EDX suggest that aSiO2, coated protected lungs from low dose CeO2-induced acute lung toxicity, but did not affect high dose- and longer term- CeO2-induced responses. A modification of the coating to improve protection of the lung from CeO2-induced lung injury is under development.

2346 Biokinetic Comparison after Single IV Administration or Chronic Aerosol Inhalation of TiO2 Nanoparticles: Impact on Blood-Brain Barrier
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Notwithstanding the translocation of reported TiO2 nanoparticles (NPs) through biological barriers, the bio-distribution of TiO2NPs to the CNS and impact on the functions of the BBB remain poorly characterized. We report such evidence. Adult rats received a single 1mg/kg iv dose of TiO2NPs or inhaled for 28 days. 5 days a week, 6hr per day a 10mg/m3 concentration of aerosol. Blood and tissues were collected at various times. Ti levels were measured using ICP-MS. In vivo permeability and transport activities of the BBB were evaluated. The expression of structural components of the BBB and the brain inflammatory profile were assessed by RT-PCR on isolated brain micro-vessels. After iv administration, data revealed a rapid bio-distribution of Ti to all the tissues with a bioaccumulation in liver up to one year; we also demonstrated a Ti uptake until 24 h followed clearance by brain endothelial cells (BECs) without translocation in the CNS. After chronic inhalation (CI), Ti was only found in the lungs. Despite lack of Ti in the brains 24h after IV and at all-time point after inhalation, modification of the BBB physiology was highlighted. In the case of iv, the BBB integrity was maintained whereas we noticed a modulation in BECs of claudin-5, occludin, IL-1 and CXCl1 mRNAs expression as well as a BCRP activity. After CI, we also noticed a reversible modu-
lotion of BCRP activity at early time point and 28 days after the end of exposition, an increase in the BBB impermeability. These data indicate a different target organ depending on the administration route. In both exposure routes, we highlighted a dose-dependent lung inflammation and cytotoxicity was observed that followed the exposure period, lung burdens were assessed and toxicological examinations were performed. (Health and Labour Sciences Research Grant, MHLW, Japan)

3.3 Taquann Dispersion Method with Direct Injection Whole-Body Inhalation System for Engineered Nano Materials

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Using Mitsui MWNT-7 as a model compound, we developed a Taquann method to generate dry aerosol of highly-dispersed MWNT-7 fibers without aggregation. Aliquots of dispersed MWNT-7 were injected into the chamber system by compressed air periodically to maintain concentration in the exposure chamber. Chamber aerosol consisted of well-dispersed fibers of their length and width similar to those in the bulk sample. In the lungs of mice exposed to 1 or 2 mg/m³ for a total of 10 hours, fibers of same length/width distribution were found at an amount of 3 μg/lung. Histologically, dispersed fibers were distributed in alveolar areas inducing patchy or diffuse interstitial pneumonia with activated macrophages; no aggregates or granulomatous changes were seen. The fibers were also found in the microscopic lesions located on the surface of the parietal pleura; similar to what is observed in human patients. Using MWNT-7, we developed a Taquann system that is relatively cheap, simple and easy to operate, and easy to keep the facility clean, and easily applicable to various types of nanomaterial; TiO₂ nanoparticles were successfully dispersed and exposed. A merit for new low-product-volume nanomaterials is that there is no sample loss after filtration and a small amount can be tested. (Health and Labour Sciences Research Grant, MHLW, Japan)

2348 Short-Term Inhalation of Nanosized Copper Oxide Results in Adverse Pulmonary Effects in Rats

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The increased production and application of nanomaterials has raised concerns about undesirable human health effects. The inhalation route is of special concern for worker and consumer safety. Short-term inhalation testing has recently been proposed as cost-effective test to generate a valuable data set for risk assessment (Ma-Hock et al., 2014). This includes apart from the generally required pathology, it also includes biochemistry and tissue burdens. Therefore, we tested nano-sized copper oxide (nano CuO), a wood protection/anti-microbial agent, in a short-term inhalation protocol (STIS). Rats were exposed by nose-only to a 6-hour equivalent concentration of 0.6, 0.6, 2.4, 3.3, 6.3 and 13.2 mg/m³ CuO, with a primary particle size of 15-20 nm and a MMAD of 1.5 μm (σ 0.38). Following a 5-day exposure period, lung burdens were assessed and toxicological examinations were performed the day after the last exposure and after a recovery period of 22 days. A dose-dependent lung inflammation and cytotoxicity was observed that followed the lung burden at day 6. These adverse effects were absent after the recovery period. Histopathological examinations indicated alveolitis, bronchiolitis, vacuolation of respiratory epithelium and epithymoma in the lung starting at 2.4 mg/m³. In the recovery groups, inflammation remnants were still observed at the highest dose groups. Degeneration of the olfactory epithelium in the nose was observed starting at 5 mg/m³ and fully recovered. No histopathological changes were detected in brain, olfactory bulb, spleen, kidney and liver. The STIS protocol proved to be a useful tool to assess the hazard of nano CuO and information can support grouping and categorization of (nano)materials for risk assessment.
concerns about possible occupational and environmental health toxicity. To investigate the potential deleterious effects of inhalation exposure to WO3 NPs, Golden Syrian hamsters were divided into three groups—a control, exposed to aerosolized sterile distilled water and two treatment groups exposed to either 5 or 10 mg/m3 of aerosolized WO3 NPs for 4 hrs/day, for 4 days in a whole body exposure chamber. WO3 NPs were characterized by TEM and the chamber concentration was monitored using the NanoScan SMPS. Animals were euthanized 24 hours post exposure and bronchoalveolar lavage fluid (BALF) was collected. Total LDH activity and TNF-α levels were evaluated biochemically while BALF macrophages were examined by SEM. After collecting BALF, lungs were snap frozen in liquid nitrogen for further analysis. Total LDH activity of control was 2431 ±169.3 uM/L while in 5 and 10 mg/m3 treated groups it was increased (p<0.05) to 2996 ±568.9 uM/L and 4280 ±887.8 uM/L respectively. Isolated macrophages from BALF of treated hamsters show presence of membrane blebbing compared to control, when observed under the SEM. Level of TNF-α of control was 79 mg/mL, while that of 5 and 10 mg/m3 treated groups were increased (p<0.05) to 105 ±4 mg/mL and 169 ±52 pg/mL respectively. Western blot analyses of tissue lyse indicated an increase of Caspase-1 and IL-1 release of IL1-β. The current study investigated the toxicity of TiO2 NP in Golden Syrian hamsters. Hamsters were exposed in a whole body chamber to aerosolized vehicle (control) or aerosolized TiO2 NP at concentrations of 0.3mg/m3, 3mg/m3 and 15mg/m3 for 4 hrs/day. Twenty-four hours post exposure, hamsters were euthanized, lungs removed and frozen for biochemical analyses. Frozen tissues were assessed by western blot analysis to detect the levels of Caspase B, NLRP3, ASC and IL-1β. The average size of TiO2 NP was determined to be 36.9 ±14.4nm by TEM evaluation. Tissue from the lungs of hamsters treated with 3 and 15mg/m3 NP had a 2-fold increase in expression of Caspase B as compared to controls and animals treated at 0.3mg/m3 NP. Lungs from hamsters treated with the above concentrations of TiO2 NP had 3 fold increase in NLRP3 protein levels when compared to control and 0.3mg/m3 treated group. Levels of ASC were 3-5 fold higher in pulmonary tissue from hamsters treated with 3 and 15mg/m3 as compared to controls. There was no significant difference in the levels of expression of IL-1β between control, 0.3mg/m3 and 3mg/m3 groups, but there was a 2 fold increase seen in 15mg/m3 NP treated group when compared to the other groups. These results indicate that inhalation of nano-sized TiO2 particles induce the formation of NLRP3 inflammasome with the subsequent up-regulation of IL-1β protein.

Assessing the mechanisms underlying adverse cardiovascular effects induced by inhaled toxins presents a substantial research challenge. We propose that blood carries an as yet unknown "inflammatory potential" consisting of modified proteins or other biomolecules and reaction byproducts that affects a pathological bioactivity which can be assessed using endothelial cells as biosensors. The approach involves applying serum from exposed animals to cultured primary endothelial cells or ex vivo isolated arteries. Mice were exposed to multi-walled carbon nanotubes (MWCNT; 0, 10 or 40 μg) via pharyngeal aspiration and serum was collected at 4 and 24 h post-exposure. Serum from exposed mice increased endothelial cell surface cell adhesion molecule-1 (VCAM-1) and intracellular adhesion molecule-1 (ICAM-1) expression and proinflammatory transcripts, and decreased ATP-stimulated nitric oxide (NO) production. The functional impact of this loss of NO bioavailability was confirmed via myography, in which serum from MWCNT-exposed mice significantly impaired vasodilation to acetylcholine. In addition, serum from MWCNT-exposed mice reduced cell migration in a traditional scratch assay experiment. To identify the bioactive circulating components in the serum, a data-independent 'omic platform enabled reproducible label-free quantitative analysis revealed a diverse set of serum factors in the size range of 500-5000 Da altered due to MWCNT exposure. Each dose was associated with its own independent set of selective factors that differentiated the two MWCNT exposure groups. More compelling was that this particular size fraction had functional effects on endothelial cell function. In conclusion, pulmonary exposure to MWCNT dynamically alters circulating factors, which promotes endothelial cell activation, decreased NO bioavailability, and altered functionality all directionally predicting adverse cardiovascular outcomes.

Quantum dots (QDs) are engineered nanoparticles commonly composed of a CdSe/ZnS core/shell and application-specific outer coatings. QDs have uses in electronics, biomedical research and medicine. However, their small size, heavy metal composition and multitude of potential uses have generated concerns regarding their toxicity. The CdSe/ZnS QDs being used in this study are 12 nm in diameter and are coated with an amphiphilic polymer (triocylphosphine oxide / poly (maleic anhydride-alt-1- tetradecane). We are investigating QD-induced lung inflammation and toxicity with a systems genetics approach utilizing recombinant inbred (RI) mouse strains from the Collaborative Cross (CC). We have observed significant heterogeneity across the RI strains in response to QD exposure 8 h after oropharyngeal aspiration. Specifically, we found significant mouse strain (p<1 x 10^-5) and QD treatment (p<0.05) related effects in the levels of total protein in bronchoalveolar lavage fluid (BALF). We also found significant background strain variation in the % neutrophils in BALF (p<0.05), levels of lactate dehydrogenase (LDH) (p<0.05) in BALF, and in levels of lung tissue heme oxygenase (p<0.002). In addition, heme oxygenase levels were significantly correlated with BALF neutrophils (r^2=0.40, p=0.005) and lung tissue glutathione (r^2=0.11, p=0.02). This study will provide insight into mechanisms of QD related toxicity.
help to identify biomarkers of susceptibility, and ultimately provide information for the design of safer engineered nanomaterials. Supported by NIEHS grants U19ES019545, P30ES007033, and T32ES007032.

2356 Glutathione Deficiency Modulates Susceptibility to Multiwalled Carbon Nanotube-Associated Acute Lung Inflammation in Mice

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Multi-walled carbon nanotubes (MWCNTs) present potential respiratory risks during manufacture in the electronics and polymer industries. Workers may be particularly susceptible if they have low levels of the major antioxidant glutathione (GSH) because of health conditions or common polymorphisms in synthesis enzymes. Therefore, we investigated if genetic deficiency in the modifier subunit of the GSH-synthesizing enzyme glutamate cysteine ligase (Gclm) modulates susceptibility to MWCNT-associated acute lung inflammation in vivo. We exposed 3 - 5-month-old female C57BL/6 Gclm wildtype (WT), heterozygous (HT), and knockout (KO) mice via oropharyngeal aspiration to vehicle or 25 μg/mouse of a non-functionalized stock MWCNT, and sacrificed the mice after 24 hours. We observed significant neutrophil influx into the lungs of exposed WT mice, but not HT or KO mice. We also observed increased eosinophils in all treated mice. Our results indicate that female glutathione-deficient C57BL/6 mice are less susceptible to MWCNT-associated acute lung inflammation than gender-matched male mice. However, significantly deregulated expression of IL-1β expression in frontal cortex may indicate that IL-1β molecules were accumulated near the surface of GNP, which reduced their interaction with interleukin receptors. Finally, it was concluded that GNP cannot be used in targeted drug delivery and therapeutic treatment of CNS diseases.

2359 Impact of Food-Grade Titanium Dioxide (TiO2) Compared to P25 Nanoparticles on the Gut Immune System after Oral Exposure in Rats

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Aim: Food Grade TiO2 used as white pigment contains until 35% of nanosized particles. Using the TiO2 P25 referent nanoparticles (NP), in vivo studies showed enhanced passage through Peyers patches (PP), a gut lymphoid tissue participating with mesenteric lymph nodes (MLN) to host defenses and oral tolerance. We compared in vivo the effects on immune system of food grade TiO2 (E171) and P25 at relevant human level through diet. Methods: Rats were given per os (1 week) water (controls), ultrasonicated E171 or P25 at 10mg/kg BW/d (n=10/group). Synchrotron imagery was used for TiO2 distribution along the gut-liver axis. Dendritic cells (DC) and T lymphocytes were assessed by flow cytometry, and secretion of IFN-γ and IL-10 by ELISA in supernatants of PP and MLN cell suspensions after in vitro CD3/CD28 restimulation. Results: In all groups, TiO2 particles were found among PP cells, and in the liver. In PP, a 3-fold increase in DC frequency was observed in both E171 and P25 rats (p<0.01 vs controls), while a concomitant decrease in MLN (p<0.05) suggests a defect in DC migration to MLN. In PP, a decrease in activated CD4+CD4+ and highCD62Llow T cells occurred in E171 rats only, as well as for CD4+CD25+Foxp3+ Treg involved in oral tolerance (~44%, p<0.05). IFN-γ secretion by PP cells was significant reduced in E171 rats (~44%, p<0.01), while an increase by MLN cells (x1.4) occurred in all TiO2 groups (p<0.01). Increased IL10 secretion was shown in supernatant from MLN cells in E171 rats only (x1.5, p<0.05). Conclusion: Food grade E171 TiO2 crosses gut barrier similarly to the referent P25, and is recovered in the liver, indicating systemic passage. E171 and P25 impact immune cell frequency in PP and MLN, together with dysregulation of cytokine secretion by T cells. These data show imbalanced immune homeostasis in the gut after oral exposure to TiO2, with marked effects for food grade E171 particles in gut lymphoid tissues.

2360 Pretreatment of Nanoparticles Alters Organ Disposition of [14C] Cholesterol in C57BL/6 Mice

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Engineered nanomaterials are extensively applied in industry, medicine and consumer products. To investigate the potential risks of nanoparticles, we assessed the effect of nanoparticles (Ag, TiO2, SiO2 and ZnO) on tissue and hepatic subcellular distribution of exogenous [14C] cholesterol equivalents 16hr after oral gavage of 5 ml corn oil/kg that contained 10 mg CH/kg. Blood, liver and bile/gallbladder total CH concentration were also measured in the same mice. Finally, plasma and hepatic apolipoprotein A1 (apoA1) and were quantified by western blotting. Treatment of nanoparticles increased biliary excretion of exogenously administered [14C]CH. These compounds did not affect total CH concentration but there was a trend of increased high density lipoprotein CH (HDL-CH) content in blood plasma. Total CH concentration in gallbladder/bile was increased in
Iron is crucial for some cellular processes and low or no consumption of foods rich in bioavailable iron such as red meat is a main cause of Iron Deficiency Anemia (IDA). In Egypt, anemia remains a problem that urges the need of innovative strategies. Exploring the advances in nanotechnology, a novel formula of iron oxide-based nanocomposite (Fe3O4) was prepared. The aim of this pilot study was to determine the median lethal dose (LD50) of this novel formula as well as to test its efficacy in correcting anemia in rodents fed on iron-deficient diet. Rats of four groups were orally administered a single dose of 667, 1000, 1500, and 2250 mg/kg rat body weight nano-sized iron oxide and mortalities were counted after 24 hours. LD50 was calculated and determined in rats as 1425.3 mg/kg rat body weight while the equivalent human LD50 is estimated to be 13854 mg/60 kg human body. The efficacy of the novel formula was tested by administration of 5, 10, 15, 20, 25, and 50 mg (equivalent human doses) of iron nanocomposite to IDA rats and monitoring the correction of hemoglobin (Hb) and red blood cells count (RBCs). Doses of 5 and 10 mg of nano iron oxide corrected Hb concentration and RBCs count starting from Day 4 to Day 7, and reached the same levels as controls within 14 days post treatment. Additionally, doses of 25 and 50 mg administration revealed the least Hb and RBCs count correction in the same period of time suggesting that availability of lower concentrations of nano-sized iron might favors its rapid and complete absorption than higher concentrations. The present results indicate that a novel effective and safe iron oxide nanocomposite possess a promising corrective effect in treatment of iron-deficiency anemia within a short period of time.

