The Toxicologist
Supplement to Toxicological Sciences

57th Annual Meeting and ToxExpo™
San Antonio, Texas
March 11–15, 2018
Important Dates and Deadlines

May 15, 2018 | Scientific Session/CE Course Proposal Submission Deadline
July 15, 2018 | Housing and Registration Open
October 9, 2018 | SOT Awards Nomination and Application Deadline
October 19, 2018 | 2019 Abstract Submission Deadline

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Preface

This issue is devoted to the abstracts of the presentations for the Continuing Education courses and Scientific Sessions of the 57th Annual Meeting of the Society of Toxicology, held at the Henry B. González Convention Center, San Antonio, Texas, March 11–15, 2018.

An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 561.

The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 584.

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Scientific Session Types:

- Continuing Education Courses
- Education-Career Development Sessions
- Featured Sessions
- Historical Highlights Sessions
- Informational Sessions
- Roundtable Sessions
- Regional Interest Sessions
- Platform Sessions
- Symposium Sessions
- Poster Sessions
- Workshop Sessions

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The Mobile Event App and Online Planner are available via the SOT website and app marketplaces. These mobile tools enable you, the attendee, to engage with organizers, exhibitors, and each other, and to manage your time and maximize your experience during the Annual Meeting. You also can access ePosters electronically via the Mobile Event App until May 15, 2018.

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This abstract book has been produced electronically by the Society of Toxicology. Every effort has been made to faithfully reproduce the abstracts as submitted. The author(s) of each abstract appearing in this publication is/are solely responsible for the content thereof; the publication of an article shall not constitute or be deemed to constitute any representation by the Society of Toxicology or its boards that the data presented therein are correct or are sufficient to support the conclusions reached or that the experiment design or methodology is adequate. Because of the rapid advances in the medical sciences, SOT recommends that independent verification of diagnoses and drug dosage be made.
Recent advances in genome editing using CRISPR-Cas9 and related technologies have revolutionized the ability to manipulate genes in a rapid, precise, and flexible manner. These advances have spurred an explosion of interest in the possible ways in which genome editing can improve human health. This course will provide an overview of CRISPR-Cas9 systems, the structure and function of CRISPR-Cas9, the repurposing of CRISPR-Cas9 for genome engineering, and recent advances in genome editing and the application of these techniques to toxicology. These include its use in screening the genome in different biological systems for gene pathways related to sensitivity or resistance to chemical toxicity, for elucidating the pathways of biological response to chemical stressors, and other applications related to the understanding of mechanisms and the environment interaction. The Regenerative Science for Environmental Health Decisions (ESEHD) Standing Committee of the National Academies of Science, Engineering, and Medicine serves as an important link between the National Academies and the Society of Toxicology. To foster this collaboration, the ESEHD Committee is pleased to sponsor this course on the use of advanced genome-editing techniques in toxicology, which follows a meeting on gene editing in toxicology and environmental health held at the National Academies’ Headquarters in January 2018.

In the last decade, the fields of toxicology and risk assessment have undergone an extensive shift towards the development of alternative testing methodologies that potentially can be used to assess the safety of chemicals and to reduce animal use in toxicological research. New approaches, including molecular biology, computational and systems biology, high-throughput screening (HTS) assays, automated analytical methods, and robotic implementation, are generating toxicological data at unprecedented speeds. Compared to traditional animal toxicity studies, advanced HTS methods, reach-across approaches, in silico tools, and other alternative methodologies hold considerable promise to define biological activity profiles of chemicals. The first step towards better understanding the application of these new methodologies and tools for safety assessment of chemicals is an interdisciplinary approach which: 1) promotes interaction among scientists from diverse backgrounds (e.g., toxicologists, chemists, biologists, mathematicians, programmers, and risk assessors) and 2) provides hands-on training to demonstrate the utility and challenges associated with the use of these alternative testing methods in different sectors. This course will provide a unique training program to equip attendees with all the necessary knowledge and know-how to use and apply data from HTS assays, in silico tools, and other emerging technologies, such as virtual embryo, in the safety evaluation of chemicals. It is a learning tool aimed at providing training to scientists interested in applying the latest approaches to the safety assessment of chemicals, as well as students and researchers interested in improving the existing methods and developing new alternative methods for toxicological research. The course will include an overview of each of the methodologies (HTS methods, in silico tools, virtual embryo) and case study exercises to demonstrate the use of data from these methods and the use of tools in different sectors, such as pharmaceuticals, consumer products, food, agricultural products, and environmental toxicants.

The immune system has long been a sensitive target of environmental pollutants, industrial chemicals, and pharmacological agents. For example, several federal laws and guidelines have data requirements for immunotoxicity. However, recent studies by an industry trade association and the US Environmental Protection Agency Office of Pesticide Programs determined that no clear signs of immunotoxicity may arise from conventional toxicity studies and that additional immunotoxicity testing may only be recommended if “primary indicators” of immunotoxicity arise from conventional toxicity studies. This approach harmonizes with the “weight of evidence” concept that is discussed in the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines S8 for human pharmaceuticals and Part 158 for pesticidal substances and certain industrial compounds. This course will provide an overview of the types of immunotoxicity tests often used to meet US data requirements for agents regulated by the US Environmental Protection Agency (EPA) and the US Food and Drug Administration. The course will address the “weight of evidence” concept by defining immunotoxicity, discussing the historic aspects of immunotoxicity testing, highlighting some of the current advances in immunotoxicology assessment, including high-throughput analysis, immunotoxicogenomics, developmental immunotoxicology, and the creation of adverse outcome pathways for immunotoxicity. The second speaker will address the particular data requirements under various laws and guidelines and their applicability to regulatory immunotoxicity. Speaker number three will go into detail about specific required tests under existing laws and guidelines, as well as novel and innovative ways of meeting data requirements. The fourth speaker will provide an overview of the utility of experimental animal models and their predictive value for understanding potential risks toward human health. Finally, the fifth speaker will delve into the information that can be gleaned from human blood samples and how these data can be used to better predict health and disease in exposed/treated humans. Each presentation will include case studies and/or examples of immunotoxicity assessment strategies applied to agents or classes of agents under study, being considered for approval, or under regulatory scrutiny.
tified nonclinically, especially with poorly designed nonclinical studies or irrelevant animal test systems. In addition to addressing the unique aspects of strategies for developing biologics, this will discuss topics such as the selection of relevant species, the role and interpretation of immunogenicity, and the current regulatory challenges. Selection and evaluation of the most relevant species for biologics programs is fundamental. Immunogenicity, the ability of a biologic to elicit an immune response leading to formation of antibodies that can bind to the biologic, can confound and cause concern as to interpretability and translatability of these findings to the clinic. A broad overview will be given of how immunogenicity and other immune responses in animals play a role in the interpretation and assessment of toxicology studies. A brief primer will be offered on current regulatory guidance, in addition to highlighting complex issues that are frequently faced when reviewing applications for biopharmaceuticals. Examples from US Food and Drug Administration submissions will be discussed to illustrate these challenges and to present scientific and regulatory strategies that have been used in the design or review of nonclinical programs that support biopharmaceutical drug development. This course will provide an understanding of the considerations of key issues for advancing these therapeutics safely in the clinic. This requires a strong understanding of the biology of the target and also a good comprehension of the caveats and limits of the current nonclinical models and an ability to design fit-for-purpose, nonclinical safety testing funnels adapted to the test agents being developed.

**1006 AM06: In Vitro Testing: Tales from the Real World**

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Advances in science and technology have paved the way for a paradigm shift in toxicity testing. We now have the opportunity to more efficiently evaluate substances and better protect human health and the environment by using approaches grounded in human mechanisms rather than animal models. These in vitro tests—namely, skin irritancy, skin sensitization, and systemic (oral, dermal, and inhalation) toxicity—are commonly conducted on medical devices, pesticides, industrial chemicals, pharmaceuticals, cosmetics, and other substances. Thus, it is important to implement rigorous alternative acute toxicity-testing approaches that will protect human health and the environment while reducing the time, cost, and animal use associated with traditional toxicity testing. The goal of this course is to teach attendees about existing in vitro, in chemico, and in silico acute toxicity tests and how they have been successfully applied in integrated approaches to evaluate the toxicity of a wide range of substances. Other approaches, such as the use of waivers or the Globally Harmonized System of Classification and Labeling of Chemicals (GHS) mixtures equation, will be discussed. The presentations will also highlight the remaining challenges that need to be overcome before alternative methods can be implemented globally and accepted by regulatory agencies. This course will be of interest to toxicologists from diverse sectors, including those from the chemical, pharmaceutical, medical device, and personal care product industries, along with others who want to learn more about currently available non-animal tests and how to use them.