**2361 Toxicity and Efficacy of Novel Formula of Iron-Based Nanocomposites in the Treatment of Iron Deficiency Anemia in Rodents**

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Many commercial feminine hygiene products are claimed to contain nanoscale materials. These nanomaterials could potentially penetrate into the vaginal mucosa and become a potential hazard to the consumer. However, there are limited data on nanomaterial penetration into the vaginal mucosal tissue. We established a vaginal penetration rodent model and evaluated penetration of Au nanoparticles (NPs) into vagina mucosa. Physicochemical properties of Au NPs (30 nm, 100 nm) coated with polyvinylpyrrolidone (PVP) or polyethylene glycol (PEG) were characterized using transmission electron microscopy (TEM), particle size analysis, UV/Vis spectroscopy, inductively-coupled plasma mass spectrometry (ICP-MS) and other methodologies. The sexually mature female rats were injected with 100 mg/kg of medroxyprogesterone to induce a synchronized estrous cycle prior to vaginal lavage of gold NPs at a dose of 100 μg/kg of body weight. Vaginal cytology examination indicated that all animals were induced an extended diestrus status. Non consistent gold element was detected in liver, lymph nodes, kidney, spleen and blood using ICP-MS at 24 hours post-Au NPs administration. However, gold nanoparticles were detected in vagina region but not in ovary or uterus region in the reproductive tract using ICP-MS. The Au nanoparticle organization has the ability to penetrate through the vaginal mucus barrier. These results suggest that the size of the particles play an important role for vaginal penetration of nanomaterials. This study provides the fundamental data on the ability of nanomaterials to penetrate vaginal mucosal tissue using a rodent model.

**2364 Role of WNT/MAPK Crosstalk in Caenorhabditis elegans Reproduction Failure Due to Graphene Nanomaterials Exposure: A Systems Toxicology Approach**

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The widespread applications of Graphene nanomaterials and its derivatives have emphasized the need for further mechanistic insight to predict the consequences of environmental health and safety impacts of its exposures. In this study, the potential hazard of graphene nanomaterials, was investigated in the nematode Caenorhabditis elegans with graphene oxide (GO) and reduced graphene oxide (rGO) using an integrated systems toxicology approach. The graphene nanomaterials, specifically GO, was found to reduce worm’s reproductive capability without affecting its survival (24 h endpoint). To unravel the underlying molecular mechanism of reproduction failure as well as the differential response of GO-rGO, we employed global gene expressions (microarrays) followed by pathway analysis. The Wnt and MAPK pathways were found as main pathways in GO-rGO comparative analysis, that is, these pathways were evoked by GO but not by rGO. With this in mind, we hypothesized that the crosstalk between Wnt-MAPK pathways are responsible for GO but not for rGO, induced reproductive toxicity in C.elegans. By targeting, with qPCR gene expressions and mutant reproduction analysis, the individual components of the Wnt-MAPK crosstalk pathway, we found that the activation of p1 (the TCF protein homologue) and subsequent repression of target genes (egl-5 and mab-5) are the central mechanisms of worm’s reproduction failure. The P0:1 possibly activated because of the suppression of modulator MAPK pathway (mom-4, lit-1). Taken together, our results highlight the key role of Wnt-MAPK pathway in regulating GO induced reproductive failure, which will be helpful for understanding the differential mode of nano-bio interaction of graphene nanomaterials, especially for its biomedical application.

**2365 Biocompatibility of Doxorubicin-Conjugated Nanoparticles for Targeted Drug Delivery in Cancer**

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Doxorubicin (Dox) is a cancer drug widely used in cancer chemotherapy, but it has heart toxicity, bone marrow suppression and other clinical toxicity. To decrease the toxicity of Dox, various carrier systems have been developed using doxorubicin-conjugated nanoparticles such as PEGylated liposomal doxorubicin (Doxil)
and doxorubicin (Dox)-linked CNT (Dox-CNT). Recently doxorubicin-conjugated nanoparticles showed to enhance the anti-cancer effect and reduce the side effect of Dox. However, the biocompatibility of these nanoparticles has not been elucidated. In this study, we demonstrated biosafety and biocompatibility of Doxil and Dox-CNT combined with free Dox in vivo. For 14 days repeated toxicity study, BALB/c mice were intravenously administered with free Dox, Doxil or Dox-CNT. Toxicity including mortality, clinical signs, body weight changes, food consumption, hematology, organ weight and histopathological findings were evaluated. No significant change between control group and drug treated group was observed in hematological values. However, there was significantly decreased in heart and liver weight of all groups, as target organs of Dox. Because intravenous administration of nanoparticles can cause acute hypersensitivity reactions (HRSs), systemic blood pressure, heart rate, serum histamine and release of IgE were tested. As results, both Dox-CNT and Doxil significantly elevated HRSS symptoms. Specifically, we found that Dox-CNT group showed a significant increase in lung weight and abnormally high histopathological findings. In addition, there was significantly decreased in body weight changes and food consumption of Dox-CNT group. The in vivo data revealed that Dox-CNT has lung toxicity and poor biocompatibility comparing Doxil. These results provide useful knowledge for development of nano-therapeutictic drug delivery.

2366 Nontoxic and Stable Nanocarriers for Delivering an Antitumor Agent In Vivo

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Statement of the Rationale and the Scope of the Study: In vivo targeted delivery of the growing number of promising novel short interfering RNA (siRNA)-based therapeutics is an important, but currently challenging, aspect of the drug development process for a variety of diseases, including cancer, viral infections, and autoimmune and neurodegenerative disorders. Our long-term goal is to develop evidence-based clinically-useful drug delivery systems to improve targeted therapies for human disease. Our objective is to develop optimized Super-Paramagnetic Nano Carriers (SPNCs) that can successfully deliver siRNAs to target tissues. These carriers consist of an iron oxide core covered by a modified polyethyleneimine shell. Their efficiency will be evaluated using two in vivo models: Droso phila melanogaster and the mouse. The central hypothesis is that SPNCs designed with modified poly(ethylene imine) (PEI) will yield maximal delivery of siRNA molecules to targeted tissues in vivo. Experimental Procedures: Different SPNCs were tested in CHO-K1 and HeLa cell lines for their transfection efficiency of firefly luciferase (GL2 + GL3) siRNA and we explored the benefits of magnetofection. Droso phila was successfully used to assess the SPNC’s toxicity and their effects on the flies circadian rhythm. Results: Reducing the polycationic character of the nanoparticles eases their toxicity while maintaining their high transfection efficiency, which is actually increased by magnetofection. Toxicity results obtained with four additional cell lines highlight that cells tolerate these new nanoparticles differently. The circadian rhythm of Droso phila melanogaster was affected, and a dependence on the SPNC’s surface groups was observed. Discussion and Conclusions: There is a fine balance between the polycationic character of a carrier to its toxicity and transfection efficiency. Magnetofection is a powerful tool to deliver the carrier-siRNA complex to a target cell or tissue.

2367 Adult Neurogenesis in Chemical-Induced Neurotoxicities: A New Frontier in Toxicological Mechanistic Investigations, Biomarker Research, and Therapeutic Targeting

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Loss of neurons in selective brain region(s) and retina is the pathological characteristic of numerous neurodegenerative diseases such as Parkinson’s disease (PD), Alzheimer’s disease (AD), and retinitis pigmentosa, among others. Classic neurobiology states that postmitotic neurons lack the ability to divide and thereby replace themselves. However, recent studies provide strong evidence that neurogenesis in the adult brain may mitigate adult neuronal loss by sustaining nonmotor function in PD and slowing cognitive deterioration and memory loss in AD. During adult neurogenesis, new neurons are generated from two primary proliferative niches in the adult brain. The subventricular zone (SVZ), nurtured by the cerebrospinal fluid (CSF) in brain ventricles, provides neural stem cells (NSC) via the rostral migration stream (RMS) to other brain regions, while the subgranular zone (SGZ) in the hippocampus produces new granule neurons for dentate gyrus. New studies suggest that toxicant exposure can alter neurogenesis, leading to compromised plasticity and neuronal dysfunction and exacerbating neuronal vulnerability to environmental toxicants. This session brings together experts in this fast evolving research field with a particular focus on how neurotoxicant exposure alters the adult neurogenesis and developmental proliferation. The goal of this session is to synthesize perspectives of critical niche areas of adult neurogenesis and its toxicology, with a focus on mechanisms that will provide new clues for potential amelioration and therapeutic intervention. This session will be of interest to those engaged in neurotoxicology related to neurodegenerative diseases, development, metal and pesticide toxicities, and systems biology.

2368 Introduction to Adult Neurogenesis

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This introduction will establish the general concept of neurogenesis, i.e., the origin of neural stem cells (NSC’s), their differentiation and migration in brain, and their roles in maintaining brain plasticity and normal function. The possible migratory pathways through which the NSC’s reach to their destinations in different brain regions are then introduced. The presentation will build the theoretical foundation for subsequent talks.

2369 Manganese-Copper Interaction: Effects on Adult Neurogenesis and Stem Cell Migration

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Brain subventricular zone (SVZ) generates neural stem/proliferating cells (NSPC), which are nurtured in the CSF secreted by adjacent choroid plexus (CP) in brain ventricles and migrate via the rostral migration stream (RMS) to the olfactory bulb (OB) and other brain regions. Recent studies by synchrotron X-ray fluorescent imaging reveal a remarkable accumulation of copper (Cu) in the SVZ. Our data along with other reports in literature also establish an altered Cu homeostasis following Mn exposure in humans and animals. Since Mn accumulates in CP and alters Cu transport, we asked if Mn exposure may affect Cu homeostasis in SVZ, leading to altered neurogenesis in brain. To test this hypothesis, rats were sub-chronically exposed to 6 or 15 mg Mn/kg as MnCl2 by ip injection once daily for 4 weeks. Mn exposure significantly increased Mn levels in the SVZ, but greatly reduced the Cu level in SVZ by 3-4 fold (p<0.05). Immunohistochemical staining revealed a significant increase of BrdU+ cells, which were co-localized with GFAP signals (stained for astrocytic stem cells: ASC) as well as DCX signals (stained for neuroublast: NB), in both SVZ and RMS; but within RMS, DCX signals appeared to be stronger than those in SVZ after Mn exposure. Interestingly, the expression of DMT1 was significantly increased in both SVZ and RMS after in vivo Mn exposure. Moreover, the DMT1 signals were co-localized with GRAP+, ASC and DCX+ NB. The in vitro neurosphere assay, however, showed a restricted proliferation of NSPCs and suppressed differentiation and migration after Mn treatment. These data suggest that Mn-induced brain damage may trigger the neurogenesis and impaired neuron precursor cells to migrate along the astrocytic “tube” surrounding the RMS in order to compensate the damage in the nearby brain regions. The presentation also introduces the nurturing function of the CP to the SVZ. The mechanism of these changes through Mn-Cu interaction is discussed in the context of Mn-induced parkinsonian injury. (Supported by NIEHS ROI-E008164)
2373 Advanced Approaches for Quantitative Risk Assessment Using Human Data with Applications Across Disciplines

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Health risk and safety assessments developed from human data are typically complicated by confounding and covariates, yet there are obvious advantages to using human, rather than animal, data for risk assessment, particularly when attempting to assess risk or set safety thresholds among sensitive individuals and/or vulnerable populations and life stages. Further, the cumulative effects of multiple stressors and the mixture of environmental exposures from multiple sources are of important public interest but not readily evaluated in animal models. Recent advances in risk assessment modeling of human data for food allergies, pharmaceutical safety assessment, and occupational and environmental health have been achieved to address sensitive subgroups, conduct dose-response analysis and assess cumulative exposures and health risks in relevant disciplines. Although the data needs vary across disciplines, the requirement to quantitatively describe variability in human response to a public health challenge is necessary across all. The objectives of this symposium are to review new approaches for risk assessment using human data and discuss case studies where these approaches have been applied, including biologically based pharmacokinetic modeling, dose-response modeling using smoothing splines, and probabilistic analysis to predict individual and population-level exposure-response relationships. The session aims to foster the use of innovative approaches across disciplines, focusing on risk assessment in sensitive/vulnerable subpopulations or sensitive life stages, and discuss strategies for improved decision-making and risk management.

2374 Evaluation of Food Allergen Exposure Risk Using Quantitative Risk Assessment Modeling


Food allergy risks differ from chemical and microbial risks in that IgE-mediated food allergy is an immune response to food proteins that can be consumed by a majority of individuals without any adverse health effects. Public health officials and food manufacturers must determine how to protect the allergic population without extensively limiting food choices and adversely affecting their quality of life. A question that many (probabilistic) food allergen risk assessment models attempt to answer is the potential risk of unintended exposure to allergen residue. Sufficient clinical threshold data from food allergic individuals (humans) does exist for specific priority allergenic foods. From the available clinical information, statistical dose-distribution modelling of individual NOAELs and LOAELs for objective responses was recorded for each subject in units of mg of total protein from the allergenic food. For each individual, the true threshold does lie between the NOAEL and LOAEL. Individual threshold data were analyzed using interval-censoring survival analysis. The data were fitted to the parametric models (log normal, log logistic, and Weibull) using the SAS LIFEREG procedure (SAS v9.3). This threshold data has been pivotal in the development of quantitative risk assessment models that utilize a number of input variables including the population dose distributions for various allergens, consumption patterns for specific foods or food categories (using dietary databases such as NHANES), and the amount of allergen potentially present in the finished food product to assess the risk of unintended food allergen exposure. Quantitative risk assessment modeling provides a key tool that industry and regulators can use for food allergen risk assessment and management. Progress has been made in the scientific evaluations of food allergen thresholds in sensitive individuals and population modeling, contributing in application to the important components of quantitative risk assessment modeling for food allergens.

2372 Gestational Lead Exposure (GLE) Increases Retinal Progenitor Cell Proliferation, Neurogenesis, and Signaling in Children and Animals

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Offspring of lead-exposed workers or lead-exposed mothers have relatively increased blood lead levels. Previous studies show that low-to-moderate level GLE in women produces a novel and selective rod pathway-mediated (scotopic) increase in the electroretinogram (ERG) of 7-10 year old children: termed scotopic ERG supernormality. In the prospective epidemiologic study, scotopic ERG supernormality was associated with lead exposure only during the first trimester in humans: the period of retinal cellular proliferation and beginning of neuronal differentiation. In rats and mice, these events occur during gestation and through postnatal day 10. Therefore, human equivalent low-to-high level GLE rat and mouse models (prenatal to postnatal day 10) were established. Low-to-moderate level GLE produced scotopic ERG supernormality in adult rats. In rats and/or mice, low-to-moderate level GLE increased the number and prolonged mouse retinal progenitor cell proliferation, delayed the onset of rod photoreceptor and bipolar cells differentiation (late-born neurons), selectively increased the number of these late-born neurons, and delayed the functional development of these neurons and their synapses. No change in normal developmental apoptosis occurred. Alterations in cell cycle entry and exit as well as kinetics underlie these proliferative changes, whereas alterations in selective proneural genes/proteins underlie the increased neuronal differentiation. In contrast, high blood lead levels during gestation, continued low-to-moderate level postnatal to adult lead exposure, or low-to-moderate postnatal only lead exposure produced rod apoptosis and photoreceptor synaptic dysfunction. The toxicological and human health relevance of the mitogenic effects of GLE on retinal progenitor cells and differentiating neurons compared to the structural and functional deficits produced by developmental lead exposure on differenti- ated retinal and brain neurons will be discussed. Supported by NIH Grants EY07551, EY07024 and ES012482.

2371 Enhanced Neurogenesis for Brain Repair following Traumatic Brain Injury

J. Chen. Indiana University School of Medicine, Indianapolis, IN. Sponsor: W. Zheng.

Traumatic brain injury (TBI) is one of the most serious injuries that humans can suffer. TBI causes significant cell death and tissue lesions in the brain, leaving patients with substantial motor disability and cognitive impairment. At present, there is no clinically demonstrated and FDA-approved drug therapy that may heal TBI. Thus, identification of neural stem/progenitor cells (NSCs) in the adult brain holds the hope for repairing the damaged brain following TBI. Our studies along with other reports have found that TBI increases NSC proliferation in the hippocampal dentate gyrus (HDG) in an attempt to initiate an internal self-repair program. Nevertheless, the newly generated hippocampal granular neurons appeared to be particularly vulnerable and prone to death before they mature. Our data indicated that newly-born neurons were significantly reduced in the adult HDG, which may contribute to the learning and memory impairment after TBI. Further studies found that the mammalian target of rapamycin (mTOR) signal pathway was required for TBI-induced NSC proliferation. Activating mTOR signaling promoted the NSC proliferation following TBI, suggesting that mTOR signaling may serve as a novel target for augmenting neurogenesis. In addition, excitotoxicity mediated by a neurotransmitter, gamma-aminobutyric acid (GABA), resulted in a selective newborn-neuron death, while systemic administration of GABA antagonist or a small molecule mimicking brain-derived neurotrophic factor (BDNF) attenuated the newborn neuron death following TBI. These results suggest that the adult neurogenesis after TBI could be enhanced. Thus, elucidation of molecular mechanisms of NSC proliferation and cell death following TBI may lead to development of therapeutic approaches to augment NSC proliferation and prevent cell death in the adult brain for promoting post-traumatic functional recovery. (Supported by NS072631 and NS075733).
assess nonlinearity and the potential for thresholds in a given dataset. In addition, the dsrmooth package contains bilinear (e.g. hockey stick) models for assessing thresholds. A live demonstration of the R tool will be provided, demonstrating the use of this new tool in quantitative risk assessment with its current applications in the study of human dioxin exposure as a case study.

2376 Application of a Probabilistic Framework to a Biologically Based Dose-Response Pregnancy Model to Evaluate Thyroidal Effects for Environmental Exposures to Perchlorate

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Disturbances in the hypothalamus-pituitary-thyroid (HPT) axis in pregnant women leading to hypothyrinemia and hypothyroidism have been shown to cause negative effects on the neurodevelopment of the fetus in utero and the neonate after birth. Iodide deficiency is a major cause for such disturbances, and exposure to perchlorate (ClO4-) and other thyroid active chemicals like thiocyanate and nitrate may predispose sensitive individuals to further alterations in thyroid homeostasis. To better characterize perturbations in thyroid hormone levels due to exposure to environmental chemicals, a quantitative model was constructed. The health endpoint of interest was maternal free thyroxine (fT4) levels, which are critical for fetal neurodevelopment, following inhibition of thyroidal iodide uptake by ClO4-.

Recently, a deterministic biologically based dose-response model was developed for the HPT axis to evaluate the effects of iodide intake and ClO4- exposure on thyroid hormone levels in the near-term pregnant woman and the fetus. In an effort to capture the response of a population of pregnant women to such perturbations, the deterministic model was extended to a probabilistic framework. Global sensitivity analysis and Monte Carlo methods were used to evaluate the effects of variability and uncertainty in the model input parameters on the predicted levels of maternal fT4. The resultant probabilistic model predictions provide a good representation of the maternal fT4 levels and urinary iodide levels observed in the pregnant population of the US and worldwide. Using ClO4- exposure distribution estimated from food sources for pregnant women in the US as input, the probabilistic model predicted urinary concentrations of ClO4- that are in good agreement with that measured in biomonitoring studies. A population-based model was successfully developed and can be used in quantitative risk assessment to better understand the risk of adverse neurodevelopmental outcomes among sensitive life-stages exposed to ClO4- and other thyroid active chemicals.

2378 United States Environmental Protection Agency’s Cumulative Risk Assessment Guidelines


The EPA is developing guidelines for cumulative risk assessment (CRA), which is defined and characterized in the EPA 2003 publication: Framework for Cumulative Risk Assessment, as “An analysis, characterization, and possible quantification of the combined risks to health or the environment from multiple agents or stressors.” An Agency Technical Panel assigned to guideline development has focused on methods to focus CRA on priority stressors, update chemical stressor additivity guidance, and to explore means to incorporate socio-economic stressors as exposure-response modifiers to pollutant stressors of concern, such as regulated chemicals. This presentation provides an update on the CRA Technical Panel’s progress. A draft outline of the CRA Guidelines has served to organize discussions on CRA methods, and the outline include approaches for more completely assessing the human health burden from multiple sources of pollution, as well as means to consider relevant and available data for assessment of health risks posed to vulnerable populations. In addition, the guidance is designed to assist agency programs and regions in the assessment of risk and decision-making, including planning and development of regulation and permits, in consideration of the health burden from multiple stressors. Current Agency CRA applications illustrate experience with CRA methods and provide the basis for the guidelines. From this guideline document, preliminary approaches and next steps for expanding the Agency’s capacity for CRA are developed. In addition, the guidelines summarize several approaches for assessing stressors with common adverse outcome pathways. In the Guideline, the Technical Panel explored the utility and complementarity of Health Impact Assessments within the CRA framework; also, linkages between human health outcomes and ecological structure and function are assessed. This abstract is the opinion of the author and does not necessarily reflect the position or policy of the U.S. EPA.

2379 Genomics of Nonrodent Mammalian Species and Impacts on Nonclinical Safety Evaluation of Pharmaceuticals and Clinical Translation

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Inclusion of a nonrodent mammalian species is required in the safety assessment of pharmaceuticals according to ICH guidelines. The nonhuman primate has been commonly used for nonclinical safety assessment. In recent years, the minipig has emerged as a viable alternative of nonrodent species. The use of nonrodent species for testing aims at limiting the uncertainty in the risk extrapolation process from animal safety data to the human situation. The uncertainty mainly originates from species variation and population heterogeneity in various biological processes. While the use of small sample sizes for nonrodent species contributes to the observed variability or precision in a nonclinical safety study, any phenotypic variability observed is largely attributed to the genetic composition of testing animals. Therefore a better understanding of genetic variation and its subsequent impact on data interpretation from nonclinical safety studies in nonrodents is important. Comparative genomic studies in nonrodent species and humans will aid in better selection of relevant nonrodent species for safety assessment and better understanding of target organ toxicity mechanisms, which should lead to better translatability to humans. In this workshop, we will discuss the use of genetic and genomic data to support understanding of safety endpoints in nonrodent mammalian species, mainly nonhuman primates and minipig. The following issues will be included: (1) an overview of nonrodent mammalian species and genomic technologies used in nonclinical animal studies; (2) a series of case studies that illustrate the application of genetics and genomics in addressing toxicity issues, including species selection, immunomodulation, and target organ toxicity; (3) challenges and future perspectives of using genetic and genomic data to support safety assessment in nonrodent mammalian species; (4) a commentary from the regulatory perspectives on the application of genomics in clinical translation and challenges in regulatory submission.
and ethical issues limit the use of primates for assessment of juvenile toxicity and minipigs, with a litter size between 6-8 piglets, are an attractive alternative. To predict drug-response we monitored global gene expression in seven toxicology-relevant tissues at several time points until young adulthood. The majority of genes are expressed as early as 2 weeks of age and reach equivalent adult tissue levels by 8 weeks of age. Development of the immune system is complete 6 weeks after birth. Testis is the only organ reaching maturity after 4 months, corresponding to young adulthood in humans. Implications for biomedical research will be discussed.

2381 Macaque Monkeys Are Vital Preclinical Models for Biomedical Research

D. O’Connor1 and R. Wiseman1. 1AIDS Vaccine Research Laboratory, University of Wisconsin-Madison, Madison, WI and 2Wisconsin National Primate Research Center, University of Wisconsin-Madison, Madison, WI. Sponsor: H. Wu.

The explosion of data on macaque genomics has profound implications for the future use of these animals. For example, cynomolgus macaques from Mauritius and Indian rhesus macaques in captive breeding centers in the United States have relatively restricted genetic diversity compared to cynomolgus macaques from Southeast Asia and rhesus macaques from China. Studies where maximizing genetic diversity is key would benefit from using more diverse populations, while the opposite is true for studies aiming for lower inter-animal variability in outcomes. In this talk we will discuss the current understanding of genetic diversity among different macaque populations, with specific emphasis on major histocompatibility complex genes that are integral to host immunity. We will also discuss how whole genome sequencing of experimental cohorts can be used to improve the interpretation of complex phenotypes, such as spontaneous control of simian immunodeficiency virus replication. Recently, we sequenced the entire genomes of 20 Mauritian macaques enrolled in an SIV pathogenesis trial and identified several genomic regions that differentiate SIV controllers from progressors. A prospective study was performed to examine the role of one of these regions, containing the immune effector gene granzyme B, in spontaneous viral control. We did not confirm our initial observation. This study led us to better appreciate how limited sample sizes in nonhuman primate studies and the current limitations of macaque genome reference sequences complicate such projects currently. In this presentation we will use this case-study to discuss the potential and challenges of nonhuman primate genomics studies.

2382 Genetics: The Underappreciated Factor in Drug Safety Assessment Using Cynomolgus Monkeys

K. Adkins, Pfizer Inc., Groton, CT.

The cynomolgus macaque (Macaca fascicularis) is a commonly used species for evaluation of toxicity prior to advancing drug therapeutics into the clinic, due largely to genetic and physiological similarity to humans. Like humans, monkeys are also genetically polymorphic, but this variability is not well understood in cynomolgus monkeys. To begin to understand genetic variability in these monkeys, DNA was collected from 100 Mauritius macaques and submitted for whole exome sequencing using a mouse exom capture array (MECA). A total of ~3 million variants were collected from 100 Mauritius macaques and submitted for whole exome sequencing using a mouse exom capture array (MECA). A total of ~3 million variants were identified with a distribution frequency of 1 per 966 bp. A large portion (82.4%) of identified variants had minor allele frequencies greater than 5%. Potential functionally important variants were identified based on in silico prediction, and enriched pathways for genes with relatively high genetic diversity were identified using pathway analysis tools. Together, these data suggest that cynomolgus monkeys have relatively high genetic variability which further suggests that phenotypic variability observed in this species during preclinical studies, may be in part due to genetic variability. In some recent toxicity studies conducted in cynomolgus monkeys at Pfizer, some animals experienced immune-mediated drug hypersensitivity reactions (IDHRs). While the mechanisms(s) and risk factors for developing an IDHR are not fully understood, an association between specific human leukocyte antigen (HLA) alleles and IDHR has been described for some drugs (e.g. abacavir and HLA B*57:01) in humans. An association between IDHR and major histocompatibility complex (MHC) alleles in cynomolgus monkeys was investigated, and data suggest that MHC M3 haplotype B alleles are associated with skin hypersensitivity reactions observed in cynomolgus monkeys. Furthermore, high sequence homology was observed between the HLA alleles associated with human drug-induced skin hypersensitivity reactions and the MHC M3 B region alleles. This illustrates the value of understanding genetic variability in monkeys, and utilizing that knowledge to improve interpretation of phenotypic variability observed in preclinical studies.

2383 Geographic Origin-Dependent Genetic Variation in Nonhuman Primates and Impact for Toxicology Programs


The presentation will introduce the need of a genetic analysis of cynomolgus monkey to improve the selection of the most appropriate species in preclinical safety studies. This is in part dictated by regulatory requirements to select appropriate species for nonclinical safety assessment. The selection of the most relevant non-human primate species requires the understanding of phenotypic differences in cynomolgus monkeys of diverse geographic origins that range from variation in microscopic findings to genetic variation on drugable targets. An overall analysis from more than 100 monkey exomes has allowed the identification of the most variable pathways (e.g. olfactory, HLA, P450 pathways). This potential impact of characterizing genotype-phenotype relationships in non-human primates will be exemplified with a safety genetic case study for drug-induced fulminant liver failure, which provides the basis for a more general strategy for assessing the impact of genetic variation of extreme phenotypes. This approach overall introduces a paradigm shift in genetic characterization of toxicity species extending to other toxicology-relevant species (e.g., dog) by focusing on the genetic variability across key pathways and paves the way for exploring the genetic basis of drug-associated safety signals.

2384 Regulatory Experiences with Submission of Genomic Data for Human Risk Assessment


Genomic technologies are often used in the discovery and development of new medicines through understanding of complex disease biology, determinants of drug response, and mechanisms of toxicity, along with identification of novel biomarkers and development of screening panels for compound selection. In 2004, the Voluntary Exploratory Data Submission (VXDS) process was developed as a non-regulatory, flexible mechanism for scientific exchange between FDA and external scientists (e.g., industry scientists, academic researchers). It was envisioned that the VXDS process would encourage a greater understanding of the state of the art, issues, and challenges of genomic data that could ultimately inform therapeutically relevant pathways for genes with relatively high genetic diversity were identified using pathway analysis tools. Together, these data suggest that cynomolgus monkeys have relatively high genetic variability which further suggests that phenotypic variability observed in this species during preclinical studies, may be in part due to genetic variability. In some recent toxicity studies conducted in cynomolgus monkeys at Pfizer, som
practices that document the impact to the students. The session should be bene-

ficial to all institutions and agencies interested in developing similar programs or 

building on their current programs. In addition, these types of initiatives not only 

provide engaged opportunities for students, but serve as demonstrated evidence of 

program development, mentoring activities, and outreach for those involved in im-

plementing and administering the programs. The workshop will conclude with an 

unstructured panel discussion where attendees can network with the speakers and 

obtain details of how these programs may be scaled to fit their needs.

2386 A Model One-Week Residential High School STEM Pre-

College Engagement Program

R. S. Polleng. Cell Biology, University of South Florida, Tampa, FL.

To respond to the national mandate regarding increasing the pipeline to STEM 

disciplines, the University of South Florida initiated a residential summer STEM 

Academy in summer of 2012. These types of programs are identified as a “high 

impact practice” to inspire potential undergraduates to STEM careers. To date over 

100 students have completed five different Academies and 100% of the participants 

have indicated that they are inspired toward a STEM degree program. Analysis 

shows that 100% of the rising seniors who completed the programs in 2012 and 

2013 enrolled in STEM undergraduate programs. The USF program is an inten-

sive six-day residential program across several STEM disciplines. Faculty from key 

research centers and colleges collaborate to immerse students in basic, clinical and 

translational research trough inquiry, discovery, creativity and hands-on research. 

Importantly, students are also exposed to the multitude of professional career op-

tions that interface with the STEM degree. Professional educators from local high 

schools and colleges build community within the cohort through individualized 

and group mentoring. The presentation will outline: i) the structure of the pro-

gram, ii) the details for the engagement exercises, iii) the cost to implement, iv) the 

connection of science to creativity, and v) program assessment instruments includ-

ing data on the enrollment in USF undergraduate programs.

2387 Designing a Laboratory-Based Summer Program in 

Toxicology and Environmental Health Sciences for High 

School Students

L. M. Alekseen. Pharmacology and Toxicology, Rutgers University, Piscataway, NJ.

As applied sciences, students are often not exposed to the fields of toxicology and 

environmental health sciences until their undergraduate or graduate studies. The 

Toxicology, Health and Environmental Disease Program at Rutgers University 

is a 1-week nonresidential program that introduces rising sophomore, junior, and 

senior high school participants to the concepts of toxicology, provides hands-on 

laboratory training, increases scientific literacy, and highlights various career paths. 

Students are exposed to the fundamentals of toxicology and experimental design 

and learn how to isolate DNA, run PCR and cytotoxicity assays, and stain and 

evaluate cells and tissues by light microscopy. This presentation will describe the 

organizational steps to developing a similar program, the resources available as well 

as the challenges that need to be considered. Attention will also be placed on the 

assessment of outcomes following engagement of high school students in didactic 

and laboratory activities in toxicology.

2388 Increasing Environmental Health Literacy: A Model for 

High School and Undergraduate Summer Internship 

Programs in Government

T. R. Collins. Office of Fellows’ Career Development, NIEHS, Research Triangle 

Park, NC. Sponsor: R. Polleng.

The Summer Internship Program (SIP) at the National Institute of Environmental 

Health Sciences (NIEHS) provides approximately 40 outstanding high school and 

undergraduate students with the opportunity to spend the summer (8-12 weeks) 

obtaining practical research training at NIEHS. These students work side-by-side 

with leading scientists on a research project devoted to environmental health re-

search. Complementing laboratory research training, the NIEHS summer intern-

ship experience also includes a structured program aimed at exposing students to 

broad topics in environmental health. To this end, NIEHS scientists provide a 

series of seminars and problem-based learning exercises focused on a central theme 

each summer; examples of past overarching themes include ‘exposure biology’ as 

well as ‘global environmental health.’ Furthermore, a range of other opportunities 

are provided, including: 1) structured advice on applying to graduate & medical 

school, 2) exposure to careers in the biomedical sciences, and 3) one-on-one career 

counseling. This presentation will cover: a) the structure of the Summer Internship 

Program and resources available from NIH, b) the structure and details of the 

problem-based learning exercises, c) the training program available to summer stu-

dent mentors, and d) key accomplishments of recent NIEHS SIP alumni.

2389 A Model for Undergraduate and Graduate Summer Student 

Programs in Industry

B. A. Pettersen. Drug Safety Research and Development, Pfizer Global Research and 

Development, Groton, CT.

The summer student program at Pfizer has proven to be a highly respected program 

among students and participating colleagues alike. Short-term benefits include 

introducing students to the pharmaceutical industry, advancing important proj-

ects and creating meaningful relationships with universities. Projects may involve 

a current Pfizer project, or may provide an opportunity for the student to ex-

plore fundamental research objectives. Researchers submit proposals by the end of 

January and they are reviewed competitively for scientific merit, business need, 

appropriateness for a summer student, and ability to complete within an 8-10 

week period. Approximately 6-8 proposals are selected for funding by the middle of 

February and the students are placed by the end of March. Undergraduate stu-

dents, who have completed 2 years of college, and graduate students are eligible for 

this program. The internships are typically 10-12 weeks and provide students with 

a positive research-intensive experience that also includes professional development 

training and networking opportunities. At the end of the program, interns have the 

opportunity to present a summary of their summer project at an in-house poster 

presentation. This provides an additional developmental opportunity for the intern 

and a chance to network with other interns from different lines at Pfizer. Colleagues 

in Drug Safety Research and Development, Groton, CT, have worked with numer-

ous students over the past decade who have gone onto careers in science.

2390 “Bridge to the PhD in Biomedical Sciences”: A Program to 

Foster Engagement of Underrepresented Minority Students 

in Biomedical Research

W. D. Atchison. Pharmacology and Toxicology, Michigan State University, East 

Lansing, MI.

A “bridge program” was developed at Michigan State University (MSU) encom-

passing the senior undergraduate year and 1st two years of the Ph.D. program. 