**1007 AM07: Physiologically-Based Pharmacokinetic Modeling to Support Modernized Chemical Safety Assessment**

M. Yoon¹ and A. Paini², ¹SciVation, Research Triangle Park, NC and ²European Commission Joint Research, Ispra, Italy.

Physiologically-based pharmacokinetic (PBPK) models have been applied to chemical risk assessment for more than three decades. Extrapolation of toxicity findings to humans has been a major application of PBPK models in risk assessment. Under the proposed new toxicity testing paradigm, which relies on data from human-relevant in vitro toxicity assays interpreted through computational approaches, PBPK models have been redefined as a critical translation tool for quantitative in vitro to in vivo extrapolation. The models would link effect concentrations in cell-based assays to equivalent human exposures. PBPK models provide a biologically relevant integration platform to describe the absorption, distribution, metabolism, and excretion of chemicals based on a wide range of in vitro, in silico, and, if available, in vivo information. This course will provide an opportunity to revisit the basic principles of PBPK modeling with a special focus on supporting chemical risk assessment under the new toxicity testing paradigm. In addition to describing the basics of model construction, recent advances in model parameterization, including in vitro to in vivo extrapolation and in silico predictions, will be presented. Evaluation of model performance and reliability along with use of available human data will be discussed. Development and application of the PBPK models to support risk-based decisions in different tiers of risk assessment will be presented. A hands-on demonstration will be provided, using a free online simulation tool (PLETHEM) to demonstrate the workflow of building and parameterizing a PBPK model, simulating different human populations, and applying the model to translate concentration-effect relationships from cell-based assays or in vivo studies to the dose-response relationship in target human populations to support chemical risk assessment. The course will address continuing challenges and future directions in PBPK modeling.

**1008 AM08: Developmental Neurotoxicity Testing: Current Practices and Latest Advancements**

K. Ryan¹ and S. Makris². ¹NIH, Durham, NC and ²US EPA, Washington, DC.

The potential for neurotoxicity in adults and children remains a high public priority due to concerns that recent increases in the prevalence of neurological disorders may in part be due to chemical effects. Guidance on developmental neurotoxicity studies in rodents currently remain the gold standard for risk assessment. However, these studies are triggered using the current guidelines due to the lack of specific requirements in the guidelines, the subjective nature of these tests causing interlaboratory variability, lack of relevance of some of the assays to human-specific outcomes, and the lack of power to capture subtle deficits. To address these issues there is a significant push to develop alternative strategies for developmental neurotoxicity testing and to recommend strategies for harmonizing guideline studies for use by regulatory agencies. This course is designed to highlight the current state of the science and recent advancements to identify an integrated strategy for developmental neurotoxicity testing. The course will begin with an introductory talk on the current practices with guideline toxicology studies, as well as developments and recommendations for them (US Environmental Protection Agency (US EPA) 870.6300, Organisation for Economic Co-operation and Development (OECD) 426, OECD 443). The next two presenters will focus specifically on the current state and advancements, and recommendations for developmental testing (learning, activity, attention) and neuropathology. The subsequent presenter will then highlight how developmental neurotoxicity is assessed in the clinic, provide case examples, and speak on the parallels between human and rodent studies. The last presenter will provide a practical strategy to rapidly screen for compounds with unknown neurotoxic potential to prioritize for further testing and how to incorporate triggers from those screens in further assessment of in vivo developmental neurotoxicity studies.

**1009 PM09: Consumer Products Safety Assessment: Progress in the Use of Alternatives to Animal Models**

K. E. Page¹ and T. Hartung². ¹The Clorox Company, Pleasanton, CA and ²Johns Hopkins Center for Alternatives to Animal Testing (CAAT), Baltimore, MD.

To meet consumer preferences and changes in the regulatory landscape, the use of animals for the safety evaluation of consumer product ingredients and formulas is decreasing across industries, including cosmetics, personal care products, and household products, and other consumer products companies are soon to follow. The purpose of this course is to provide an encompassing overview of the progress in the field of safety evaluation of consumer products ingredients using alternative approaches. The introductory presentation will map the current landscape of alternative methods for assessing consumer product safety, including highlighting the holes still remaining in the field. Occasional resistance to change by regulatory agencies can be frustrating to the industry at large. However, recent government-industry collaboration has shown the potential for helping the progress. The first presentation will provide insight into the current regulatory landscape of the US Consumer Product Safety Commission (CPSC) animal testing policy, including what methods are approved and recommended, as well as the struggles faced in getting there. A roadmap of where testing and regulations are likely headed will also be presented, including what challenges await. Several alternative methods have been developed and validated to assess product safety and are now considered acceptable by regulatory agencies. However, the path to their integration into safety assessments and communication to scientists-at-large is still a work in progress. The second presentation will showcase available options for alternative testing strategies, as well as struggles and triumphs in getting methods accepted by regul-
latory agencies. The final two presentations will provide case studies of how alternative testing is used in the cosmetics and cleaning products industries, as well as specific examples from L’Oréal as a trendsetter in the field and regarding method development and from Clorox about how to navigate the current regulatory requirements for cleaning products and animal testing. The course will provide a sound understanding of the current and proposed future state of alternatives to animal testing in consumer product safety assessment.

### 1010 PM10: Evaluation of Leachable Substances from Materials with Applications in Foods and Pharmaceuticals: Science- and Risk-Based Approaches

G. L. Erexson1 and K. L. Li2, 1AbbVie, North Chicago, IL and 2Amen Inc., Thousand Oaks, CA.

Polymeric materials commonly used in food and pharmaceutical manufacturing and packaging components are known to leach chemical substances into the final products. The leachable substances may present potential safety risks to consumers and patients. The goal of this course will be to provide an overview of scientific and technical considerations relevant to the assessment of leachable substances covering historical and current context on patient safety and product quality, collaboration between chemists and toxicologists, best practices for deriving chemical-specific safety margins, and use of in vitro and in vivo tools to apply the 3R principle to replace, reduce, and refine animal testing. This course will provide a comprehensive overview of the risk assessment process for leachable compounds from food contact and pharmaceutical materials.

### 1011 PM11: Lead Optimization of Therapeutic Small Molecules: From Drug Target to Clinical Candidate Selection—Strategies and Decision Making

D. Diaz1 and D. Misner2, 1Denali Therapeutics Inc., South San Francisco, CA and 2Atos Biopharma, South San Francisco, CA.

From the decision to drug a chosen target with a small or large molecule to the selection of a lead candidate to take into Good Laboratory Practices (GLP) toxicology studies, discovery toxicologists engage an arsenal of tools and strategies with the objective of selecting molecules with a safety profile that provides an optimal chance of clinical success. This is best achieved by early safety involvement in target selection and target de-risking, selection of the best possible chemical matter with minimal off-target effects through lead optimization, and robust safety characterization and investigation of safety issues as they arise. The premise is that robust and thoughtful early safety involvement would reduce attrition in later phases of drug development. This course will provide a comprehensive overview of the strategies and approaches leading from drug target selection to lead identification, optimization, and selection of clinical candidates for first-in-human studies. The first presentation will address the safety considerations in target selection, to ensure that the intended targets are tractable from a safety perspective, and that the relevant questions are addressed and the proper lead optimization paradigms are in place for the particular program. The second presentation will tackle the critical aspects of selectivity for small molecules, including related and unrelated off-targets, and how to ensure that the molecules that are progressed are screened appropriately to ultimately have minimal off-target effects. The next two presentations will focus on two essential aspects of lead optimization: 1) cardiovascular safety and 2) genotoxicity, for which in vitro models have been particularly effective in minimizing liabilities. The session will continue with a presentation focused on particularly promising in vitro models and strategies to minimize hepatotoxicity: these approaches are becoming increasingly important in drug discovery since preclinical in vivo studies poorly identify human-relevant hepatotoxins. The course will conclude with a presentation that integrates the different aspects of lead optimization discussed previously with in vivo data generated in pilot toxicity studies and will discuss how to incorporate these data into deciding whether to move into GLP toxicity studies. All presentations will focus on the practical aspects of implementing thoughtful de-risking strategies and on how data-driven decisions are made with the data outputs; presenters also will provide relevant examples to illustrate these approaches. The course will provide understanding of how to address safety for small molecules from the inception of a program to candidate selection, into investigative new drug (IND)-enabling studies, and provide to evaluate and integrate relevant data, and how to make good decisions related to compound progression.