It initially entailed partnership between MSU and the University of Puerto Rico-

Caray (UPR-C), but spontaneously expanded to other UPR colleges, in part due to 

interactions of the PI with student participants at the SOT Undergraduate Student 

Education Session at the Annual Meeting. Ultimately, participants from five UPR 

campuses were included. Annual support was provided for four Ph.D. students in 

Neuroscience-related projects and four undergraduates. Undergraduate students 

spent two summers at MSU doing research after which they spent 1-2 semesters 

at MSU, taking 9 credits of undergraduate courses in neuroscience, continuing 

with the research project begun during the prior summer, and make application 

to grad school. Additional work on science writing skills and enrichment activities 

were provided during the first two years of graduate study. Over five years, 22 un-

dergraduates participated: 19 females and 3 males. Sixteen are now in Ph.D. programs, 
one in an MD/PhD and three in professional school. Two have not yet graduated; 
one is employed in the biotech industry. This program has successfully advanced 

Hispanic students to Ph.D studies in biomedical sciences.

2391 Integrating Gene Expression Profiling into High-

Throughput Toxicity Testing

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NC.

Coordinated programs in high-throughput toxicity testing (HTT) (e.g., Tox21 and 

ToxCast screening programs) currently use assays that evaluate a limited number of 

potential molecular targets. Because of their complete coverage of the genome, 

microarrays have the potential to evaluate the underlying network of most, if not 

all, targets simultaneously. This workshop addresses the growing recognition in the 

toxicology community that technologies that measure global gene expression can 

be adapted for HTT. Significant advantages for integrating these technologies into 

HTT include simultaneous assessment of a greater diversity of potential chemical 

targets, linkage to ongoing large-scale efforts that examine gene expression changes 

after chemical and genetic perturbations in multiple in vitro systems (in particular, 

the Library of Integrated Network-based Cellular Signatures [LINCS] project), 

and the potential for using in vitro transcript profiling as a first step in HTT prior 

to more targeted in vitro assays. This workshop brings together a balanced repre-
sentation of experts working in the field who will address challenges and provide solutions for using these global technologies in HTT and interpreting the results to inform risk assessment. The first speaker will present a comprehensive strategy for how expression profiling can be integrated into HTT, allowing the audience to understand the context of the following talks. The second and third speakers will describe two technologies (RASL-Seq and L1000 platforms) that have promising applications to HTT, highlighting how differences in platform performance can impact interpretation of chemical effects. The fourth speaker will discuss how transcript profiling results derived from cell cultures can be extrapolated to potential dose-relevant effects in the tissues of humans. The last speaker will describe the LINCS project as a model of HTT, in which transcript profiling of ~8000 chemicals was carried out across 17 cell lines. This workshop will be of broad interest to SOT members including scientists interested in the application of in vitro assays to regulatory decision-making.

W 2392 Strategies for Integrating Transcript Profiling into High-Throughput Toxicity Testing
R. S. Thomas, NCCT, US EPA, Durham, NC.

The release of the National Research Council’s Report “Toxicity Testing in the 21st Century: A Vision and a Strategy” in 2007 initiated a broad-based movement in the toxicology community to re-think how toxicity testing and risk assessment are performed. ToxCast and the associated Tox21 effort has screened thousands of chemicals across hundreds of high-throughput in vitro screening assays in concentration-response format to identify potential biological targets and the concentrations at which they are affected. Although this paradigm has been useful for evaluating chemical effects, there is recognition that, as a whole, the suite of ToxCast and Tox21 assays cover a limited slice of biological space. New high-throughput transcriptomic technologies have been developed that could be used to cover a broader slice of biological space for less cost. These technologies could be deployed in a tiered fashion to complement the existing suite of high-throughput in vitro screening assays; however, a number of challenges must be addressed prior to using these technologies. The challenges include what in vitro systems to use that provide the greatest representation of potential biological targets for chemicals, what platform is most appropriate for this application, what subset of genes should be measured, and how test to interpret the profiles. A strategy for integrating these new transcriptomic technologies into high throughput toxicity testing will be presented and the challenges discussed.

W 2393 A High-Throughput Gene Expression Approach to Identify Toxicity Mechanisms

The expression levels of approximately 1400 genes are being determined using a technology called RASL-Seq. This technology is high-throughput, allowing analysis of hundreds of thousands of samples per year. This throughput allows the evaluation of multiple cell lines, exposure levels and time points, and facilitates the generation of a reference dataset of chemicals with known modes or mechanisms of toxicity. This multidimensional exploration enables us to clarify cellular mechanisms of toxicity and adaptation. As an example, environmental contaminants that lead to mitochondrial membrane depolarization are compared to benchmark compounds. For these compounds, RASL-Seq reveals disparate pathways that are disrupted to trigger mitochondrial membrane depolarization. This method can be used to ‘bin’ compounds by ‘toxicity modes’ and associate uncharacterized toxicants with characterized toxicants. As a second example, RASL-Seq is used to compare members of a single drug class to identify off-target specificities, and assess whether they could ‘predict’ clinical adverse events. Several approaches for drawing usable information from gene expression data will be discussed. High-throughput gene expression quantitation provides an opportunity to improve risk assessment approaches for environmental toxicology, and to improve our understanding of adverse events by drugs.

W 2394 Comparison of a Full-Genome Microarray with the L1000 Platform
G. P. Daston, Procter and Gamble, Cincinnati, OH.

Whole genome microarrays provide a great deal of information but are low throughput and high cost, both of which have been serious impediments to the development of large databases for toxicants. The L1000 platform offers a potential solution, in that it uses cell lysates rather than purified RNA (a significant time and labor savings), and measures expression of only about 1000 genes (significant cost savings, at the possible expense of resolving power). We carried out a systematic comparison of in vitro dose-response gene expression results derived from identical sample sets from a number of human cell types using the Affymetrix U133A and the L1000 gene expression platforms. Although there were sensitivity differences, the overall ability to identify large differences in action and identify compounds with similar modes of action was satisfactory in both platforms. These results indicate the feasibility of using a targeted set of genes to generate a large connectivity map database for toxicants at a reasonable cost and timeframe.

W 2395 From Cell Lines to Tissues: Extrapolation of Transcriptional Effects to Human Tissues
J. F. Wambaugh, NCCT, US EPA, Durham, NC.

A new suite of assays in the metabolically-competent, human hepatocyte-derived HEPaRG cell line has been added to the ToxCast screening suite. For 1066 chemicals we have evaluated the chemical treatment-induced changes in expression for a diverse set of 93 genes representative of various biological processes and disease states including: cell proliferation, survival, and death; nuclear receptor (NR) mediated metabolism and transport; oxidative stress; steatosis and fibrosis. Many of the genes are known to be transcriptionally regulated by key NRs, including CAR, PXR, PPARa, and HNF4a. The profiled chemicals include industrial compounds, pesticides, reference in vivo toxicants, and a mix of failed and successful pharmaceuticals. Many of the chemicals are found in the environment and have little or no in vivo data. Through statistical analysis we assess both likely NR activation (as a molecular initiating event) and likely in vivo toxicity assay result (as an adverse outcome) to putatively assign chemicals to adverse outcome pathways wherever possible. For a subset of chemicals for which in vitro toxicokinetic data exists, we predict in vivo doses that produce plasma concentrations corresponding to in vitro bioactive concentrations, which we then compare to in vivo data for the onset of adverse outcomes. This abstract does not necessarily reflect U.S. EPA policy.

W 2396 Using the Library of Integrated Network-Based Cellular Signatures (LINCS) to Characterize the Mechanism of Action of Small-Molecule Therapeutics
A. Subramanian, Broad Institute, Cambridge, MA. Sponsor: C. Corton.

The Library of Integrated Network-based Cellular Signatures (LINCS) is an NIH program that aims to create a network-based understanding of biology by cataloging changes in gene expression and other cellular processes that occur when cells are exposed to a variety of perturbing agents (e.g., chemicals and shRNAs). As part of the LINCS program the Broad Institute has generated ~1.5M gene-expression profiles from the effects of 4,000 small-molecule compounds and 3,000 genes on a diversity of cancer cell lines and primary cells. These reference signatures provide a systematic, unbiased approach to relate novel compounds to established pharmacological classes, gene targets and mechanisms of toxicity. This talk will describe the LINCS resource (data and analytical tools) and demonstrate how they are being used to characterize query signatures. Importantly, as the number of signatures has grown dramatically, it has become vital to use readouts from complimentary assays including phenotypic measurements to prioritize hypotheses for laboratory testing. Analytical approaches to data integration will be described as well as a cloud-based informatics platform that allows analysis within web-browser based user interfaces and programmatic access via APIs. Finally, example applications of these resources for identification of putative gene targets and characterization of efficacy and toxicity biomarkers will be described.

W 2397 Strengths and Weaknesses of Mouse Models in Studies of Immunological Effects of Drugs and Chemicals
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Recent results with high-throughput datasets have raised new interest in discussions on the suitability of mice as models for immunology, inflammation, and immunotoxicology in humans. Mouse models are routinely used in immunotoxicology safety testing, efficacy testing for new drugs, and in basic immunology research. Recent studies from the Inflammation and Host Response to Injury, Large-scale Collaborative Research Program compared humans to mouse models with regard to trauma and sepsis, and raised questions about the validity of mice as a model to study acute inflammatory responses in humans (www.pnas.org/cgi/doi/10.1073/pnas.1222878110). This and other publications and original data from the presenters will provide an opportunity to discuss evidence for and against
mice as suitable models for humans with regard to inflammation, immunology, and immunotoxicology. Data from industry and academia will be presented, along with evidence from various murine strains currently used in the study of acute inflammatory responses and immunity to attempt to identify key differences between mice and humans with regard to inflammatory and immune responses. Presenters will discuss how increased knowledge of these differences could increase the value of the mouse as an animal model for immunotoxicity studies.

W 2398 Mice As an Animal Model in Immunology: Regulatory and Industry Perspectives

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Currently, mice are the best model available for genetic manipulation to study specific issues in toxicology. This is especially true for immunologic parameters: we probably know more about the immunogenetics of mice than any other species (including man). In addition, "humanization" of mice has become a relatively routine method used in drug development, especially for biologics. Although there are significant physiological differences between mice and man, when understood and taken into account, valuable information can be obtained using transgenic mice.

W 2399 A Reassessment of Mice As a Model for Sepsis in Humans

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A recent paper by Seok and colleagues in the Proceedings of the National Academy of Sciences is receiving considerable attention, because its major conclusion is that mice are very poor models for humans in cases of burn injury, trauma and sepsis with regard to correlation of changes in gene expression. However, other reasons for imperfect correlation between mice and humans have been proposed, and we have reported results indicating that some aspects of sepsis in humans are recapitulated reasonably well in mice. In addition, preliminary results based on analysis of variance (ANOVA) of IL-6 concentrations from humans and mice with sepsis reported in the literature, indicated that species (mouse vs. human) had no effect while agent (type of microbe or material that induced sepsis) had a significant effect on the IL-6 protein concentration. On the basis of these and other considerations, we would suggest that abandoning mice as an animal model for immunopathology is not the optimum solution and that a better understanding of the differences between mice and humans would be more useful and cost effective. This work was supported by NIH grant R01AA009505 and SBP is also supported by NIH grant P20GM103646.

W 2400 Xenobiotic Effects on Immunoglobulin Expression in a Humanized Mouse Model

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There are significant genetic differences between the mouse and human transcriptional regulatory regions within the immunoglobulin heavy chain (IgH) gene that may lead to species differences in xenobiotic susceptibility and immune dysfunction that may not be adequately reflected or difficult to model in a humanized mouse model. With in vitro mouse and human B cell models we have seen species differences in transcriptional activity of an IgH enhancer shown to be polymorphic in humans (not mice) and to be associated with various autoimmune diseases. These species differences appear to be partly sequence dependent and partly dependent on differences in the cellular model (i.e. human vs. mouse B cell line). This work was supported by NIH grant R01ES014676.

W 2401 Autoimmune-Prone versus Normal Mice As Models for Toxicant-Mediated Autoimmune Disease

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The concordance rate for developing an autoimmune disease in identical twins demonstrates the etiological contribution of genetic susceptibility. To mimic this requirement “autoimmune-prone” mice have been used to test the ability of toxicants such as mercury and trichloroethylene to promote autoimmunity and/or other types of hypersensitivity. However, the results from these experiments can be confounded by inherent baseline markers of autoimmunity. In addition, some investigators believe that a toxicant cannot be described as immunostimulatory unless it promotes autoimmunity in a “normal” mouse strain. This presentation addresses the pros and cons of selecting “autoimmune-prone” mice for testing potentially immunostimulatory toxicants. This work was funded by a grant from the National Institutes of Health 1R01ES017286.
Epidemiologic evidence played a critical role in IARC’s decision to classify air pollution in general, and PM in particular, as a known human carcinogen. This talk will summarize the epidemiologic studies on which IARC’s decision was based, and discuss issues relevant to public health, such as interaction of air pollution with tobacco smoking and exposure-response relationships for quantitative risk assessment.

The “mechanistic” section of the IARC monograph on the carcinogenic risk to humans posed by outdoor air pollution reviewed, evaluated and summarised all the available information on genetic and related effects observed in experimental organisms exposed in situ, in vitro or in vivo. These included rodents, birds, cattle, plants, Drosophila, and a wide range of eukaryotic (i.e., human, animal, plant, yeast) and prokaryotic cells (i.e., Salmonella). The endpoints examined included mutations, cytogenetic abnormalities, oxidative DNA lesions, DNA strand breaks, stable bulky adducts, and a range of epigenetic (e.g., hypo- and hyper-methylation) and gene expression changes. The detailed summary of the published data reveals that outdoor air pollution, or components of outdoor air pollution (e.g., PM, PM extracts, SVOC, concentrates) readily induce genetic and related effects in a wide range of experimental systems. Moreover, the observed experimental effects correspond to effects observed in humans exposed to outdoor air pollution that are empirically and/or mechanistically linked to an elevated risk of cancer. The wealth of Salmonella mutagenicity data, over 2000 observations of mutagenic potency (per cubic meter of air) collected from over 250 publications, permitted a thorough examination of spatial (e.g., urban/industrial locations versus residential/rural locations) and temporal (e.g., seasonal, diurnal) trends, as well as trends in the “atmospheric burden of mutagenicity” related to PM size, meteorological conditions, traffic density, and contributions from noteworthy sources (e.g., biomass fires, large industrial operations).

More than 20 carcinogenicity studies involving exposures of rodents to outdoor air or extracts/fractions of outdoor air particulate matter were reviewed by IARC. Nearly all of these studies found carcinogenic effects in a variety of strains via a variety of routes (inhalation, IP, SC, intratracheal installation, skin painting) from more than 20 different air sheds around the world. Thus, these rodent carcinogenicity studies were consistent with and supportive of the human epidemiological studies—both of which were supported by a vast number of mechanistic studies. The animal studies bridged and linked the human and in vitro mutagenicity studies, providing additional mechanistic insights. This presentation expands upon earlier published reviews of these data with more recent studies that were used by IARC in their analysis, specifically focusing on the potential for animal models to help provide some of the mechanistic information needed for a better understanding of the carcinogenicity of polluted outdoor air to humans. The views expressed in this abstract are those of the author and do not necessarily reflect the views or policies of the U.S. Environmental Protection Agency.

Endocrine disruption (ED) has become an important topic of public concern. Despite increasing attention, little consensus exists about if/ how low doses of ED chemicals affect homeostasis or even how these activities should be measured or regulated. Critics of current methodologies suggest that gaps in standard developmental and reproductive toxicology studies result in insufficient prediction of human safety in cases with an ED mode of action, arguing that nontraditional study designs and systems biology endpoints should be incorporated into regulatory decision-making. An increasing number of ED research and regulatory studies are now conducted using protocols modified to include ex vivo and in vitro assays, and kinetic modeling, as well as omic and epigenetic assessments. These enhancements may provide many new avenues for scientific discovery, but come at a price of increasing complexity. In this workshop, the strengths, weaknesses, and experimental pitfalls of these techniques will be presented and compared with conventional approaches to assess the consequences of ED. Advantages of such modifications for the detection and assessment of ED compounds will be weighed against their drawbacks using real-world example studies. The studies will first be presented, addressing what modifications were used and whether the “add on” parameters helped with hazard assessment. After each speaker has presented, the workshop will then conclude with a panel discussion session covering the good, the bad, and the ugly—which endpoints are useful and which are too complicated to make work, or generate uninterpretable data. The session will be of broad interest to academic, industry, regulatory, and consultant toxicologists concerned about the current status of ED testing and/or advances in nonclinical ED safety assessment.
Endocrine disruption (ED) is a mode of action which is typically identified by the demonstration of characteristic adverse apical reproductive toxicity findings during a regulatory animal study. As a result test guidelines have been adapted to include a number of estrogenic-sensitive endpoints. These adaptations are very resource-intensive, yet may still be of limited value in understanding the human-relevant ED-associated adverse outcome pathway(s) (AOPs). Thus, there is renewed stakeholder interest in the identification of novel methodologies able to address AOPs. A reduction in animal testing would also be desirable. This workshop introduction will provide an overview of some of these proposed additions (e.g. fetal testis xenographs, steroid receptor reporter assays, kinetic modeling, and assessments of transcriptome, metabolome, and epigenetic changes) and how they might offer increased toxicologic understanding of ED in comparison to classical study designs.