### 1012 PM12: NGS-Based Technologies Enable Biomarker Development and Discovery in Toxicology

B. A. Merrick1 and A. B. Nixon2, 1NIHES, Research Triangle Park, NC and 2Duke University Medical Center, Durham, NC.

Next-Generation sequencing (NGS) represents a series of powerful platforms that have revolutionized DNA and RNA analysis. The simultaneous sequencing of millions of DNA molecules can rapidly provide mechanistic insights into toxicology and biomarker discovery. Evolution of NGS technologies has improved the overall sensitivity and accuracy of these sequencing platforms, allowing for the development of new biomarker assays from tissue, blood, and other biofluids. NGS technologies can identify potential molecular indicators at the cellular and toxicopathological level in response to xenobiotics. The goal of this course is to review NGS-based technologies, demonstrate how they can be used as tools for target discovery in tissue and blood, and suggest best practices for optimal sample analysis and processing in the toxicology setting. The technological transition from microarray toward NGS platforms in toxicology will be briefly reviewed. In the first presentation, a broad overview will be provided on the development, validation, and implementation of circulating biomarkers from a clinical perspective. Emphasizing lessons learned from the clinical oncology field, where biomarker development is key to disease treatment, the implementation of NGS and its potential use in toxicology will be discussed. The second presentation will describe the roles of non-coding RNAs as potential biomarkers. RNA-Seq data will be presented on long-noncoding RNAs (lncRNAs) and microRNAs (miRNAs), which are small ~22nt RNAs and represent promising new biomarkers; the isolation, NGS analysis, species, cell and tissue specificity, and toxicological responses in humans and animal models. IncRNAs are an exciting and emerging area in toxicology. The third presenter will focus on another type of noncoding RNA, microRNAs (miRNAs), which are small ~22nt RNAs and represent promising new biomarkers; the isolation, NGS analysis, species, cell and tissue specificity, and toxicological responses in vitro in tissues and biofluids will be discussed. The fourth presentation will highlight best practices for optimal sample collection to ensure success with downstream NGS applications, followed by an introduction to whole exome sequencing (WES) in toxicology. WES provides the coding and noncoding genome to discover new sequence variants. A new WES platform, which is designed for rats, with future directions toward analysis of circulating, cell-free DNA (cfDNA) as a potential biomarker for targeting specific biological processes, such as inflammation, will be described. The final presentation will involve NGS methods in epigenetics, using whole-genome bisulfite sequencing (WGBS), as well as other approaches to assess changes in DNA methylation after chemical exposure. Case studies will include the valuable role of zebrafish as an alternative animal model in chemical toxicity screening for NGS-based environmental epigenetics. This course will be of broad interest to investigators in academia, pharma, and government wishing to explore and expand their interest in novel NGS-based approaches with an emphasis on the development and discovery of biomarkers for the detection of xenobiotic toxicity and exposure.


K. Muldoon Jacobs1 and A. Richarz2, 1NIEHS, Research Triangle Park, NC and 2European Commission Joint Research Centre, Ispra, Italy.

Understanding, describing, and, if possible, quantifying uncertainties is an essential part of risk assessment which needs to be communicated clearly and in an informed decision-making context, with the provision of a transparent statement of the likelihood of possible outcomes as a basis of building confidence in decisions taken. This is particularly true for risk assessments that rely on new toxicological methods with which the risk assessment community does not have the benefit of historical experience. The course will clarify the nature and sources of uncertainties and variability and give an overview of existing initiatives and available guidance for uncertainty evaluation for chemical risk assessment in the regulatory context. Current practice in regulatory review will be discussed, as well as challenges in application for the risk assessor/manager (often the same person) in industry. The importance and challenges of communicating uncertainties also will be addressed. A special focus of the course will be the characterization of uncertainties for alternative methods used for chemical hazard and risk assessment, bearing in mind that the incorporation of new toxicological methods into risk assessment is still happening, and that we still need to describe and assess the associated different uncertainties. The new methods, for example, combine in silico, in vitro, and high-throughput toxicokinetics approaches to predict hazards and to provide quantitative estimates of effect levels and are then combined into models to predict in vivo effect levels, such as lowest-observed-adverse-effect levels (LOAELS).
Approaches will be shown to quantify uncertainty in these individual inputs, as well as methods to combine uncertainty across all inputs in the final models. Furthermore, uncertainty and variability are compared with those in the in vivo databases that are used as benchmarks for the new models. Another example will include consideration of uncertainties for the non-chemical-specific Threshold of Toxicological Concern (TTC) approach as compared to traditional blood-based assessments. The course will further describe state-of-the-art mathematical, statistical, and other methods, such as expert elicitation, to characterize and quantify uncertainty and tiered approaches to handling uncertainty in risk assessment. Examples will illustrate qualitative, deterministic, and probabilistic uncertainty assessment. Two case studies will show how these methods can help risk assessors and support decision-making: 1) the use of Bayesian-belief networks to quantify the uncertainty in the potential of a chemical being a skin sensitizer in the light of competing evidence and 2) a mathematical model for an adverse outcome pathway (AOP)-based risk assessment, defined and parameterized by vitro data sources. Overall, the purpose of the course is to give an overview of the concept of uncertainty and to identify existing resources on uncertainty characterization and reporting in guidance related to hazard assessment, as well as available mathematical methods. Practical examples based on case studies from practice in various sectors will illustrate alternative methods supporting hazard assessment. Thus, the course will present concrete methods to characterize uncertainty in the context of chemical risk assessment, in particular, how to pragmatically apply them in a tiered approach, while gaining more confidence in assessing alternative methods.

**1014 PM14: Xenobiotic Pharmacokinetics during Pregnancy and Lactation**

N. Catlin¹ and A. Slitt², Pfizer, Inc., Groton, CT and ¹University of Rhode Island, Kingston, RI.

Exposure to xenobiotics during pregnancy and lactation is unavoidable and can be either intentional with pharmaceuticals or unintentional as contaminants in air, food, and water. During this critical time period of development, xenobiotic exposure can result in deleterious effects to the developing fetus. A number of critical physiological and molecular changes occur in the pregnant woman or animal that will influence the disposition and toxicity of chemicals, often in a gestational age-dependent manner. These changes range from altered blood flow to changes in metabolic pathways, as well as enhancement of renal filtration. In addition, the placenta is considered a selective barrier to protect the fetus against xenobiotics. However, some pharmaceuticals and toxicants can readily cross this placental barrier through active transport or diffusion, leading to exposure in utero. After birth, lactation represents a new route of xenobiotic exposure for the infant. The goal of this course is to provide participants with an introduction to the maternal and perinatal pharmacokinetics of xenobiotics during pregnancy and lactation. A variety of approaches will be covered, including animal studies, physiologically-based pharmacokinetic (PBPK) modeling, and clinical pharmacology studies in pregnant women. Case study examples will also be discussed in each presentation to illustrate these different approaches. The first presentation will provide an overview of the maternal pharmacokinetics and disposition associated with pregnancy, as well as the metabolic capabilities of fetal and neonatal rodents and humans. The second lecture will discuss placental morphology and the transfer of nutrients and foreign chemicals. The third presentation will cover the application of PBPK modeling to understand how physiological and biochemical processes change, and how they impact xenobiotic disposition in the postpartum period, as well as the early-life stage development. The final presentation will describe clinical pharmacology examples of pharmacokinetic changes during pregnancy and the postpartum period and will provide an overview of xenobiotic transfer through breast milk. This course also will cover changes in pharmaceutical drug labeling related to pharmacokinetics and pharmacology during pregnancy and lactation.

**1015 Advanced Imaging and Microscopy for Retinal Disease and Toxicity**

M. Rios Blanco, Allergan, Irvine, CA.

The three major challenges in the study of acquired and innate diseases, as well as drugs and toxicants that affect the retina in man and experimental animals, are difficulty in early noninvasive detection of decreased visual function and/or loss with conventional tools and techniques; determination of the cellular site(s) and mechanism(s) of action; and monitoring of progression and/or repair following different toxic or therapeutic regimens. Advances in optical coherence tomography (OCT) techniques, for man and experimental animals, provide the ability to noninvasively image individual retinal cells, the optic nerve, and retinal vasculature in living eyes. These new noninvasive techniques enable the clinician and scientist to detect, monitor, and treat retinal disease and injury earlier; to visualize the site of action with greater specificity; and to follow the progress of treatment longitudinally. Moreover, the visualization and determination of the cellular, subcellular, and ultrastructural site and mechanisms of injury in retinas from donated human eyes and experimental animals require a variety of different sophisticated imaging techniques. A variety of new noninvasive and experimental techniques are readily applicable to the study of drugs and toxicants that produce pathophysiological alterations similar to known retinal and neurodegenerative diseases, or that exacerbate such existing conditions. The objective of the session is to present state-of-the-art research approaches to clinical and experimental animal model imaging and their utility in toxicological research and to show how the obtained data can be utilized for translational research. The presentations will cover the study of different cell populations within the retina and of the supporting vascular networks. Each presentation will share the fundamental aspects of the imaging modality, illustrate their application to model organisms, and highlight the utility of imaging other techniques to study this phenomenon in experimental animals. Overall, the session will accomplish three goals. First, it will introduce and educate the scientific community on the use of state-of-the-art approaches to noninvasive ocular imaging for the early detection, assessment, progression, and treatment of retinal damage in man and experimental animals. Second, it will enhance the understanding of retinal sites and mechanisms of action of injury. Third, it will provide a basis for determining the translatability of experimental data to humans. The session will be of interest to basic scientists, clinicians, and researchers engaged in drug development and testing.