The emerging field of epigenetics has introduced a new layer of complexity in hazard identification. The point of departure of the molecular/epigenetic dose-response relationship is left-shifted, right-shifted, or overlays the point of departure for apical endpoints. In order to begin to answer these questions, our laboratory has performed studies designed to identify chemicals and mixtures that induce the Phthalate Syndrome in vivo. The main part of this presentation will describe a short-term in vitro protocol, termed the Fetal Phthalate Screen (FPS), designed to identify chemicals and mixtures that induce the Phthalate Syndrome (PS) in male rat offspring, by examining fetal testosterone production and testis gene expression. KeIs in the PS AOP. Using this protocol, we were able to correctly classify all the known positives and negatives for PS induction (n~ 30) based upon reductions in testosterone production and reductions in the expression of 20-25 genes. In addition, we found that the magnitude of the fetal testis alterations is predictive of the potency of the chemical to produce PS malformations in F1 males. Phthalates that are not active in this protocol fail to produce the phthalate syndrome in the male offspring, eliminating the need to conduct a postnatal study for these effects. The final segment of the presentation will discuss the utility and limitations of this approach and the use of AOPs in general for hazard identification.

Endocrine disruption is an important topic of public concern with little consensus as to its measurement and regulation. Critics of current practices suggest that the standard parameters measured in regulatory developmental and reproductive toxicity studies are insufficient measures of an endocrine disrupting mode of action, resulting in under-prediction of human hazard. Transcriptomics, miRNomics and metabolomics have been proposed as more suitable alternatives. To test this hypothesis, we performed pre/post-natal reproductive toxicity studies to measure the developmental toxicity of low single- and mixed-doses of three anti-androgens at both classical and molecular endpoints. Doses were selected to represent a LOAEL, resulting in under-prediction of human hazard. Transcriptomics, miRNomics and metabolomics have been proposed as more suitable alternatives. To test this hypothesis, we performed pre/post-natal reproductive toxicity studies to measure the developmental toxicity of low single- and mixed-doses of three anti-androgens at both classical and molecular endpoints. Doses were selected to represent a LOAEL, a NOAEL for endocrine effects, and an ADI. While female offspring demonstrated findings related to anti-androgenicity, the male offspring demonstrated findings related to anti-androgenicity across a variety of classical animal and pathological endpoints in the single- and mixed-LOAEL dose groups only. Metabolomics was found to be more sensitive than classic toxicology; this concurs with our prior observations. The outcomes of the mRNA and miRNA transcriptomic analyses were heavily influenced by the analytical methodologies (e.g. stringency cutoffs) and tools (e.g. databases) selected during analysis, suggesting that methodological standardization is necessary for these datasets to be fit for use in a regulatory context. Moreover, findings from these datasets required additional confirmation of a biological effect, implying that little novel toxicological understanding was gained for the significant regulatory cost. Thus, we believe that the regulatory use of these system biological techniques is premature.

The emerging field of epigenetics has introduced a new layer of complexity in how interactions among the genome, epigenome, transcriptome, proteome, and exposome can manifest as toxicological responses. As connections between alterations in the epigenome/transcriptome/proteome and apical clinical and pathological effects begin to be made, a fundamental question about whether the point of departure of the molecular/epigenetic dose-response relationship is left-shifted, right-shifted, or overlays the point of departure for apical endpoints.

In order to begin to answer these questions, our laboratory has performed studies on estrogenic molecules (DES and E2) across an array of animal platforms from zebrafish to rodent models. Molecular, epigenetic, and apical endpoint responses have been characterized across these species in an attempt to understand epigenetics in the context of product safety assessment. Additionally, the available primary literature was examined for case study molecules that had a wealth of epigenetic as well as classical toxicity data, such as vindoline, DES, and butadiene. We concluded that risk assessments conducted utilizing current points of departure based upon the lowest overall no-observed-adverse-effect level (NOAEL) would also be protective of an epigenetic mechanism. Importantly, the analysis also revealed the challenges in assessing epigenetic changes; the epigenome is in a constant state of flux due to development, aging, and exposure to nutrients, that a single change by itself cannot currently be contextualized as adverse in the absence of a phenotypic anchor. More research, especially additional epigenetic studies which include apical end points, is needed in order to gain a more comprehensive understanding of epigenetic dose-response curves. Therefore, we recommend focusing future research towards 1) examining potential causal relationships between epigenetic alterations and adverse apical endpoints, and 2) understanding the dose-response relationship of epigenetic alterations in comparison to those of the apical endpoints.
2415 Combining an In Vitro Developmental Toxicity Assay with In Vitro Placental Transfer Increases Predictive Value

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For animal welfare reasons an increasing number of compounds are initially assessed for developmental toxicity by *in vitro* tests (zebrafish, whole embryo culture, embryonic stem cell test (EST)) which can be performed rapidly, require only minimal amounts of test compound and directly assess developmental toxicity. However, the kinetics of a compound are not taken into account. In this presentation, the *in vitro* developmental toxicities of five antifungal compounds, all known developmental toxicants and probable EDGs (expressed as BMD50 values in the EST), will be compared to the *in vitro* developmental toxicities in rats (BMD10 values), which were reasonably correlated (R=0.57). However when placental transfer was taken into account using the BeWo transport model, the correlation improved to R2=0.95, thereby demonstrating that kinetics must be considered when assessing developmental toxicity in *vitro*.

2416 World Trade Center Dust and Health—Short- and Long-Term Effects of Exposure


Nearly 3,000 people perished in the attacks on September 11th 2001, 87% of which were in New York City (NYC) alone. More than a decade later people in and around NYC continue to suffer from the effects of this catastrophic event and are still inflicting further damage caused by dust and debris exposure from the collapse of the World Trade Center (WTC). While the etiologies of some illnesses are known, due to the unique set of circumstances and confounding effects, the definitive cause of many are not. The present study is investigating the toxicity of WTC dust exposure in order to help find the cause of the array of chronic illnesses associated with it. F344 rats (8 week old males) were given a single highly concentrated dose of WTC dust. Tissue samples harvested two hours post exposure showed an increased expression of the genes that are related to inflammation and oxidative stress. Metal distribution/persistence studies harvested tissue samples between 0 to 360 days post exposure (DPE). Control (Naïve: N7-DPE and air exposed: A7-DPE) and WTC dust exposed rats lung tissue samples (E7-DPE, E120-DPE, and E360-DPE) were sliced, mounted on Kapton film, and analyzed using the µ-XRF and µ-XRD at Beamline X-26a at the National Synchrotron Light Source in Brookhaven National Laboratory. The elements of interest were As, Bi, Ca, Cr, Cu, Fe, Pb, Mn, Ni, Ti, V, and Zn. E360-DPE has similar characteristics as A7-DPE which includes sporadic, small flecks of Ti and Zn throughout. E7-DPE has the highest background levels of Zn, Pb and As, as well as the largest and most complex hotspots containing AsFeNiTiZn, CuGrFeMnNiPiZn and FeTiVZn. E120-DPE has high background levels of Zn and a ZnFeAs hotspot. Although preliminary, the tissue analyses show that the metal distribution is time dependent and that WTC dust is inflammatory. Future studies aim to address the importance of concomitant subchronic PM exposures on WTC dust toxicity.

2417 Toxicity of Subchronic Aspergillus fumigatus Exposures in BALB/cj and B6C3F1/N Mouse Strains

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Recent natural disasters have increased public awareness of the potential health effects of toxic exposures. Associations between mold and cough, wheeze, and hypersensitivity pneumonitis in susceptible individuals, and asthmatic symptoms in sensitized persons have been reported. However, there is limited toxicological data on the consequences of long-term respiratory exposures to molds. To address this, we have developed an acoustical generation system to deliver fungal spores to mice housed in a nose-only exposure chamber in an effort to replicate environmental exposures. BALB/c (a Th2 skewed strain) and B6C3F1/N (a mixed Th1/Th2 strain) were exposed to 105 *Aspergillus fumigatus* spores twice a week for 13 weeks. Cumulative weight gain was observed in both strains and no differences were observed between the control and fungal exposed groups within the same mouse strain. Histopathology showed comparable pleocellular inflammation, goblet cell metaplasia, and arterial remodeling between the two mouse strains. Flow cytometry analysis of bronchoalveolar lavage (BAL) showed that both mouse strains develop a mixed Th response (Th1, Th2, Th17 and Th22). CD4+ T cells expressing the allergy-associated cytokine IL-13 dominated responses in both strains, although the kinetics of peak expression differed. Kinetics of Th1 responses, which are involved in the clearance of inhaled fungal spores were similar in both strains. Interestingly, eosinophils constituted a higher proportion of leukocytes in BAL from B6C3F1/N mice (36%) compared to Balb/cj mice (9%). Fungal debris and fungal spore genotyping were observed in the lungs more than in the nasal passages of mice. This data suggests that long-term respiratory fungal exposures result in inflammatory pathology in the lung, which does not appear to be dependent on the immunological predilection of the mouse strain.

2418 Functional Characteristics of Immune Cells in Peripheral Blood of Individuals with Pleural Plaque and Patients with Malignant Mesothelioma

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Our previous studies have revealed that asbestos exposure causes decreased functions in T and NK cells, some of which patients with malignant mesothelioma also show, including decrease in cell surface expression of NKp46 on NK cells. The present study examined functional characteristics of immune cells in peripheral blood of individuals with pleural plaque (PL) and patients with malignant mesothelioma (MM), compared with healthy volunteers (HV). The mRNA levels in CD4/CD8 T, CD4 CD8+ CTL, CD56+ NK and monocytes, sorted from PBMC were examined by realtime PCR and compared statistically among the three groups of HV, PL and MM. Some parts of cell population were stimulated with PMA and ionomycin before the analysis. T-bet, Foxp3 and GATA3 mRNAs in Th cells did not differ among the groups, but RORC was low in MM. Both of stimulated Th cells from PL and MM showed high IL-4 mRNA, whereas those of HV showed high TNFα mRNA. Granuzyme B mRNA in stimulated CTL was high in both PL and MM, whereas TNF-α mRNA in those cells was high in MM but not in PL. NKp46 mRNA in NK cells was clearly low in MM but not in PL. NK cells in MM also showed low granuzyme B mRNA. Both monocytes in PL and MM showed high IL-18 mRNA, whereas IL-1β was high in PL but not in MM. The high expression of granuzyme B by both CTL of PL and MM suggests that there might be some kind of abnormal “non-self” cells even in PL. Additionally, high IL-18 mRNA in monocytes by both monocytes of PL and MM suggests inflammatory response related to asbestos exposure. In contrast, the high expression of TNF-α by Th and CTL and the low expression of NKp46 and granuzyme B by NK cells in MM suggest chronic inflammation and impaired natural cytotoxicity related with the pathology of MM following exposure to asbestos.

2419 Metformin or Rosiglitazone Prevent Fine Particulate Matter (PM2.5) Exposure-Induced Vascular Insulin Resistance and EPC Retention

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Exposure to ambient air fine particulate matter (PM2.5) is associated with an increase in the risk of developing cardiovascular disease (CVD) and type 2 diabetes (T2D). Our previous studies have shown that in mice short-term (9days) exposure to concentrated PM2.5 (CAP) induces vascular insulin resistance and the depletion of circulating endothelial progenitor cells (EPCs), while longer CAP exposures (30 days) enhanced diet-induced systemic insulin resistance. Feeding mice HFD (6-12 weeks) led to similar vascular defects. Because treatment with an insulin sensitizer, rosiglitazone or metformin, has been shown to improve vascular insulin sensitivity and dysfunction (including changes in EPC) in diet-induced obesity, we tested whether treatment with either insulin sensitizer had beneficial vascular effects against CAP exposure. For this, male C57BL/6j mice were exposed to air or CAP for 9 days, treated simultaneously with either metformin (graduated up to 300 mg/kg) or rosiglitazone (1 mg/kg) in drinking water, and then vascular insulin sensitivity and inflammation as well as changes in circulating and bone marrow resident EPCs were measured. Interestingly, we found that treatment with either metformin or rosiglitazone prevented CAP-induced vascular insulin resistance, vascular inflammation as well as EPC retention. While CAP exposure decreased levels of phosphorylated Akt (insulin-stimulated) and IkBα in aortas of vehicle-treated (water) mice, insulin-induced Akt phosphorylation and IkBα levels were restored in aortas of CAP exposed mice co-treated with either metformin or rosiglitazone when compared with air controls. Similarly, metformin- or rosiglitazone-treatment also prevented CAP-induced decrease in circulating EPCs and the increase in bone marrow EPCs. Moreover, we found that CAP-induced vascular insulin resistance was linearly related to both CAP-induced depletion of circulating EPCs and the retention of bone marrow EPCs indicating that these effects could be mediated by a common (perhaps pro-inflammatory) mechanism.
Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental contaminants that are a human health concern due to exposures such as second- and thirdhand smoke (SHS). High molecular weight (HMW) PAHs act as complete carcinogens, however, recent evidence suggests that the low molecular weight (LMW) PAHs, typically more prevalent than HMW species, may act through distinct mechanisms to contribute to lung injury, inflammation, and potentially tumor promotion. In C10 cells (a mouse, non-tumorigenic type II cell line), we evaluated two secondhand smoke PAHs in vitro (1-methylnaphthalene (1-MeN) and fluorethene (Flthn)) for their ability to dysregulate gap junction intercellular communication (GJIC), decrease connexin 43 (C43: primary lung gap junction protein) expression and influence localization, activate MAP kinases, and induce several inflammatory markers. Novel in vitro studies used Flthn that was oro-pharyngeally aspirated into C57BL/6 (B6) and BALB/c mice to validate in vivo pulmonary inflammation 1 and 3 days following exposure using bronchoalveolar lavage analysis. In vitro methods used: scalpel-loaded/dye transfer assays (GJIC), immunoblotting (C43 localization), immunocytochemistry (C43 localization), 1-MeN and Flthn dysregulated GJIC and decreased C43 expression. Both p38 and ERK MAPK were activated in response to 1-MeN and Flthn and several inflammation pathways induced, however only p38 inhibition reversed the GJIC dysregulation and inflammation pathways. In vivo, Flthn induced significant inflammatory cell infiltration in both strains, including alveolar macrophages and PMNs, which supports a role for these PAHs in SHS-induced pulmonary inflammation. Future work will address the in vitro mechanisms of PAH-induced lung injury, how these mechanisms may relate to our in vitro data, and the potential for tumor promotion.
to clarify which epigenetic changes are involved directly in the pathogenesis of exposure-related disease and which are the results of the pathological state, they might be useful in safety assessment of chemicals, including pharmaceuticals. The application of epigenomic profiling technologies to chemical safety assessment has great potential for providing novel mechanistic insights into the molecular basis of long-lasting cellular perturbations, including increased susceptibility to disease and/or toxicity. However, a better understanding of some of the key questions, including the following: (i) can we design screening systems to identify epigenetic perturbations that are actual predictors of toxicity?; (ii) what type(s) of epigenetic alterations should be used as biomarkers of exposure?; (iii) can appropriate trans-species epigenomic biomarkers be identified?; (iv) how does the inter-individual variability of the epigenome affect risk assessment?; (v) can these biomarkers and evaluations improve the risk assessment process?; and (vi) what needs to be acquired prior to incorporation of an epigenetic evaluation into the overall chemical safety assessment process?

2425 The Future of Carcinogenicity Testing

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The current carcinogenicity testing scheme was developed in the 1930s and has undergone modifications in the years since. However, considerable advances in biology and knowledge of cancer mechanisms have also occurred over this time, and our increased mechanistic understanding of cancer development and progression coupled with the need to increase the science base of risk assessment warrant a re-evaluation of cancer testing approaches. This roundtable will examine current and future cancer assessment. Specifically, the roundtable will address how improvements in the detection and identification of carcinogens and the utility of this information for human risk assessment should be incorporated into the evaluation of potential carcinogenic risk. For example, recent proposed changes to the ICH S1 cancer risk guidelines have sparked a discussion of the utility of the rodent bioassay. The format of the roundtable will consist of three speakers providing an overview of the current and anticipated future approaches to carcinogen testing. In addition, a panel consisting of the three speakers and three additional toxicologists involved with carcinogen testing from the academic, industry, and regulatory sectors will be convened and will address the future testing paradigm for cancer.

2426 Crafting High-Impact Manuscripts: The Process from Hypothesis through Review and Publication

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Publications are an essential component for a successful career across all sectors of toxicology, including industry, academia, and government. Although mentors provide informal guidance, students and postdoctoral fellows rarely receive formal training on how to develop a high-impact manuscript. Therefore, trainees still have questions regarding the publishing process. A complete understanding of the publication process will benefit junior scientists in formulating research plans, preparing manuscripts, in developing manuscript submission strategies, and in effectively serving as a reviewer—all important elements in a successful career. This session is designed to provide early-career toxicologists with insight into the publication process from the journal’s perspective. Speakers will focus on (1) how to craft a high-impact manuscript; (2) the role of the associate editor, strategies of selecting reviewers, the expectations of a reviewer, and responding to reviewers’ comments; (3) maintaining scholarly productivity in nonacademic careers; and (4) publishing in top-tier journals. Each speaker will also highlight what led to some of his or her most significant publications. Attendees will learn the benefits of publishing in the Society of Toxicology’s journal, how this may help in their unique career path, and define the roles of key players of the publication process. As well, Dr. Marcia McNutt, the editor in chief of Science, will share her insights on what it takes to publish in high-impact journals. This discussion is pertinent to all junior-level toxicologists who are in the process of publishing, undergoing revisions, and reviewing manuscripts. This career-development session will provide the formal training to understanding the entire process of creating a high-quality manuscript.

2427 Chromatin Structure, Genomics, and Transcriptional Responses to Environmental Insults

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Exposure to many environmental toxicants has been associated with epigenetic changes, which can affect gene expression patterns and likely contribute to disease or other phenotypes associated with exposure. The information on the mechanisms by which chemicals may impact gene expression is rapidly evolving, and recent discoveries show how exposures perturb the proteins and processes upstream of DNA methylation and other epigenetic marks. The transition from using epigenetic signatures of exposure as potential biomarkers to identifying their mechanisms allows for better characterization of the biology of how environmental insults are involved in establishing and maintaining gene expression patterns and chromatin state. The participation of the proteins that act as “readers,” “writers,” or “erasers” of the epigenetic code, depositing/removing epigenetic marks or binding to them and recruiting other proteins, as well as other factors such as noncoding RNAs, chromatin remodeling complexes, inter- and intra-chromosomal interactions, and functional genomic elements, is now possible to elucidate using the latest genomic technologies. This symposium will explore how transcriptional regulation may be controlled by the developmental cues and/or environmental stimuli through a series of complex mechanisms which fall under the heading of epigenetic processes or modifications. Through a series of case studies, the basic mechanisms of the environmental control of epigenetic mechanisms will be illustrated. The linkages among exposure, genome, epigenome, and the host genetics will be addressed through data from in vivo and in vitro model systems.

2428 Epigenetic Programmers Targeted during Developmental Reprogramming

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Development appears to be an especially sensitive window of exposure to environmental toxicants. These early life exposures often have profound effects on the health of exposed individuals throughout their lifetime, increasing risk for many adult diseases such as cancer. Although the primary mechanism by which these developmental exposures increase susceptibility to disease in adulthood is thought to be by altering the epigenome, until recently, little was known about how early life environmental exposures disrupted the epigenetic machinery to reprogram the epigenome. In the case of endocrine disrupting compounds (EDCs) that engage nuclear hormone receptors such as the estrogen receptor (ER), we now appreciate that non-genomic signaling downstream of ER activation is a key mechanism for developmental reprogramming. Kinases activated in these signaling cascades inappropriately phosphorylate epigenetic “readers, writers and erasers”, modifying their activity, and altering epigenetic programming. As a result, epigenetic programs installed during development become disrupted, causing changes in the epigenome that persist for the life of the individual and increase susceptibility to disease across the life course.

2429 Epigenetic Dysregulation by Oxidative Stress from Chemical Exposures

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Oxidative stress is a common effect of a number of environmental stressors. Hyoxia can also produce superoxide radicals when the mitochondrial electron transport has been disrupted by lack of oxygen. Recent work related to 2-oxoglutarate dependent dioxygenases makes it clear that there are additional effects of hypoxia and oxidative stress beyond those mediated through Hyoxia Inducible Factors. The 2-oxoglutarate dependent dioxygenases, which regulate epigenetic parameters, include oxidative histone demethylases and TET proteins, the 5-methylcytosine (5mC) hydroxylases. These enzymes require oxygen to function and are also affected by oxidative stress. We believe that the inability to hydroxylate 5mC in DNA (which eventually leads to demethylation of 5mC) and demethylation of a number of histone lysines is due to the inactivation of 2-oxoglutarate dependent dioxygenases, and is likely to have major effects on a cell’s epigenetic program resulting in inherited aberrations in gene expression. We hypothesize that oxidative stress (and hypoxia) induced by a variety of environmental insults is upstream of persistent and inherited epigenetic changes that can lead to diseases such as cancer, via their ability to transiently inhibit the activity of the 2-oxoglutarate dependent histone demethylases and TET proteins. Environmental insults such as arsenic exposure and other agents that produce oxidative stress cause the oxidation of Iron (Fe2+ to Fe3+) in the active site of these enzymes as well as the oxidation of
been linked individually to both genetic and epigenetic changes, understanding including response to environmental exposures. As environmental factors have populations. This model provides a mechanism by which genetic variation or mu-
crease in transcriptional activity. Genome-wide chromatin assays allow for the sub-
tions, and can ultimately affect the expression of regulated genes. For example at substitutions where transcription factors bind can interfere with these interactions and chromatin structure at multiple related regions in the genome. Nucleotide studies demonstrate that single DNA variants can influence epigenetic patterning have generally viewed as independent of the underlying DNA sequence. Recent
w shown that tamoxifen up-regulates immune-response pathways in the breast, and steroi
dial and proliferative pathways in the endometrium. Taken together these studies yield insights into the mechanisms responsible for the clinical efficacy of this important drug, and may elucidate breast cancer drug design in the future.

Tamoxifen is a selective estrogen receptor modulator used for adjuvant therapy and chemoprevention of breast cancer. It also increases the risk of endometrial and myometrial cancer in women. Underlying mechanisms may involve DNA damage, as well as tissue-specific tamoxifen-induced gene expression alterations. Examination of uterine tissues from two species of primates and breast cancer patients receiving tamoxifen, as well as unexposed controls, revealed evidence of tamoxifen-DNA adduct formation in women and both species of monkeys, but not in corresponding unexposed controls. Evaluation of tissue-specific differences in normal human mammary epithelial cells and human endometrial stromal cells showed that tamoxifen up-regulates immune-response pathways in the breast, and steroi
dial and proliferative pathways in the endometrium. Taken together these studies yield insights into the mechanisms responsible for the clinical efficacy of this important drug, and may elucidate breast cancer drug design in the future.

Cancer arises through a complex and multifactorial progressive process of trans-
formation of normal cells into malignant cells caused by a continuous exposure to certain natural and man-made chemicals, including lifestyle and occupational chemicals. It is a complicated multistage process of interconnected causes, events, and various molecular and cellular deregulations driven by the mutational and/ or non-mutational (i.e., epigenetic) events that cooperate and complement each other at every stage of tumor development. In a broad sense, carcinogenesis may be induced through either genotoxic or non-genotoxic mechanisms; however, both genotoxic and non-genotoxic carcinogens also cause prominent epigenetic changes. Through examples of epigenetic alterations caused by exposure to model genotoxic and non-genotoxic chemicals, this presentation will highlight the role of epigenetic events in the carcinogenic process. In addition, this presentation will demonstrate how carcinogen-induced epigenetic alterations may be used in the evaluation of the carcinogenic potential of chemical agents.

Epigenetic mechanisms are known to control heritable gene expression but have been generally viewed as independent of the underlying DNA sequence. Recent studies demonstrate that single DNA variants can influence epigenetic patterning and chromatin structure at multiple related regions in the genome. Nucleotide substitutions where transcription factors bind can interfere with these interactions resulting in the repositioning of nucleosomes, changes in histone tail modifications, and can ultimately affect the expression of regulated genes. For example at the CAMK1D locus, a type-2 diabetes-associated variant rs11257655 in an islet hepatocyte enhancer affects binding of FOXA1/FOXA2 leading to a two-fold increase in transcriptional activity. Genome-wide chromatin assays allow for the detection of genetically-driven epigenetic variation across phenotypically diverse populations. This model provides a mechanism by which genetic variation or muta-
tions in non-coding genomic regions contribute to variability in complex traits, including response to environmental exposures. As environmental factors have been linked individually to both genetic and epigenetic changes, understanding phenotypic effects of the environment will require teasing out the relationship between all three factors.

The fascinating optoelectronic and chemical properties of noble metal nanopartic-
les led to their significant application in nanotechnology and biomedicine. The silver nanoparticles have found widespread use in consumer products ranging from disinfectant, antifouling agents, and textiles, to nutraceuticals, biosensing, diag-
nostic imaging, and therapeutics. This widespread use of nano silver has potential to contribute to human health effects and raise environmental safety concerns. Hence, there is a need to develop an integrated and coordinated approach to gain a comprehensive understanding on the potential toxicity to better guide safe and sustainable use of nanotechnology. The toxicology data available to date on nano silver produced by diverse methods, physiochemical properties (size, shape, and surface coatings), and systems (in vitro, in vivo) used to investigate toxicity out-
comes suggest the issue of dissolution of nanoparticles and silver ions and incom-
plete characterization data. As part of ongoing research efforts within the NIEHS Centers for Nanotechnology Health Implications Research Consortium, investiga-
tions are carried out to understand the biological interactions and response to silver nanomaterials of defined shape, structure, and surface coating. These materials were commercially procured, extensively characterized, and investigated using cell culture systems (human, mouse, rat) representative of diverse organ systems and multiple rat and mouse models (wild type, knock out, collaborative cross strains, physiological states). The biological, physiological endpoints, and toxicokinetics from different routes of exposure indicated toxicity outcomes are dependent on the physicochemical properties, silver dissolution kinetics, cell culture system, or animal strains used. The presentations in this session will share with the scientific community the issues and scientific challenges in integrating this information into predictive health effects risk assessment models and the benefits of collaborative consortium efforts in addressing Nano EHS issues identified by NNI.

Increasing use of silver nanoparticles (AgNPs) in consumer and biomedical products as an anti-microbial agent has led to active investigation of the potential for toxicity and inflammation. Through the NIEHS Centers for Nanotechnology Health Implications Research (NCNHIR) Consortium, we have examined toxicity of AgNPs across >15 cell types representing multiple organ systems and have identified susceptible and resistant cell types. Much of the effort has focused on toxicity of AgNPs mediated by silver ion (Ag+) dissolution, a known toxic ion. However, the contribution of ionic silver vs. nanoparticulate silver to toxicity within a cellular environment is unclear. Further, little effort has been devoted to understanding the role of protein binding (i.e. protein corona) to ion dissolution and toxicity of AgNPs, which likely confounds interpretation of in vitro to in vivo correlation of AgNP toxicity. This presentation will first focus on in vitro studies of AgNP uptake and toxicity across multiple cell types based on a consortium effort. Second, the role of Ag and Ag+ dissolution and metallothionein expression across cell types and within simulated cellular environments including the impact of protein corona formation on dissolution will be discussed. In addition, we will provide evidence of a role for AgNP size and surface coating in modulating formation of a protein corona using proteomics approaches as well as the impact of AgNP-protein corona on cellular uptake and toxicity. Overall, this presentation will provide new compre-
hsive data based upon the NCNHIR consortium effort to study in vitro AgNP toxicity and improvements for in vitro assessment of metal based nanomaterials.

The widespread use of silver nanoparticles raises questions of environmental and biological impact. Since both silver nanoparticles, and silver ions formed by particle dissolution, may impact biological systems, understanding and quantifying toxicity of silver nanoparticles requires knowledge of both the nature of the particles as they interact with the biological system and the concentration of ions formed as a result of particle dissolution. Many factors combine to influence these parameters including: the physical and chemical properties of the nanoparticles as-synthesized,

S 2430 Transcriptional Effects of DNA Damage: Gene Expression Changes Associated with Tamoxifen Exposure in Humans and Nonhuman Primates

M. C. Poirier, NIH/NICI, Bethesda, MD.

S 2431 Genotoxic and Epigenotoxic Effects of Chemical Exposures: One Side of the Same Coin?

I. Pogribny, NCTR/US FDA, Jefferson, AR.

S 2432 Genetics Driving Epigenetics Associated with Altered Complex Phenotypes

T. Furey, Genetics, University of North Carolina at Chapel Hill, Chapel Hill, NC. Sponsor: J. Rusyn.

S 2433 Comprehensive Analysis of Nano Silver Toxicity Profiles: Known, Unknown and Surprise!

S. S. Nadalur and L. S. Birnbaum, Division of Extramural Research & Training, NIEHS, Research Triangle Park, NC.

S 2434 Improving In Vitro Assessment of Silver Nanoparticle Toxicity through Understanding of Ion Dissolution and Protein Corona Formation

J. M. Brown, Pharmaceutical Sciences, University of Colorado, Aurora, CO.

S 2435 Important Characteristics of Silver Nanoparticles and Particle Transformations in Biological Systems

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The widespread use of silver nanoparticles raises questions of environmental and biological impact. Since both silver nanoparticles, and silver ions formed by particle dissolution, may impact biological systems, understanding and quantifying toxicity of silver nanoparticles requires knowledge of both the nature of the particles as they interact with the biological system and the concentration of ions formed as a result of particle dissolution. Many factors combine to influence these parameters including: the physical and chemical properties of the nanoparticles as-synthesized,
the nature of surface coatings added deliberately or due to interaction with the environment, changes the particles may undergo during storage or processing, and the media or method associated with delivery of particles for biological testing. Studies undertaken as part of the NIEHS Centers for Nanotechnology Health Implications Research consortium examined the impacts of particle size and shape, structure, structural defects, the nature of surface coatings, method of particle dispersion and the cell culture media on important physico-chemical endpoints: particle aggregation, the rate and extent of particle transformation and the resultant ion concentrations. The nanoparticles used in these studies were characterized by a wide variety of analytical methods and the transformations examined by in situ and ex situ spectroscopies. Significant results include: defects present in as-prepared particles influenced dissolution and toxicity; proteins in fetal bovine serum increased the ability of nanoparticles to remain suspended in cell culture media, but significantly increased the rate of silver dissolution; the presence of significant sulfur in the biological media enabled the formation of stable secondary silver-sulfide nanoparticles.

2436 The Role of Genes in the Susceptibility to Inhaled Nanoparticles
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Investigators within the NIEHS nanoparticle research Consortium (NCHIR) have collaborated to explore a number of innate factors, including age, pre-existing disease, genetics, and gender, that may contribute to the pulmonary and systemic response to nanoparticles. In particular, this talk will discuss the contribution of genetic background to an adverse biological response which may be more easily determined and partitioned from environmental influence in test animals than in more heterogeneous species such as humans. Such animal research tools have been utilized extensively in the environmental health field and are now being used to explore the role of genetic susceptibility in the toxicity of inhaled nanoparticles. Significant inter-strain differences in response to inhaled nanoparticles were observed in inbred mice and rats exposed to silver nanoparticles by inhalation or oropharyngeal aspiration (OPA). In 28 inbred strains of mice, a wide range of pulmonary inflammation and injury was observed after OPA treatment with 20 nm silver citrate nanoparticles. Similarly, a strain-dependent difference in response was observed in mice treated by OPA with quantum dots and significant differences in the pulmonary response were also observed in 2 strains of rats treated with silver nanoparticles by tracheal instillation. To test the relevance of these studies to the inhalation route of exposure, a subset of 8 strains of mice was exposed to spark-generated silver nanoparticles and again a strain-dependent difference in pulmonary effects, although there were strain differences in the relative response between the OPA and inhalation experiments. Additional collaborative studies have also been conducted to determine whether differences in delivered dose (by inhalation), macrophage function, or activation of the inflammasome might be underlying contributors to the observed inter-strain differences in response. Thus, the collaborative efforts of the NIEHS Consortium have made significant headway in our understanding of nanoparticle toxicity.

2437 Pulmonary Responses to Silver Nanoparticles: Role of Rat Strain, Particle Type, and Route of Exposure

The lung is a major site of exposure to engineered nanomaterials. Exposure can be through the circulation as well as by inhalation. Because the lung is composed of more than 40 cell types, it is critical to identify the location of administered nanomaterials as well as resident or recruited cells that are affected. This was measured in bronchoalveolar lavage fluid (BALF) and lung tissues. Recent use of autometallography to localize silver to specific cells and regions of the lung has expanded our understanding of where silver nanoparticles (AgNP) may impact the pulmonary system. Using well characterized AgNPs supplied by the NCHIR, differences in lung responses were found based on particle size and surface coating, route of exposure (intravenous, intratracheal instillation and inhalation of defined aerosols) and rat strain (Brown Norway vs Sprague Dawley). PVP or citrate coated spherical AgNP of either 20 or 110 nm size were used. Dose responsive increases in BALF macrophages at all timepoints examined (out to 56 days post exposure) and ICPS of lung tissue following inhalation or instillation indicates significant persistence of silver in the lung tissue especially of the 20 nm citrate coated particles, which were also most potent at producing BALF inflammation. Support: NCHIR research consortium, NIEHS grants U01 ES020127, U19 ES019525, U19 ES019536.