**1016 Cellular and Structural Imaging Techniques in Glaucoma Diagnosis and Treatment**

S. McKinnon, Duke University, Durham, NC. Sponsor: D. Fox

Glaucoma is a potentially blinding neurodegenerative disease that affects millions of people worldwide. The disease is diagnosed by assessing function (peripheral visual field testing) and structure (optic nerve appearance). Unfortunately, these standard techniques are subject to large variabilities that make the diagnosis and/or staging of glaucoma a clinical challenge. Imaging modalities such as Optical Coherence Tomography (OCT) and Adaptive Optics (AO) improve glaucoma diagnosis by allowing objective imaging of retina and optic nerve structure. Clinical OCT provides data concerning abnormalities in RGC axonal distribution, and volume images can now be obtained using Swept-Source Spectral-Density (SDOCT) in a matter of seconds. Enhanced Depth Imaging SDOCT can measure retinal nerve fiber layer and axonal loss, allowing earlier risk assessment in glaucoma. Polarization-sensitive OCT (PS-OCT) measures axonal birefringence, providing data concerning microtube stability and structure that are surrogates of RGC health. AO is able to image individual retinal cells, and recently RGCs in monkeys and humans have been imaged for the first time in the near future. AO is highly probable that RGC maps of individual retinal neurons can be created and followed longitudinally for disease progression. Combining AO and OCT allows assessment of retinal vasculature and blood flow using Sensorless AO-OCT Angiography. These seemingly constant improvements in resolution and speed of retinal imaging are changing the way we think about the potential to revolutionize glaucoma assessment and treatment outcomes.
Adaptive Optics Imaging to Study Retinal Degeneration and Response to Treatment


Retinal degenerative diseases cause progressive vision loss, and ultimately blindness. Retinal degeneration can be caused by mutations in over 200 genes, and therefore cause photoreceptor death through a variety of mechanisms that affect photoreceptors, retinal pigment epithelial cells, and choroidal or retinal perfusion to varying degrees. Visual function measures provide information about how the patient experiences the world. In this poster, we present clinical outcomes and how adaptive optics can be utilized to image the retina at cellular and sub-cellular level.

Mitochondria-Mediated Retinotoxicity: Determining Pathophysiological Mechanisms Using Multifaceted Image Analysis and Biochemical Techniques

D. Fox, Robson Forensic, Inc., Philadelphia, PA.

Half of all neuroactive/neurotoxic chemicals adversely affect sensory functions. Of these, the initial and most frequent alterations following chemical exposure occur in the retina, suggesting that the retina is especially vulnerable to chemical insult. Several distinct tissue-, layer- and cell-specific biochemical, metabolic, physiological, and structural, characteristics underlie this vulnerability. The aim of this talk is to provide a comprehensive understanding of the differential pathophysiological responses of rod and cone photoreceptors with intact inner and outer segments that are in contact with retinal pigment epithelial cells. Split detection AO systems utilize non-confocal scattered light to image structures that are not waveguiding, such as photoreceptors that have disrupted or missing outer segments and retinal pigment epithelial cells. Finally, AO can be used to precisely deliver visual stimuli to individual photoreceptors, such that visual function and retinal structure can be studied with cellular resolution. AO imaging offers new insights into retinal cell structure and function on a cellular level, non-invasively, in patients with retinal degenerative diseases. Application and interpretation of these advanced technologies in healthy and diseased retinas is presented.

Cancer Risk Assessment of PAH Mixtures: Current and Future Directions

M. Pratt. US EPA, Washington, DC.

The cancer risk posed by exposures to polycyclic aromatic hydrocarbons (PAH) has long been a public health concern. The two-year rodent cancer bioassay remains the definitive source of information employed for cancer risk assessment, and, using such conventional approaches, the US Environmental Protection Agency (US EPA) is actively working to address the need for an updated and expanded document that serves as the agency’s approach for assessing cancer risk from exposure to PAH mixtures. However, the time and resources required to perform two-year rodent bioassays for the range of environmental PAH mixtures are significant and serve as a disincentive to the development of these kinds of data. Recent trends in toxicology and risk assessment offer opportunities to explore less costly and more rapid alternative approaches using non-apical endpoints for estimating the cancer risk posed by PAH mixtures; however, alternative approaches require critical evaluation to ensure that they represent an improvement from the current approach. The approaches that will be discussed in this session include PAH whole mixtures risk assessment, component-based approaches, use of shorter-term in vitro and in vivo assays to provide cancer-relevant data, and a greater focus on adverse outcome pathways to understand cumulative risk. This will be followed by a panel discussion of the merits of these alternative approaches and what steps are needed to move toward validation. Disclaimer: The views expressed are those of the author and do not necessarily represent the views or policies of the US EPA.
relevant exposure routes, use of tumor bioassay data only, and using an intermediate index chemical to expand the database if benzo[a]pyrene, the index chemical, was not tested concurrently. In addition, methods to compare the cancer potency predicted by the RPF approach with those reported in the few available whole mixtures studies will be discussed. The views expressed are those of the author and do not necessarily reflect the views or policies of the US EPA.

The Genetic Toxicity of Complex Mixtures of Polycyclic Aromatic Hydrocarbons: Evaluating the Dose-Additivity Assumption Using a Transgenic Mouse Model

P. White. Health Canada, Ottawa, ON, Canada. Sponsor: M. Pratt

Over 3,000 federally-owned contaminated sites in Canada contain complex mixtures of polycyclic aromatic hydrocarbons (PAHs). Consequently, the Federal Contaminated Sites Action Plan (FCSAP) funded work aimed at evaluating the assumption of component additivity that is commonly employed for regulatory evaluations of PAH mixtures. Initial work evaluated the excess lifetime cancer risk (ELCR) posed by 10 PAH-contaminated soils using (i) the currently advocated, chemical-specific RPF approach; and (ii) a bioassay-based approach that employs the in vitro mutagenic activity of the soil fractions to determine levels of benzo[a]pyrene (BaP) equivalents and, by extension, ELCR. The results show that ELCR values for the PAH-contaminated fractions, determined using the chemical-specific approach, are generally (i.e., 8 out of 10) greater than those calculated using the bioassay-based approach; most are less than 5-fold greater. Follow-up in vivo work employed subchronic oral exposures of the transgenic Muta Mouse to determine tissue specific mutagenic activities, and, by extension, levels of BaP equivalents in several coal tar derived test articles. Similar to the in vitro work, the results were compared to BaP equivalent levels determined using the RPF approach. The results showed that BaP equivalent concentrations based on experimentally-observed in vivo responses in tissues that are distal from the exposure (i.e., bone marrow), were lower than levels determined using the RPF approach. However, the converse was true for proximal tissues (e.g., small intestine). Nevertheless, in both cases the differences between levels based on experimental observations, and those based on the RPF approach, were within 5 fold. The in vitro and in vivo bioassay-based approaches, which permitted estimation of BaP equivalent concentrations without a prior knowledge of mixture composition, demonstrated that experimentally determined levels are surprisingly close to those determined using the targeted RPF approach that is based on only 7-8 carcinogenic PAHs. The results indicate that the targeted RPF approach for PAH mixtures provides a reasonable estimate of BaP equivalent concentrations, and, by extension ELCR estimates.

Mechanism-Based Classification of PAH Mixtures to Predict Carcinogenic Potential

S. Tilton. Oregon State University, Corvallis, OR.

One of the most difficult challenges for risk assessment is prediction and evaluation of health hazards from environmental chemical mixtures. In particular, polycyclic aromatic hydrocarbons (PAHs) are a major group of over 1,500 chemicals measured in complex mixtures from petrogenic or pyrolytic processes. Humans are primarily exposed to PAHs as mixtures, which include both class 1A (known) and 2A (probable) human carcinogens, through multiple exposure routes. Unfortunately, current reference approaches for assessing PAH risk are inadequate for complex environmental mixtures of unknown composition. To successfully distinguish early regulatory events during initiation and progression, RSP agonism, ER antagonism, and GR antagonism. Based on a comparison of candidate screening assays to in vivo potencies data from the literature, cytotoxicity in diverse cell lines was selected for PAH compounds and prioritized for in vivo testing. A 28-day immunotoxicity study in female B6C3F1/N mice was selected to generate potency data and evaluate application of the relative potency factor approach. The results showed that BaP equivalent concentrations, and, by extension ELCR estimates.