2438 Disposition and Toxicokinetics of Nanosilvers
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The effects of particles in biological systems may depend on particle properties (e.g. size, shape, aspect ratio, surface charge), as well as the stability of particles in biological systems. This has been a focus of the NCHIR Consortium investigations of nanotoxicity. As part of the NCHIR consortium investigations, we examined the disposition and kinetics of four different silver nanoparticles administered to female and pregnant female Sprague Dawley rats, administered by IV injection or oral, and either collected blood at various times out to 7 days, or tissues at 24 and 48 hours. The silver nanoparticles were 20 nm and 110 nm particles with either a PVP coating or a citrate coat, and were administered at 1 mg silver/kg by iv injection or 5 mg/kg by gavage. An equivalent dose of silver acetate (1 mg silver/kg) was administered to additional animals. Concentrations of total silver in blood and tissues were determined by ICP-MS. Silver from each nanosilver was rapidly removed from circulation on IV injection, and then released into blood. Differences between particles administered IV were observed for retention in tissues at 24 and 48 hours. Total silver in liver ranked 110 nm citrate > 20 nm citrate > 110 nm PVP > 20nm PVP > silver acetate. In spleen, recovery of both the 110 nm particles were substantially higher than silver acetate or 20 nm particles. Silver was extensively excreted in feces by 48 hours with PVP nanosilvers administered orally, with silver acetate administered iv and orally. PVP coated nanosilvers were more extensively excreted in feces than citrate nanosilvers. For nanosilver, it appears that particulate silver is extensively taken up into tissues, and dissolution to soluble silver may be a driving factor in its elimination. Supported by Grant NIEHS U19ES019525

2439 Epigenetics, Developmental Programming, and Immune Function: Where Do We Go from Here?
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The epigenome is most vulnerable to disregulation during the prenatal/fetal period as certain transient environmental influences can lead to persistent changes in epigenetic marks. These changes can adversely affect human development and health in childhood. Importantly, epigenetic changes that occur early during development may persist throughout life and, in some cases, may result in transgenerational impact on disease susceptibility. Many of the environmental factors that are implicated in infectious and noncommunicable disease risk are known to influence epigenetic programming of immune-related genes. This symposium is aimed at exploring the interaction between epigenetic, environmental, and developmental factors and its implications for childhood, lifecourse, and transgenerational disease risk, and also for immune disregulation and inflammation. In order to achieve this aim, the symposium will start with an overview of the role of both microbial- and mammalian-generated epigenetic marks on immune development and dysfunction and will discuss related health risks and specific vulnerabilities. Next, the impact of prenatal and early postnatal environmental exposures on asthma and allergy risk will be discussed in the context of epigenetics, including alterations in DNA methylation, histone modifications, and microRNA expression of candidate genes. This will be followed by multigenerational and transgenerational outcomes of gestational arsenic exposure on tumor incidence in relation to epigenetic changes and the role of immune-related epigenetic regulation on tumor susceptibility. The symposium will end with a presentation discussing environmental epigenetics, the opportunity for development of epigenetic biomarkers of exposure and disruption of immune cell functions that are associated with disease development, and epigenetic potencies of individual pollutants. Novel, high-throughput technologies for examination of the epigenome as a whole will be leveraged to illustrate these concepts.

2440 Environmental Epigenetics: Pivotal Factors for Programming Immune Function vs. Dysfunction

The generation of epigenetic marks involves exposure to pollutants, drugs, dietary or infectious factors and/or psycho-social conditions that result in: 1) metabolite-driven cellular alterations, 2) the production of cellular intermediaries (e.g., microRNA, methylating enzymes) and 3) ultimately, chromatin restructuring. These
chromatin alterations provide an imprint that not only establishes same-generation, later-life health risks, but also a potential legacy of health risks extending across multiple generations. Immune- and inflammatory-linked gene targets are often affected in the resulting phenotype following exposure, and these same genes are pivotal for facilitating the occurrence of and/or severity of both infectious and non-communicable diseases. Knowledge of pivotal factors such as life stage, sex, and ancestral vulnerabilities can help to identify those populations at greatest risk following exposure. Additionally, because the microbiome is a front-line sentinel for problematic environmental exposures and also contribute heavily to the overall metabolome, their role in epigenetic-programmed disease needs to be included. This presentation will examine the landscape of both microbial- and mammalian-generated epigenetic marks that program immune and inflammatory dysfunction and impact health risks. In particular, it will focus on the questions of who, when, and where as relates to specific vulnerabilities. Finally, the presentation will consider options for prioritized, toxicology-driven risk reduction.

**2441 Prenatal and Postnatal Environmental Exposures and Epigenetic Influences in Asthma and Allergy Risk**


This presentation will discuss the epigenetic regulation following environmental exposures that may underlie the interface between prenatal and early-life environmental exposure and asthma susceptibility. Epigenetic regulation and the effect of environmental exposures in asthma and allergic disease is an exciting topic that has gained a great deal of scientific momentum in recent years. In this presentation, we will review the latest published research on exposures to pollutants, allergens, chemicals and stressors and their emerging influence on DNA methylation, post-translational modifications of histones, and microRNA of candidate asthma and allergy genes. Special attention will be given to environmental exposures during the prenatal and early postnatal period. The pathways important to the allergic immune response that are epigenetically regulated, the key environmental exposures associated with epigenetic changes in asthma genes, and newly identified epigenetic biomarkers that have been linked to clinical asthma will be discussed. The inherent plasticity of epigenetic regulation following environmental exposures offers opportunities for prevention using environmental remediation, measuring novel biomarkers for early identification of those at risk, and applying advances in pharmacoepigetics to tailor medical therapies that maximize efficacy of medical treatment of asthma.

**2442 The Effects of Gestational Arsenic Exposure on the F2 Generation: Role of Epigenetics**

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Recent studies have reported multigenerational and trans-generational outcomes of gestational exposure to a variety of chemicals through epigenetic changes. Elucidation of the mechanism should be required to understand the phenomenon. We have recently found that gestational arsenic exposure increases adult-onset hepatic tumor incidence not only in the F1 male offspring but also in the F2 male offspring of C3H mice (arsenite-F2). The results of reciprocal crossing experiments suggested that some modifications of F1 male germ cell genome by the gestational arsenite exposure augmented tumor incidence in the arsenite-F2 male. The gene expression analyses of the hepatic tissues of the arsenite-F2 male indicated changes in a variety of tumor-related pathways. Current studies are focused on microRNA analysis and genome-wide DNA methylation analysis to explore the involvement of epigenetic modifications. Epigenetic regulation in immune genes/pathways might play a critical role in tumor increase. This talk will focus on the latest data and discuss potential mechanisms of this novel arsenic-induced effect on the F2 generation.

**2443 Early-Life Exposure to Environmental Pollutants and Epigenomic Programming of Immune-Related Diseases**

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Evidence is mounting to support the hypothesis that early life exposure to environmental pollutants such as polycyclic aromatic hydrocarbons, particulate matter, heavy metal ions, environmental tobacco smoke, endocrine disruptors and products from incomplete combustion is associated with increased risk for the development of immune-related diseases. Epigenetics serves as an important mechanism to translate the interaction between the inherited genome and the environment stimuli into phenotypes. Since epigenetic biomarkers persist for long periods in target and surrogate cells, they can serve as “memories” of environmental exposures and predictors of disease susceptibility in later-life. In the context of clinical and public health relevance, epigenomic marks in bodily fluids have shown great promise as biomarkers for exposure and may help uncover disease regulatory pathways. In this presentation, we will focus on: (1) early-life stages as windows of epigenetic reprogramming; (2) the challenges of using genome-wide approaches to interrogate epigenetic reprogramming; (3) the applicability of data from studying surrogate cells; and (4) current technologies used to profile epigenetic marks in different immune cells. The important question of determining the sensitivity and specificity of these biomarkers as tools for monitoring exposure and/or medical intervention will be addressed. Finally, the ability of “ranking” the epigenetic potency of individual pollutants will be discussed.

**2444 Exposure Assessment in the 21st Century: Needs and Challenges Facing High-Throughput Exposure Modeling**

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The release of Toxicity Testing in the 21st Century: A Vision and a Strategy generated a great deal of interest in assessing the utility of high-throughput (HT) in vitro assays in chemical hazard identification. Dosimetric adjustment of in vitro bioactivity data allows the derivation of oral equivalent doses that, on a mg/kg/day basis, provide a comparator to external exposure, allowing generation of putative margins of exposure (MOEs) that may then be employed in prioritization strategies. As federal HT hazard assessments transition to chemicals lacking exposure estimates, developing HT exposure prediction tools becomes increasingly important and is key to inserting risk relevancy into the process. While recent HT modeling efforts have yielded promise, they have concomitantly identified key needs that will require resolution to reduce model uncertainty. Biomonitoring data can play an important role in ground-truthing models, but ongoing surveys only offer limited data relevant for this task. This session will provide an update on the HT exposure modeling efforts currently underway, challenges identified, and additional needs to support realistic estimates of exposure variability, including identification of sensitive populations. This symposium will also provide perspective on the use of such tools in a regulatory setting.

**2445 Evaluating Rapid Models for High-Throughput Exposure Forecasting**


High-throughput exposure screening models can provide quantitative predictions for thousands of chemicals; however these predictions must be systematically evaluated for predictive ability. Without the capability to make quantitative, albeit uncertain, forecasts of exposure, the putative risk due to an arbitrary chemical cannot be rapidly evaluated. We use a statistical evaluation framework to evaluate and calibrate predictive models with exposures inferred from monitoring. This evaluation framework provides three important results 1) the calibrations to exposure data assess the predictive ability of the available models, 2) the scatter of the exposure data about the calibrated predictions is an empirical measure of uncertainty, and 3) the calibration and uncertainty can be extrapolated from limited sets of monitoring data (100s of chemicals) to the much larger (1000s) chemical sets with no monitoring data. For 7968 chemicals tested by the U.S. Federal Government Tox21 consortium, we have made exposure forecasts using use information compiled from multiple databases and production volume. A reverse pharmacokinetic model was used to infer multiple chemical exposure combinations that would be consistent with biomarkers measured in urine samples and reported by the Centers for Disease Control (CDC) National Health and Nutrition Examination Survey (NHANES). For thousands of chemicals with no other source of exposure information, these methods predict human exposure for various demographic groups surveyed by NHANES, including women of child-bearing age and children aged 6-11. This abstract does not necessarily reflect U.S. EPA policy.
Chemical risk estimation requires quantitative information on exposures and toxicological effects. Quantitative exposure information can include chemical intake rates and (bio)monitoring data; however, such information does not exist for the vast majority of marketed chemicals. In addition to limited exposure data there is limited information on chemical use patterns and production and emission quantities. These data gaps require the application of mass balance, statistical and quantitative structure-activity relationship (QSAR) models to predict exposure and exposure potential for humans and ecological receptors. Models and modeling frameworks that can be parameterized and used for high-throughput screening (HTS) with the currently available (limited) chemical information are being developed and evaluated to obtain essential estimates of exposure for data poor chemicals. This presentation provides an introduction to underlying principles of some models used for exposure- and risk-based HTS for chemical prioritization for human health, including tools used in the ExpoDat project (USEtox, RAIDAR, CalTox) and other initiatives (SHEDS-HT). Case study examples of HTS include (i) model applications for screening thousands of chemicals for far-field human exposure, (ii) comparisons of far-field and near-field human exposure model results, and (iii) model evaluations with biomonitoring and monitoring data. These illustrations show how the current tools can be used in a regulatory setting and what improvements in the models and chemical information used to parameterize the models are needed to address uncertainty in HTS exposure estimation.

Biomonitoring provides an integrated assessment of chemical exposure, reflecting internal dose encountered from all exposure routes, but is resource intensive and focused on a relatively small universe of "well-studied" chemicals. Creative approaches to design of biomonitoring studies, interpretation of in vitro toxicologically relevant exposure levels in the context of biomonitoring matrices, and development of broader tools for prediction of toxicokinetic properties of data-poor chemicals are needed to enhance the utility of biomonitoring in population chemical exposure and risk assessment in an HTS framework. In vitro tested concentrations have been compared to blood concentrations in previous assessments, supporting that blood-based concentrations of HTS compounds can be compared to in vitro active concentrations as a prioritization tool. Strategies for use of biomonitoring can move away from comprehensive population-based sampling to use of pooled serum sampling for screening and prioritization. Targeting analysis of pooled serum samples to achieve detection limits below active biological concentrations can provide screening-level information for prioritization of chemicals for further exposure and dose-response evaluation. Study designs using pooled serum samples with replicate pools per demographic group can provide information on both central tendency and population variation of biologically relevant internal exposure levels for comparison to in vitro active concentrations. Empirical examination of existing biomarker datasets can inform likely population variation from the central tendency as a function of toxicokinetics for biomarker concentrations. For example, for persistent compounds, the ratio of 95th percentile population concentration to the population mean is seldom greater than 4; for non-persistent compounds this ratio can approach 30 or more. This talk will address challenges and outline approaches to further integration of biomonitoring in HTS-based exposure and risk assessment.

Comparing high-throughput exposure predictions with National Health and Nutrition Examination Survey (NHANES) data shows that consumer use is an influential variable, even when defined crudely. It follows that more sophisticated modeling of consumer exposure will reduce uncertainties in risk estimates, and so “near-field” exposure models are being developed. This talk will illustrate the use of environmental and biological measurement data to develop generalizable relationships that should be reflected in these models. First, we will demonstrate validation of indoor partitioning models for semivolatile organic chemicals based on simultaneous measures of 60 analytes, including a variety of chemical classes, in indoor air and house dust from 170 homes in two geographic regions. For phthalates, pesticides, PCBs, and flame retardants, simultaneously collected biomonitoring data allows us to identify key exposure determinants. Second, we will use NHANES and environmental measurements to describe population exposure variation and explore how to balance reducing model uncertainty with accurately predicting the highest-exposed subpopulations, since exposure distributions are often markedly skewed and can include extreme values. Finally, many consumer product chemical exposures are correlated, either because the chemicals co-occur in products or because of consumer behaviors. We will present key examples of mixtures at multiple levels, including from measurements of consumer products, indoor media, and biomonitoring, and discuss implications for exposure modeling and risk assessment.
example, in the developing nervous system, multiple cell types including neurons, astrocytes, and oligodendrocytes, interact in the presence of growth factors, cytokines, and other hormones to function within a 3D spatial configuration that can reflect normal biological functioning in a predictive manner. The purpose of this workshop is to take a close look at the novel approaches being applied for biologically-driven assembly, in which exploiting the capacity of an embryo to build tissues and organs from scratch, and the multicellular response dynamics in biologically-driven assembly are facilitating “human-on-a-chip” microscale systems and other cellular-complex culture models for evaluating developmental neurotoxicity. The individual topics will address the progress that has been made concerning how the cellular microenvironment dictates tissue morphogenesis and the importance of 3D cellular architecture in cellular function; identification of signaling pathways that contribute to exogenously-induced developmental neurotoxicity; mini-brain organoid platforms to study complex cellular networks and disease models for drug development, toxicology, and medicine; and the requirement for quantitative outcome measures that are essential to the overall success of the organotypic culture approach in order for it to be predictive of the human situation. Standard approaches will be outlined with the use of positive and negative test agents to allow confirmation of the reproducibility of these in vitro test systems in different laboratory environments. The views expressed in this abstract do not necessarily reflect US EPA or US FDA policy.

W 2451 Engineered Microphysiological Systems for Cell-Based Predictive Models of Developmental Neurotoxicity and Teratogenicity

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The need for human, organotypic culture models coupled with the requirements of contemporary toxic screening (i.e. reproducibility, high throughput, transferability of data, clear mechanisms of action, defined adverse outcomes) frame an opportunity for a paradigm shift. The next generation of toxicity testing formats will require a broadly applicable set of tools for human tissue assembly and wholistic analysis. Toward that end, we have recently focused on: i) generating iPSc-derived cells that properly represent the diverse phenotypic characteristics of developing or mature human somatic cells; ii) assembling organotypic cell culture systems that are robust and reproducible; iii) translating organotypic cell culture models to microscale systems for HTS; and iv) combining genomic analyses with bioinformatics to gain insights into organotypic model assembly and the pathways influenced by toxins. This talk will emphasize recent studies in which we have achieved biologically driven assembly of organotypic vascular and neural tissues. These tissues mimic critical aspects of human tissues, and can be used for predictive neurodevelopmental toxicity, and for vascular toxicity.

W 2452 Probing Signaling Pathways in Developmental Neurotoxicity with Human 3D Neurospheres

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Neurospheres are three dimensional (3D) cell culture models consisting of neural progenitor cells (NPCs), which proliferate in culture and migrate and differentiate into neurons and glia cells thus mimicking basic processes of brain development in vitro. With regards to timing of development, they represent early developmental events occurring within the 2nd trimester of human gestation. Rodent neurospheres are time-matched to the human spheres by the translatingtime algorithm (www.translatingtime.net). We investigate mechanisms of action of selected chemicals for the identification of relevant DNT ‘Toxicity Pathways’ contributing to the ‘Adverse Outcome Pathway’ (AOP) concept. By employing different compounds in the neurosphere assay, we so far identified active cellular signalling pathways like the arylhydrocarbon receptor (AhR), thyroid hormone, Nr62, gap junction intercellular communication, integrins and epithigenic modification by histone deacetylases in NPCs modulating certain developmental processes. Some of those, like the AhR, are specifically active in rodents, but not in humans, while others do not exert species-specificities. Species-specific algorithms for high content image analyses are established to increase the image-based experimental throughput. Knowledge on signaling pathways essential for neurodevelopmental processes will feed into AOP building and thus hazard and risk assessment of DNT compounds.