Use of Non-Apical Assay Data in an Integrated Approach to Testing and Assessment of Chemical Mixtures in the Environment: the Advent of Adverse Outcome Pathway Footprinting

J. Lambert. US EPA, Cincinnati, OH. Sponsor: M. Pratt

A key challenge in the risk assessment of environmental chemical mixtures is the availability of sufficient apical endpoint data to inform hazard and dose-response assessment. The virtual absence of whole mixtures data at environmentally relevant exposure levels or proportions, and limited availability of traditional toxicity assay information for individual chemicals present in mixtures, is a challenge for human health risk assessment. This is particularly difficult in the case of cancer risk assessments, where data from two-year rodent bioassays are often not available. With the advent of 21st century toxicity testing and approaches such as adverse outcome pathway (AOP) testing, opportunities to evaluate hazards associated with exposure to “data-rich” mixture chemicals have advanced significantly. Potential health impact(s) of mixture chemicals may be informed using an integrated read-across approach that includes AOP “footprinting” when adverse outcome data derived from traditional bioassays are lacking. In brief, AOP footprinting makes use of key event data to inform qualitative and quantitative relationships between mixture components. This presentation will provide an overview of AOP footprinting and how it complements an integrated approach to testing and assessment (IATA) of both data-poor and data-rich chemicals in environmental mixtures. The views expressed in this abstract are those of the author and do not necessarily reflect the views or policies of the US EPA.

Novel Insights on Chemical-Induced Immunotoxicity: Microvesicles and microRNA Dysregulation

E. Corsini. University of Milan, Milan, Italy.

Eukaryotic cells contain extracellular organelles named microvesicles (e.g., exosomes, nanovesicles) that are released into the microenvironment. Microvesicles and their main content microRNAs are believed to play a central role in many physiological and pathological processes, including inflammation, autoimmunity, atherosclerosis, and cancer. miRNAs, a class of non-protein-coding RNA molecules negatively regulating mRNA translation, have been shown to be involved in several cellular processes, and their role in toxicology is emerging. The miRNA-mediated coordinated control of gene expression has been shown to be crucial in immunity, promoting and finely regulating appropriate immune responses. Both microRNA and microvesicles are very promising tools in identifying early alterations induced by chemical exposure,
which can revolutionize both monitoring and toxicological assessment.

The aim of this session is to provide novel insights on the mechanisms of action of immunotoxic compounds focusing on microRNA and microvesicles. Recently, differential expressions of miRNAs and association with several immunologic and inflammatory disorders have been reported, which have important implications in immunotoxicology assessment. miRNAs influence regulatory mechanisms of inflammation and can induce and contrasting acute and chronic inflammation. In addition, research on microvesicles also is an emerging and developing field. Studies available to date identified several exposures or lifestyle factors able to modify the trafficking of microvesicles, including air pollutants, cigarette smoke, and oxidative stress. The first speaker will guide the audience in the world of microRNAs from discovery to their role in physiological and pathological conditions, with emphasis on tumors and immunosurveillance, to their use as biomarkers. The second speaker will present data showing influences of environmental exposures on EV-encapsulated RNAs and potential links with several adverse health outcomes, including immunotoxicity. The last two speakers will focus on the role of microRNAs in allergic phenomena both in humans and in experimental models. Challenges, limitations, and opportunities in this emerging field in environmental health sciences will be discussed.

**1028 Effects of Environmental Exposures on Microvesicles Release and Their Contents**

A. Baccarelli. Columbia University, New York, NY. Sponsor: E. Corsini

Interest in intercellular communication has risen in recent years, with an increasing awareness of the complexity of its contributions to diverse physiological processes. In particular, the identification of extracellular vesicles (EVs) as novel mediators of intercellular communication has re-focused research efforts in the field. The number of human studies of EVs-using a number of body fluids including blood, urine, saliva, amniotic fluid, and breastmilk, have grown exponentially in the past few years. Several investigations have focused on EV-packaged non-coding RNAs, which are released into EVs by the cell of origin and may reprogram gene expression in recipient cells. EVs have been linked to environmental exposure pathways and the infiltration of the immune system in cancer. MicroRNA and other short or long non-coding RNA alterations are identified in the initiation, progression, and metastasis of human cancer. The main molecular alterations are represented by variations in gene expression, usually mild and with consequences for a vast number of target protein coding genes. The causes of the widespread differential expression of non-coding RNAs in malignant compared with normal cells are still not clear. These findings by the location of these gene in cancer-associated genomic regions, by epigenetic mechanisms, and by alterations in the processing machinery. MicroRNA and other short or long non-coding RNAs expression profiling of human tumors has identified signatures associated with diagnosis, staging, progression, prognosis, and response to treatment. In addition, profiling has been exploited to identify non-coding RNAs that may represent downstream targets of activated oncogenic pathways, or that are targeting protein-coding genes involved in cancer. Recent studies proved that miRNAs and non-coding ultraconserved genes are main candidates for the elusive class of cancer-predisposing genes and that other types of non-coding RNAs participate in the genetic puzzle, giving rise to the malignant phenotype. Last but not least, the shown expression correlations of these new ncRNAs with cancer metastatic potential and overall survival rates suggest that at least some members of these novel classes of molecules could potentially find use as biomarkers or novel therapeutics in cancers and other diseases.

**1029 Circulating microRNAs and Prediction of Airway Hyperresponsiveness**

K. G. Tantisira. Harvard Medical School, Boston, MA. Sponsor: E. Corsini

Airway hyperresponsiveness is a cardinal feature of asthma. While clinically measured via non-specific agents such as methacholine and histamine, other agents, including pollen, smoke, and chemical inhalation, can cause significant airway hyperresponsiveness. Identification of miRNAs associated with airway responsiveness may have biologic, prognostic, and therapeutic relevance. Murine models have identified several miRNAs associated with airway hyperresponsiveness, including miR-126 and let-7. Examination of these murine phenotypes is hindered by the lack of equivalent murine asthma models. MicroRNA expression profiling of human asthma patient samples has revealed a number of the miRNAs with methacholine-induced PC20 changes in a cohort of childhood asthmatics has been reported. Two of these miRNAs, miR-16-5p and miR-30d-5p, form central hubs in miRNA-predicted gene networks and are associated with changes in airway smooth muscle growth and size. MicroRNAs have also been used to provide a prognostic signature for the natural history of asthma with good predictive power for resolution of airway hyperresponsiveness over time. Thus, miRNAs in general, and circulating miRNAs specifically, appear to have an evolving role in the understanding of airway hyperresponsiveness and may shed future light on inhalational and toxicologic lung disorders.

**1030 microRNA in Experimental Models of Chemical Sensitization**

E. Anderson. NIOSH, Morgantown, WV.

Allergic disease is an important occupational health concern, with work-related asthma and allergic contact dermatitis being the most frequently diagnosed occupational illnesses. Understanding the mechanisms behind allergic disease is critical for treatment and prevention. The regulatory potential of microRNAs (miRNAs) has been recognized in a variety of disease states, including allergic disease; however, the roles of miRNAs in chemical sensitization are largely unknown. Increased expression of multiple miRNAs during toluene 2,4-diisocyanate (TDI) sensitization was observed, and several putative mRNA targets identified for these miRNAs were directly related to regulatory T-cell (Treg) differentiation and function, including Foxp3 and Runx3. Specifically, miR-210 expression increased in the mouse draining lymph node (dLN) and Treg subsets following dLN TDI sensitization. Alterations in dLN miRNA and protein expression of Treg-related genes/putative miR-210 targets (foxp3, runx3, cplt, and cdx2) were observed at multiple time points following TDI exposure and in ex vivo systems. A Treg suppression assay, including a miR-210 mimic, was utilized to investigate the suppressive ability of Tregs. Cells derived from TDI-sensitized mice treated with miR-210 mimic had less expression of miR-210 compared to the control, suggesting other factors, such as additional miRNAs, might be involved in the regulation of the functional capabilities of these cells. These novel findings indicate that miR-210 may have an inhibitory role in Treg function during TDI sensitization. Because the functional roles of miRNAs have not been previously elucidated in a model of chemical sensitization, these data contribute to the understanding of the potential immunologic mechanisms of chemical-induced allergic disease.

**1031 Toxicological Implication of Copper in Neurodegenerative Diseases**

M. Kitazawa. University of California Irvine, Irvine, CA.

Copper (Cu) is an essential transition metal and required for many normal physiological functions, including energy production, free radical scavenging, connective tissue production, iron mobilization, and neurotransmission. However, excessive intake due to occupational or environmental exposure to divalent Cu(II) has been implicated as a risk for various human diseases. When administered, almost all Cu ions are bound to ceruloplasmin (Cp), and the remainder, non-Cp-bound Cu (fabile Cu), is bound to albumin, transcuprein, various peptides, and amino acids in plasma. For the normal level of free Cu is tightly controlled by the above-mentioned Cu-binding proteins. Recent studies have clearly indicated that environmental exposure to Cu in adults accelerates cognitive decline and may increase the risk of developing Alzheimer’s disease (AD)-like neuropathology by elevating non-meroplasmin-bound Cu in plasma. This session will bring together the experts who are actively engaging in investigating chemistry of Cu in biological systems, Cu neurotoxicity, and its underlying...
cellular and molecular mechanisms to discuss the toxicological implications of Cu in neurodegenerative diseases. After a brief introduction of Cu in health and human diseases, the first speaker will highlight the mechanisms by which Cu is transported in and out of the brain through the blood-brain barrier (BBB) and blood-CSF barrier and how the altered Cu transport processes in brain barriers may cause Cu dysregulation, leading to Parkinsonian disorders (PD). The second speaker will present new evidence that Cu controls MMP activity and CLUT-1 levels and how these changes lead to BBB dysfunction in the aged brain in conjunction with the role of Cu in regulating the endothelial lipoprotein receptor-related protein 1 (LRP1) as one possible mechanism increasing the risk for AD.