W 2453 Biological and Medical Applications of a Brain-on-a-Chip


With limited understanding of the complexity of brain development, simple in vitro systems do not represent form and function. Moreover, the difficulty of studying interactions between human genetics and environmental factors leads to lack of knowledge about the events that induce neurological diseases. Rat primary cells such as reaggregating neonatal brain cell cultures have allowed the creation and study of 3D cultures of all brain cell types. Human tissue is rare but most recently the advent of stem cell technologies and especially induced pluripotent stem cells, generated from donor fibroblasts, creates an avenue for human neurospheres in unlimited quantity and of high quality. We have developed such an iPSc derived model: Differentiating iPSc into neural precursor cells and then changing them into shaker cultures allows the production of hundreds of neurospheres of about 250um of diameter of different types of neurons as well as astrocytes and oligodendrocytes over the course of eight weeks. These “mini-brains” allow various studies of cell functionality and substance testing. The unique opportunity to replicate this model from donor cells with specific genetic or neurologic diseases opens up to study gene environment interactions. We have demonstrated this first as proof of principle for Down syndrome cells, currently expanding to autism spectrum disorders. One goal of Toxicology for the 21st Century (Tox-21c) is the deduction of Pathways of Toxicity. The complex mixture of cell types in the mini-brain model and the ongoing maturation of the system can here be a disadvantage. We therefore developed in parallel a model of 3D shaker cultures of dopaminergic neurons derived from LUMHES cells. This model has been used to identify PoT of toxins such as MPP+ and rotenone. Microphysiological systems based on different perfusion platforms are under development and the human mini-brain model is currently combined with some. These examples show that Tox-21c also needs a cell culture for the 21st century.

W 2454 Standards and Minimum Requirements for Validation of Complex Organotypic Culture Model Systems

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The objective quantitation of effect is fundamental to good science. The challenge for in vitro systems is to measure something which drives a phenotype in an animal. Then we need to show that the model system responds the same as the whole animal. The surest way to do this is to use exposures which produce well-characterized effects in animals. In short, one needs at least two rounds of work: the first to develop and characterize the test system and to define the endpoints, and then a second round with known positive and negative exposures to test the response of the model. It is critical that the exposures used to test the model "not" be the same ones which were used to develop the model in the first place. You will have more confidence that you know the true performance of an in vitro model when you have tested many compounds. More compounds give more confidence. Experience with alternative developmental tox models suggests that the closer to 100 exposures you tested many compounds. More compounds give more confidence. Experience with alternative developmental tox models suggests that the closer to 100 exposures you can come (50 positives, 50 negatives), the better. This will pose a real challenge for DNT, a field with many chemicals of concern but relatively few known positives. An even bigger challenge will be measuring effects in the range of human exposures. The current broad crisis of replicability in science suggests that confidence in a method will be best conferred by its simultaneous use in multiple laboratories who work together, compare notes, and consense on a broadly workable and reliable method. Pre-competitive consortia play a key role here. Ultimately, the standards will be largely driven by regulatory authorities, the final consumers of this information. An 80% likelihood of a correct response (a common outcome for new assays) may be sufficient for internal corporate candidate selection, but may be too uncertain for public health decision-making. This continues to be an exciting time, full of promise and huge challenges.

W 2455 Painting the Future of Repeat-Dose Systemic Toxicity Testing: Progress from the European SEURAT-1 Project

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In 2011, a public-private partnership between the European Commission and Cosmetics Europe funded the “Safety Evaluation Ultimately Replacing Animal Testing” (SEURAT-1) cluster of six research consortia, with the goal of filling scientific knowledge gaps and accelerating the development of nonanimal test methods for repeat-dose toxicity testing. Replacement of repeated-dose testing with alternative approaches is a daunting task and will require a complete shift in para-
digm toward a new definition of “adversity” defined at the molecular and cellular level, rather than by traditional apical endpoints. To demonstrate and evaluate the applicability of the alternative methods developed within the consortium, a series of cross-cluster case studies were initiated to (i) develop a series of adverse outcome pathways (AOPs) that can be used for designing integrated test systems, (ii) demonstrate various AOP-based systems for quantitatively predicting repeat-dose toxicity, and (iii) apply information from the predictive systems to a chemical safety assessment. The purpose of this workshop is to present, for the first time, the results and lessons learned from a complementary set of these case studies. The workshop will be of high interest to a broad audience, including industry representatives whose products are affected by the European Union ban on cosmetic animal testing, government regulators interested in in vitro alternatives to animal tests, and academic researchers investigating the mechanisms of chemical toxicity.

**2456 Predictive Power and Robustness of an AOP Construct for Bile Salt Export Pump Inhibition to Cholestatic Injury**

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As a part of the SEURAT-1 program, an AOP framework from bile salt export pump inhibition to cholestatic is was developed. For this purpose, an in-depth survey of relevant scientific literature was carried out to identify key events, including bile accumulation, the induction of oxidative stress and inflammation and the activation of specific nuclear receptors. Collectively, these mechanisms drive both a deteriorative cellular response, which underlies directly caused cholestatic injury, and an adaptive cellular response, which is aimed at counteracting cholestatic insults. AOP evaluation was performed according to OECD guidance. In order to test the predictive power, robustness and reliability of the established AOP, 3 liver-based in vitro models, namely primary human hepatocytes, human liver hepatoma HepaRG cells and human skin-derived precursor differentiated to hepatic progenitor cells are exposed to bosentan, a prototypical cholestasis-inducing drug. In a first instance, it is investigated whether the different AOP information blocks, in particular the key events, can be reproduced in the 3 in vitro settings. In a second instance, potentially new AOP building blocks are identified by applying a number of “omics”-based technologies. This is anticipated to yield novel biomarkers of cholestasis, being a common manifestation of repeated dose systemic toxicity induced by drugs and cosmetics.

**2457 Chemotypes for Mitochondrial Toxicity Prediction**

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In silico models play a key role in the SEURAT-1 program. Specifically they provide the basis for the computational prediction of adverse effects and interpretation of toxicological knowledge. Guided by mitochondrial-related Adverse Outcome Pathways (AOPs), a computational profiler has been developed for mitochondrial toxicity. The profiler is a compilation of chemotypes, developed from the AOPs, which allows compounds to be screened for toxicity and grouped for read-across. The chemotypes represent the next generation of structural alerts which combine not only information on the molecular fragment responsible for toxicity, but also further information to refine the alert which may include properties, descriptors and even local QSARs. The chemotypes developed are based around the molecular initiating events for AOPs relating to mechanisms for uncoupling of oxidative phosphorylation, inhibition of the electron transport chain, induction of membrane permeability transition, alternative electron acceptor and initiation of the death receptor pathway. The funding of the EU COSMOS Project is gratefully acknowledged.

**2458 AOP-Based Classification Model for Repeat-Dose Liver Toxicity**


The objective of this case study is to develop and compare the performances of several Adverse Outcome Pathway (AOP)-based classification models aimed at distinguishing between hepatotoxicants and non-hepatotoxins, with hepatotoxicity being mainly related to three major liver adverse outcomes associated with repeated dose exposure: cholestasis, fibrosis and steatosis. Within SEURAT-1, a well characterised in vitro liver system, HepaRG, was exposed to 90 selected reference compounds (75% known hepatotoxicants and 25% known non-hepatotoxins) and the knowledge of the key events of the three AOPs was used to select the in vitro endpoints to be measured. High-throughput screening (using a 96-well plate format) was employed to test the reference chemicals and the read-outs of the selected in vitro endpoints is performed using high content screening based mainly on automated imaging. The selection of reference chemicals is based on clear and robust evidence that they cause/do not cause hepatotoxicity based on animal and/or human data. The reference chemicals are selected to cover the cosmetic structural space but also to have a high structural diversity. To this extent, along with cosmetic chemicals also pharmaceuticals, pesticides and environmental chemicals are included in the dataset. The prediction goal is to minimise false negative predictions while ensuring an adequate discrimination between hepatotoxic and non-hepatotoxic chemicals. It is foreseeable that the resulting classification model(s) will be useful for the hazard profiling of large chemical sets and priority setting (for further testing).

**2459 Development of a Liver Co-Culture System for Evaluating Adverse Outcome Pathways Leading to Fibrosis**


Chronic liver diseases lead to liver fibrosis and subsequent cirrhosis of the liver. Hepatic Stellate Cells (HSCs) have been identified as key regulators in several adverse outcome pathways (AOPs) leading to fibrosis. In the quiescent state, HSCs store vitamin A and have a balanced liver fibrosis production, but upon (chronic) injury these cells differentiate into myofibroblasts, lose the vitamin A and increase proliferation, motility and ECM deposition. In certain AOPs, this activation can be the result of direct activation of the HSCs, but in majority of the cases it is a response to hepatocyte injury. To develop an in vitro system capable of capturing multiple liver fibrosis-related AOPs, conditions were identified that allow primary human HSCs to be cultured in 3D spheroids in co-culture with hepatocytes (HepaRG) for 21 days. These conditions repressed the natural activation of HSCs normally observed in regular 2D HSC mono-cultures. At 21 days, HSC/HepaRG 3D co-cultures, when compared with HSC and HepaRG 3D mono-cultures, behave better with respect to HSC activation and hepatocyte functionality. Treatment of HSC/HepaRG co-cultures with acetaminophen, a model compound that induces hepatocyte death, induced concentration-dependent HSC activation and showed higher sensitivity when compared to HepaRG 3D cultures alone. The results suggest that the 3D HSC-HepaRG co-cultures may be used within SEURAT-1 as a part of an integrated test system for repeat dose liver toxicity by capturing fibrosis-related AOPs. Further characterization of the model as well as validation with additional model chemicals is underway. The co-culture system is also being converted into a bioreactor equipped with sensors that permit continuous and real-time monitoring of glucose and oxygen consumption, lactate production, lactate dehydrogenase and alanine transaminase production.

**2460 Case Studies on Using In Vitro Molecular Screening, ’Omics, and Computational Models to Support a Quantitative Chemical Risk Assessment and Chemical Read-Across**

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A flexible ’conceptual framework’ has emerged from SEURAT-1 that can be used as a basis for rational combination of information derived from predictive tools to support a safety assessment process or decision to achieve a stated protection goal in the context of repeated-dose systemic toxicity. A full chemical risk assessment requires a comprehensive characterization of the potential hazards to assess and identify the primary endpoints or molecular initiating events that would lead to adversity, and that would drive a subsequent exposure based risk assessment. Two case studies are being developed as proof of concept of the SEURAT-1 approach at the application level: (a) an ab initio assessment as a ’stretching target’ that will highlight gaps for future development and illustrate overall progress made in SEURAT-1 and (b) to use information from the SEURAT-1 predictive tools to support ’read-across’ from a substance of known toxicology to target substance(s) as a practical outcome from SEURAT-1 that demonstrates a particular application of the approach of the ’conceptual framework’, thus giving reassurance that broader application to ab initio prediction of toxicological properties will be feasible. The ab initio case study will show translation of findings and data from in vitro molecular screening and omics studies within the SEURAT-1 consortium for a quantitative mechanistic safety assessment. The prediction goal is to determine a safe dose of an ingredient within a consumer use scenario. The output will be benchmarked against published adverse data for a selection of well-studied pharmaceutical compounds to assess accuracy of predictions; subsequently the approach can be applied to a cosmetic ingredient to bridge from pharmaceutical space to cosmetic space.
Perfluoroalkyl sulfonates (PFSAs) are fatty-acid analogues widely used in the industry as surfactants. Because of their chemical stability and limited renal and fecal elimination, some PFSAs can accumulate in the environment, wildlife, and human serum and liver. Our previous results demonstrated that uptake of certain PFSAs such as perfluorobutanesulfonate (PFBS), perfluorohexanesulfonate (PFHxS) and perfluorooctanesulfonate (PFOS) into human hepatocytes can be inhibited by bromosulfophthalein (BSP), indicating carrier-mediated transport. We recently identified that these 3 PFSAs are substrates of the Na+/taurocholate co-transporting polypeptide (NTCP) expressed at the basolateral membrane of hepatocytes. In addition there is also a sodium-independent portion of PFSA uptake in hepatocytes. Therefore, we evaluated other human liver transporters, such as the organic anion transporting polypeptides (OATPs), for their abilities to mediate the uptake of PFSAs into human hepatocytes. We used CHO cells stably expressing OATP1B1, OATP1B3 or OATP2B1, and demonstrated that there was OATP-mediated uptake of PFBS, PFHxS and PFOS. Time-course studies showed that uptake by all three OATPs was linear for all three PFSAs at least to 1 minute. We are further characterizing this OATP-mediated transport by performing concentration-dependent uptake studies. Taken together, we have demonstrated that OATP1B1, OATP1B3 and OATP2B1 can transport and contribute to the hepatic uptake of PFBS, PFHxS, and PFOS.

**2462 Effects of PFOA and PFOS on Cholesterol Efflux and Gene Expression in THP-1, Huh-7, and Caco-2 Cells**

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Some epidemiological studies suggest that perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic (PFOS) exposure is associated with increased cholesterol levels, although this is inconsistent with studies performed with laboratory animals. Reverse cholesterol transport (RCT) is a coordinated process involving multiple tissues that is involved in maintaining cholesterol homeostasis. Previous studies show that cholesterol efflux from macrophage-derived foam cells (MDFC), an important step in RCT, is increased by treatment with PFOA and PFOS. The purpose of the present studies was to examine the effects of PFOA and PFOS on cholesterol efflux by examining its role on cholesterol transport in MDFC (THP-1, Huh-7 and Caco-2 cells). By understanding the effects of PFOA and PFOS on these in vitro model systems, we will be able to address if there is indeed a mechanistic link between exposure to these compounds and cholesterol concentrations.

**2463 Transcriptomic Effects of Ortho-PCBs on Developing Zebrafish**

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Developmental effects of PCBs are among the most important and least well-understood concerns in PCB toxicology. Ortho-substituted PCBs are far more abundant than the dioxin-like PCBs but effects, especially on development, are poorly known. Zebrafish embryos were exposed to 3 μM of the poly ortho-PCB P153 for two different lengths of time during development, beginning at 48 hpf and extending 6 hrs or 24 hrs. Transcriptomic responses were examined using Illumina RNA-Seq of poly-A selected RNA. After mapping to the zebrafish genome, differential gene expression analysis found that 1182 genes were significantly differentially expressed after 6 hrs of exposure (186 up- and 996 down-regulated), while 265 were differentially expressed after 24 hrs of exposure (90 up- and 175 down-regulated). CYP activity, glycolysis/glucogenesis, and lipid binding and transport were among the downregulated functional gene clusters at both timepoints. Wound-healing, immune function, and endopeptidase inhibitor activity genes were downregulated at short time scales, while crystallins and cation homeostasis was downregulated only after 24 hrs of exposure. CYP2AAs were prominently downregulated at both time periods, as well as in independent experiments over longer exposure times. In contrast, thioeir oxidin redox homeostasis and iron dependent oxygenases (not P450s) were upregulated at short and longer timescales, respectively. Similar exposures to the poly ortho PCB95 and PCB52 are underway. (NIH SP2ES007381, The Belgian-American Education Foundation).

**2464 Toxicological Responses following Oral Exposure to Dechlorane Plus in Zebrafish (Danio rerio)**

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Dechlorane Plus (DP) is a widely used chlorinated flame retardant, which has been detected in various environmental matrices and biota including human samples. Although DP is globally distributed in high frequency, there is little information on toxicity of DP. In order to identify possible toxicological responses of DP in zebrafish, adult male zebrafish were exposed to DP-25, a commercial product of DP. Since DP is insoluble in water, DP-25 was delivered via oral gavage feeding with doses of 0, 0.5, 1, and 3 μg/zebrafish wet weight. Gavage feeding was done twice (day 0 and day 2). After 6 days post first exposure (day 6), blood, liver, testis, and brain were collected to evaluate oxidative stress, DNA damage, and endocrine disruption. There was no effect of DP on mortality. Among oxidative stress markers, catalase activity in liver showed a significant dose-dependent increase after DP exposure while DP did not affect hepatic superoxide dismutase activity. Although plasma thyroid hormone levels were not changed significantly, T4 level showed a decreasing trend following DP exposure. Transcriptions of ocr and shpβ in brain increased in a dose-dependent manner (1.6 and 3.7 fold change for the highest dose group, respectively), partially explaining increased plasma T4 level. Transcription of foxp4 in adult zebrafish was up-regulated in the highest dose with 1.7 fold change. This observation implies that DP may alter sex steroid hormone regulation. The results of this study suggest that DP can induce not only hepatic oxidative stress but also endocrine disruption, particularly in thyroid hormone and sex steroid hormone system. In our knowledge, this study is the first attempt to deliver environmental toxicants to zebrafish via gavage feeding. Successful oral dosing shows that this method will help improve usefulness of zebrafish as a model vertebrate in environmental toxicology researches especially on insoluble persistent chemicals.
Segmented filamentous bacteria (SFBs) are important commensals that are known to respond to changes in the intestinal micro-environment and help modulate immune responses. The environmental contaminant, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) has been widely studied as a potent toxin that suppresses immune responses. TCDD dose-population abundance studies were conducted to observe changes in the abundance of SFBs within the intestine of mice that have been exposed to TCDD. We studied SFB abundances before and after TCDD dosage (30 μg/kg) to mice and found an increasing trend in SFB populations after dosage. Also, fecal samples were collected weekly from mice treated with 0, 0.01, 0.03, 0.1, 0.3, 1, 3, 10 or 30 μg/kg TCDD every four days over a period of 90 days. The animals exhibited a dose-dependent increase in SFB abundance, with mice exposed to higher doses (3, 10 and 30 μg/kg TCDD) showing a significant increase in SFB numbers in their intestines. Therefore, certain bacteria in the mouse intestinal tract are responsive to environmental contaminants and may participate in immune and metabolic responses in the host. Other changes in the intestinal bacterial community structure may have occurred, e.g. Clostridia cluster IV and cluster XIVa, and other bacterial species that may impact the gut immune system.
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