The third speaker will further expand the subject by addressing the immunomodulatory and multifactorial influences of Cu on Aß clearance in the brain and how microRNAs play a critical role in the Cu-mediated down-regulation of LRP1 in the endothelial cells. The fourth speaker will focus on the unique structural chemistry of Cu in its interaction with Aß and how copper positions within the fibrillar structure of Aß promote its assembly by using highly sophisticated nanoscience technology. The final speaker will introduce the role of Cu in adult neurogenesis in the subventricular zone. New data unveiling the mechanism by which Cu regulates the critical steps leading to migration and differentiation of neural stem cells in the SVZ-rostral migratory stream pathway, and possible implication in the involvement of Cu in PD, will also be presented.

Overall, this session will present the latest findings on the structural, genetic, cellular, and molecular mechanisms of Cu neurotoxicity linking neurodegenerative diseases such as AD and PD. The session will capture the broad interest of those engaged in toxicological research of neurodevelopment and neurodegenerative diseases, neuroscience, neurotoxicology, metals biology, and nanoscience.

1032 Regulation of Copper Homeostasis by the Brain Barrier Systems: Implications in Neurodegenerative Diseases


Copper (Cu) is essential to neuronal function; excess or deficiency is known to underlie the pathophysiology of several commonly known neurodegenerative disorders. This delicate balance of Cu in the central milieu is maintained by the brain barrier systems, i.e., the blood–brain barrier (BBB) between the blood and brain interstitial fluid and the blood–cerebrospinal fluid barrier (BCB) between the blood and cerebrospinal fluid (CSF). This talk will provide a concise description on the structural and functional characteristics of the brain barrier systems supported by unpublished and new data. Current understanding of Cu transport across the brain barriers will be examined, with major focus on whether the BBB and BCB coordinate the direction of Cu fluxes between the blood and brain, and the blood and CSF. In particular, the mechanism by which pertinent metal transporters in the barriers, such as divalent metal transporter (DMT1), copper transporter (CTR1), ATP7A/B, and ferroportin (FPN), regulate metal movement across the barriers is explored. Finally, the detrimental consequences of dysfunctional metal transport by brain barriers, as a result of endogenous disorders or exogenous insults, are discussed. Understanding the regulation of Cu homeostasis in the central nervous system aids in the design of new drugs targeted on the regulatory proteins at the brain barriers for the treatment of metal's deficiency or overloading related neurological diseases.

1033 Brain Capillary Copper and the Accumulation of CNS Proteins Associated with Neurodegeneration and Dementia

R. Deane. University of Rochester, Rochester, NY. Sponsor: M. Kitazawa

Excess free Cu, a powerful pro-oxidant, is toxic, and thus, tightly controlled. Imbalance in cerebral Cu homeostasis plays a role in the pathogenesis of Alzheimer’s Disease (AD), and possibly other neurodegenerative diseases and neurotoxicity, by altering APP processing and Aß deposition and clearance. Brain Aß levels are regulated by its rate of production from APP and by its rate of clearance. Clearance of Aß from the brain involves enzymatic degradation by proteases, including insulin-degrading enzyme (IDE) and neprilysin (NEP), bulk flow of interstitial fluid, and mainly transport across the BBB via low-density lipoprotein receptor-related protein 1 (LRP1). LRP1 is expressed in brain endothelium, and a reduced expression is observed in aging rodents and in patients with AD. In the sporadic form of AD, Aß production is unchanged while its clearance is reduced. Thus, faulty clearance in the aging brain is the major contributor to Aß neurotoxicity. In wild type mice, Cu is accumulated in brain capillaries but entry into the brain is restricted. Consequently, this is associated with a reduction in LRP1 levels at the BBB and higher brain Aß levels. These effects can be reproduced by chronic dosing with low levels of Cu via the drinking water without changes in Aß synthesis or degradation. In human brain endothelial cells, Cu, at its normal labile levels, caused LRP1-specific down-regulation by inducing its nitrotyrosination and subsequent proteosomal-dependent degradation due in part to Cu/(cellular pri) protein/LRP1 interaction. However, in APP mice (Tg2576, another mouse model of AD), Cu down-regulates LRP1 in brain capillaries and also increases Aß production and neuroinflammation. These effects are due to Cu accumulation in brain capillaries and, unlike control mice, in the parenchyma, possibly due to the dysfunctional BBB. New data will be presented to include Cu’s role in controlling MMP activity, CLUT-1 levels and CBF, which could explain its contribution to BBB dysfunction in the aging brain. These findings should provide unique therapeutic approaches to control neurotoxicity in the aging brain.

1034 Multifactorial Role of Copper Toxicity on Modulating Amyloid-Beta Clearance via microRNA and Inflammation

M. Kitazawa. University of California Irvine, Irvine, CA.

While copper is an essential transition metal, a growing body of evidence strongly implicates that excessive intake of inorganic copper, particularly through environment, drinking water, and supplements, accelerates cognitive decline and the development of Alzheimer’s Disease (AD). In fact, elevated levels of total non-ceruloplasmin bound free copper levels in serum have been reported among patients with mild cognitive impairment and AD. However, detailed molecular and cellular mechanisms by which excessive copper exerts neurotoxicity remain an open discussion. Here we demonstrate that exposure to copper significantly alters pro-inflammatory responses and attenuates Aß-induced phagocytosis in murine monocyte cells and an animal model of AD. Additionally, copper directly and indirectly reduces the expression of low-density lipoprotein receptor-related protein 1 (LRP1) in primary human endothelial cells via microRNA-200 and -205. Inhibition of these microRNAs markedly prevents copper-mediated loss of LRP1 in endothelial cells. Both microglial phagocytosis and LRP1 have been documented to play a key role in the clearance of Aß in the brain. Our results strongly imply that copper targets these mechanisms to impair Aß clearance and promote accelerated buildup of Aß in the brain. Thus, our finding provides a critical molecular link to copper as a potential environmental risk factor for AD. In this talk, multifactorial mechanisms of Aß clearance by various brain cell lineages, and how copper differentially impacts these processes, will be integrated.

1035 Amyloid-Copper Complexes and Approaches to Heterogeneity in Biological Systems

P. Weiss. University of California Los Angeles, Los Angeles, CA. Sponsor: M. Kitazawa

Many important biomolecular structures and biomolecular assemblies have varied structures and functions. Many are not amenable to crystallography. We have developed novel nanoscale approaches to measuring the structures of these molecules and assemblies that do not rely on averaging and also elucidate the intrinsic heterogeneity of the systems. In Cu-amyloid systems, we determine the binding sites of the metal ions and the effects that this coordination has on the structures. Two binding sites are found in Aß1-42, and assembly structures are changed even when the metal ion sparsely populate these sites. No equivalent information is available through other methods, and these determinations are enabling, in terms of theory and simulation. Additional metal-amylloid structures are being determined, and using approaches from the fields of sparsity and compressive sensing, we have accelerated our measurements and analyses by orders of magnitude. Next, we will study the positions and roles of the molecular modulators that catalyze and induce misfolding in these systems.

1036 Does Copper Play a Role in Adult Neurogenesis in the Subventricular Zone?

W. Zheng. Purdue University, West Lafayette, IN.

Neural stem/progenitor cells (NSPCs) in the adult subventricular zone (SVZ) have the capability to self-renew and to migrate via the rostral migratory stream (RMS) to reach their final destination, i.e., the olfactory bulb, where they mature and integrate into the local neuronal circuitry. Adult neurogenesis plays an important role in the etiology of neurodegenerative diseases. This presentation will provide a concise review on
the current understanding of how Cu plays a role in regulating the adult neurogenesis in the SVZ. The finding of extraordinarily high levels of Cu in the SVZ will be first introduced. To investigate the role of Cu in adult neurogenesis, we have used the intra-cerebral-ventricular (ICV) infusion technique to deliver Cu ions directly into rat brain lateral ventricle. The proliferating cells in the SVZ were then counted and analyzed. Our data indicate that Cu surges after星光出现了 about a 43% increase in proliferating cells in the SVZ. However, infusing Cu to the ventricles significantly suppressed the activated cell proliferation to the level of non-surgical controls, suggesting that a high level of Cu in the CSF may inhibit the proliferation of NSPCs in the SVZ. Our new evidence from in vivo and in vitro further revealed a significant reduction or increased proliferation of NSPCs in the SVZ in the Cu-overloaded or in Cu-deficient animal models, respectively. In summary, these data indicate that the homeostasis of Cu in blood and/or CSF regulates the adult neurogenesis in the SVZ. I will discuss the implication of our recently identified mechanisms by which Cu regulates the adult neurogenesis in Parkinson’s disease and its possible environmental etiopathogenesis.

1037 Understanding the Molecular Mechanisms of Zika Virus Reproductive and Developmental Toxicity

P. Del Valle. US FDA, Silver Spring, MD.

Zika virus (ZIKV) is related to the Yellow fever, West Nile, Japanese encephalitis, and Dengue fever viruses, all classified under the genus Flavivirus that belongs to the family Flaviviridae. Symptoms of infection may include a rash, itching, fever, muscle pain, conjunctivitis, nausea, vomiting, and headaches. ZIKV was isolated from a rhesus macaque in the Marzamemi area in Italy in 1967, from mosquitoes in Uganda in 1974, from pregnant women from Asian countries in the 1960s, and reached the Americas in 2015. Outbreaks have been reported in Micronesia (2007), French Polynesia (2014), and Brazil (2015). By January 2016, autochthonous cases of ZIKV were reported in more than 50 countries in South, Central, and North America, and the Caribbean, with most ZIKV-related outbreaks in the mosquito-borne disease in the United States. ZIKV in pregnant women is reported to cause a wide spectrum of fetal malformations, collectively called congenital Zika syndrome (CZS), that include microcephaly, absent or poorly developed brain structures, retinal damage, hearing deficits, and impaired growth. This session will explore recent findings that have begun to link ZIKV infection with male testicular damage and potential human male infertility and with congenital malformations, microcephaly, and other brain malformations and birth defects. The session will also include an integral discussion of preclinical trials and the role of vector control approaches to reduce/prevent ZIKV infection. The introduction will include a brief overview of the ZIKV infection during outbreaks, with emphasis on the current status of the outbreak in the Americas. ZIKV is primarily spread by the daytime-active female Aedes aegypti mosquito, and several mechanisms of ZIKV replication and tissue tropism have been investigated. Evidence that ZIKV requires the ubiquitin-proteasome system for replication and the hijacking of the host ubiquitin system will be discussed. Understanding the molecular mechanisms of replication is critical to identifying existing or developing new drugs that can prevent or stop viral infection. ZIKV can inhibit the proliferation of NSPCs in the SVZ will be first introduced. To investigate the role of Cu in adult neurogenesis in the SVZ. The finding of extraordinarily high levels of Cu in the SVZ will be first introduced. To investigate the role of Cu in adult neurogenesis, we have used the intra-cerebral-ventricular (ICV) infusion technique to deliver Cu ions directly into rat brain lateral ventricle. The proliferating cells in the SVZ were then counted and analyzed. Our data indicate that Cu surges after星光出现了 about a 43% increase in proliferating cells in the SVZ. However, infusing Cu to the ventricles significantly suppressed the activated cell proliferation to the level of non-surgical controls, suggesting that a high level of Cu in the CSF may inhibit the proliferation of NSPCs in the SVZ. Our new evidence from in vivo and in vitro further revealed a significant reduction or increased proliferation of NSPCs in the SVZ in the Cu-overloaded or in Cu-deficient animal models, respectively. In summary, these data indicate that the homeostasis of Cu in blood and/or CSF regulates the adult neurogenesis in the SVZ. I will discuss the implication of our recently identified mechanisms by which Cu regulates the adult neurogenesis in Parkinson’s disease and its possible environmental etiopathogenesis.

1038 Introduction: A Brief Overview of Zika Virus Infection and Current Magnitude of Infection

P. L. Del Valle. US FDA, Silver Spring, MD.

The ZIKV has been isolated from countries located mainly within the equatorial regions. Imported cases through travelers into other countries in the world occurred occasionally. However, for more than 50 years, ZIKV was only associated with mild cases of infection, and the outbreaks in the French Polynesia, where more than 30,000 cases (11.5% of the population) of ZIKV fever occurred. Severe neurological complications were reported, including association of ZIKV infection with Guillain-Barre syndrome (GBS). It is estimated that 2-5 patients for every 100,000 infected with ZIKV will go on to develop GBS. The outbreak in Brazil attracted the most attention because this country hosted the 2016 Olympic Games. It is estimated that 440,000-1,300,000 suspicion cases of ZIKV disease occurred in Brazil in 2015. Microcephaly was suspected in 5,909 cases reported during the outbreak; however, microcephaly was confirmed in only 641 cases, and it was discarded in 4,222 cases. More than 2,000 cases of pregnant women with any lab evidence of ZIKV infection in the US was 1,793, and in the US territories up to 3,700. The Vital Signs report from the CDC also reported that of the 250 pregnant women who had confirmed Zika infection in 2016, 24, or about 1 in 10 of them, had a fetus or baby with birth defects. These figures and reports illustrate the magnitude of the ZIKV effects on health in the Americas and around the world. Infants born to women with laboratory evidence of ZIKV infection during pregnancy are now subject to a postnatal neuromaging, testing for ZIKV, and a comprehensive newborn physical examination, because the fetus or baby with birth defects of congenital Zika virus infection is not yet known. This presentation will provide updated figures and statistics on ZIKV infection, GBS, and developmental and reproductive adverse outcomes reported for newborn infants to women with ZIKV infection during pregnancy.

1039 The Role of the Host Ubiquitin System in Zika Virus Replication and Tissue Tropism

R. Rajsbaum. University of Texas Medical Branch, Galveston, TX. Sponsor: P. Del Valle

ZIKV is a mosquito-borne, positive-sense single-stranded RNA virus from the genus Flavivirus (family Flaviviridae). ZIKV is closely related to other flaviviruses that cause important human disease, including Dengue virus (DENV), West Nile virus (WNV), and Yellow fever virus (YFV). In contrast to other flaviviruses, there is evidence that ZIKV replicates more efficiently in reproductive tissues, can be sexually transmitted, and is associated with congenital neurologic disorders, indicating that the mechanisms of ZIKV tropism and transmission may be unique. The molecular mechanisms underlying these differences are currently not known. Previous studies suggest that replication of DENV, a close relative of ZIKV, requires the ubiquitin-proteasome system for replication. Similarly, we found that pre-treatment of a placenta-derived cell line (JEG-3) with the proteasome inhibitor MG132 also reduced ZIKV replication, suggesting that the ubiquitin-proteasome system may be important for ZIKV replication and tissue tropism. The host ubiquitin system is a conserved cellular pathway important in many functions, including innate immune signaling and virus replication. By using mass spectrometry analysis of ZIKV infected cells, we identified specific sites of ubiquitination on critical viral proteins. We generated recombinant infectious ZIKV mutants lacking ubiquitination sites and observed reduced virus replication in placenta-derived cell lines, consistent with a role for the ubiquitin system in promoting ZIKV replication and cell tropism. Here, we will provide an overview of ZIKV infections, geographical distribution, and viral pathogenesis, and will discuss potential mechanisms of virus replication and tissue tropism via hijacking of the host ubiquitin system. We will also discuss potential use of US FDA-approved drugs against ZIKV infections and the possibility of targeting the host ubiquitin system to prevent ZIKV replication.
system as a potential therapeutic approach to control ZIKV infections. The identification of host cellular factors required for Zika virus replication may provide novel antiviral targets for future drug development, with a minimal inherent risk of viral escape mutants, and similar antiviral approaches could be used against other flaviviruses.

1040 Zika Congenital Syndrome in Murine Experimental Model
J. P. S. Peron. University of São Paulo, São Paulo, Brazil. Sponsor: P. Del Valle

Brazil has recently gone through an unprecedented public health crisis due to the Zika virus epidemics. As many other flaviviruses, it has never been correlated with human morbidity or mortality. Unfortunately, it has changed dramatically, as the virus is responsible for more than 2,300 babies born with microcephaly. The so-called Zika Congenital Syndrome has, besides microcephaly, many other relevant features, such as retinal damage, intra-uterine growth restriction, and arthropathy. In fact, babies born without microcephaly, but with significant neurologic and retinal lesions, have also been reported. In this context, the development of an experimental model is of great relevance for the studies on the pathogenesis of microcephaly. We demonstrated that the infection of pregnant SJL mice with ZIKV results in severe damage of the pups. Most prominently was the significant reduction in size and weight associated with high viral titers in the brains. This was accompanied by high level of apoptotic cell death, probably of neuronal precursor cells (NPCs). In order to confirm that hypothesis, we infected NPCs and human brain organoids with ZIKV, and observed a significant reduction in the number of NPCs, corroborating the apoptotic cell death hypothesis. Currently we are focused on several aspects of the brain inflammation during microcephaly. Interestingly, many pro-inflammatory cytokines are down-regulated, as well as viral receptors and signaling transduction molecules. This is consistent with clinical findings, showing no sign of inflammation, at least through cerebrospinal fluid protein and cell counts. Our data may indicate that the virus controls inflammation in situ. The elucidation of such a mechanism would greatly contribute to the understanding of the viral biology in the central nervous system and also of the pathogenesis of microcephaly.

1041 Mouse Model of Zika Virus Infection in Testis and Its Potential Relevance to Mechanism of Infection in Human Testis and Male Infertility
P. Esaky. Washington University School of Medicine in St. Louis, St. Louis, MO.

ZIKV in pregnant women causes a wide spectrum of fetal malformations, collectively called CZS, that includes microcephaly, intra-uterine growth restriction and intrauterine death syndrome. Several animal models have been developed to study ZIKV pathogenesis in parents and offspring. We developed a male mouse model of ZIKV infection using a mouse-adapted African strain (Dakar 41519). Results indicate that ZIKV-mediated damage in male reproductive system occurs primarily by destroying testicular germ cells, and noticed that the ZIKV-mediated tissue damage the male reproductive tract, reduced testosterone and inhibit B levels, sperm count, and fertility. In addition to infiltrating inflammatory cells, ZIKV preferentially infected spermatogonia, spermatocytes, and Sertoli cells in the testis, resulting in cell death and destruction of the seminiferous tubules, the basement membrane, and blood testis barrier. Although further studies are needed to monitor ZIKV infection in affected human males, the establishment of a model to evaluate male reproductive tract damage by ZIKV will allow rapid testing of new classes of therapeutic agents to mitigate or prevent disease. As our data has demonstrated an unanticipated clinical manifestation of ZIKV infection, the ZIKV mouse models strongly support the notion that humans are more susceptible than mice. Being the only sexually transmitted arbovirus in humans, ZIKV is well adapted to generate higher levels of viremia, haematospermia, dysuria, and perineal pain. Recent studies have demonstrated the presence of ZIKV on human sperm head that last for more than 30 days in semen, blood, and urine, similar to Ebola virus. Thus, ZIKV persistence in semen relating to viral tropism in male germ cells is a major threat to male fertility. So far, the available experimental, clinical, and epidemiological data lead us to conclude that the effect on fertility caused by non-obstructive azoospermia as demonstrated in animal models could be less of a problem in humans, whereas fertility in men might be at risk mainly owing to transmission of the disease by ZIKV-infected spermatooza during natural conception or assisted reproductive technologies. Our current and future studies are directed toward understanding the translational aspects of ZIKV infection in human subjects with respect to male reproductive health.

1042 Protective Efficacy of Multiple Vaccine Platforms against Zika Virus Challenge in Rhesus Monkeys
R. A. Larocca. Harvard Medical School, Boston, MA. Sponsor: P. Del Valle

ZIKV is responsible for a major ongoing epidemic in the Americas and has been causally associated with microcephaly. The development of a safe and effective ZIKV vaccine is therefore an urgent global health priority. Here we demonstrate that three different vaccine platforms protect against ZIKV challenge in rhesus monkeys. A purified inactivated virus vaccine induced ZIKV-specific neutralizing antibodies and completely protected monkeys against ZIKV strains from both Brazil and Puerto Rico. Purified immunoglobulin from vaccinated monkeys also conferred passive protection in adoptive transfer studies. A plasmid DNA vaccine and a single-shot recombinant rhesus adenovirus serotype 52 vector vaccine, both expressing ZIKV premembrane and envelope, also elicited neutralizing antibodies and completely protected monkeys against ZIKV challenge. These data support the rapid clinical development of ZIKV vaccines for humans.

1043 Integrated Pest Management: A Multifaceted Approach to Vector Control
E. Mendez. US EPA, Washington, DC.

As the Zika virus crisis unfolded, the federal government was faced with the need to swiftly develop a strategy to control the spread of infection. With the Centers for Disease Control and Prevention (CDC) as the lead agency, the coordinated federal response included the Department of Housing and Urban Development (HUD), the Department of Homeland Security (DHS), National Institutes of Health (NIH), the Department of Health and Human Services (HHS), Federal Emergency Management Agency (FEMA), and the US Environmental Protection Agency (US EPA), among others. As the primary mode of Zika virus transmission, mosquito control became a critical aspect of the response to this health crisis. Under the Federal Insecticide Fungicide and Rodenticide Act (FIFRA), the US EPA is charged with regulating the marketing of pesticides in the US; the US EPA conducts a comprehensive evaluation that considers the toxicological profile as well as potential exposure patterns to the chemical in order to assess the potential impact it may have on human health, non-target species, and the environment prior to marketing. The US EPA’s role in responding to this public health crisis, however, goes beyond the regulation of pesticides to incorporate Integrated Pest Management (IPM) strategies. IPM is a comprehensive, sustained effort encompassing a wide-range of mosquito control methods, such as personal protective (e.g., insect repellent), physical control (e.g., breeding environment reduction), larval control (e.g., copepods/fish), biological adulticides (e.g., Wolbachia), and chemical adulticides (e.g., insecticides) measures. This presentation will discuss the risk assessment process undergone by mosquito control methods currently available, as well as new tools under development and their role within an IPM strategy.

1044 Assessing the Dose of Particles in Toxicological Studies: Advances in Dosimetry Models for In Vitro and In Vivo Applications in Light of Risk Assessment
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Emerging hybrid, experimental/computational approaches to cellular dosimetry can be used by particle toxicologists to accurately calculate the delivered dose to cells for various particles and under different in vitro experimental conditions as a function of exposure time. Likewise, in vivo lung dosimetry models allow researchers to estimate the delivered particle dose in any region of the respiratory system, as well as study the implications of particle properties and breathing parameters for diverse animal species. Moreover, knowing the deposited dose also will facilitate the extrapolation from experimental animals (rat, mouse, rabbit, pig, and monkey) to humans of all ages. Most importantly, incorporating such dosimetric methodologies in the study design enables particle toxicologists to bring in vitro and in vivo doses to the same scale, an important step towards the development and validation of in vitro cellular screening assays.
The number of (complex) nanomaterial and other types of particles that are being developed and put on the market is creating a problem for risk assessors as all of these have to be tested for their safety aspects. Standard procedures require mainly in vitro testing, whereas the bulk of the toxicological tests are nowadays performed in vivo. This is partly a result of the societal need to reduce the use of experimental animals. However, it is far from obvious for risk assessors to rely on in vitro data. For particles there is also the issue of the difference between an exposure concentration and the dose that is delivered to the cell in the in vitro model, irrespective of submerged or air-liquid interface systems. The purpose of the session is to bring together risk assessors, modelers, and toxicologists to exchange the needs and possibilities and to funnel down towards an agenda for an efficient development of both in vivo and in vitro that meets the needs of risk assessors and allow for a more accurate read-across and extrapolation of information from experimental studies in human risk assessment. This presentation will focus on the needs from and the data requirements of risk assessors.

A major obstacle in the development of accurate cellular models for investigating nanomaterial interactions in vitro is determination of physiologically relevant measures of dose. Comparison of biological responses to nanoparticle exposure typically relies on administered dose metrics such as mass concentration of suspended particles, rather than the effective dose of particles that actually comes in contact with the cells over the time of exposure. Adoption of recently developed dosimetric methodologies will facilitate determination of effective dose delivered to cells in vitro, thereby improving the accuracy and reliability of in vitro screening data, validation of in vitro with in vivo data, and comparison across multiple datasets for the large variety of nanomaterials currently in the market.

Evidence continues to grow of the importance of in vitro and in vivo dosimetry in the hazard assessment and ranking of engineered nanomaterials (ENMs). Accurate dose metrics are particularly important for in vitro cellular screening to assess the potential health risks or bioactivity of ENMs. To ensure meaningful and reproducible quantification of in vitro dose, with consistent measurement and reporting between laboratories, it is necessary to adopt standardized and integrated methodologies across the ENM dispersion preparation-colloidal characterization-cellular dosimetry. Such integrated methodologies need to include three interconnected steps: (i) Generation of stable ENM suspensions in cell culture media; (ii) colloidal characterization of suspended ENMs, particularly of properties that determine particle kinetics in an in vitro system (size distribution and formed agglomerate effective density); and (iii) robust numerical fate and transport modeling for accurate determination of the ENM dose delivered to cells over the course of the in vitro exposure. Here we will present an integrated comprehensive protocol based on such an integrated methodology for in vitro dosimetry, including detailed standardized procedures for each of these three critical steps/aims.

Respiratory tract deposition and retention kinetics of inhaled particles are determined by their behavior due to physics and chemistry in air, in physiological fluids, and by physiological clearance mechanisms. Dosimetric modeling of deposition requires input of several aerosol characteristics, including density, and modeling retention involves knowledge about particle bio-dissolution. Particle dissolution rates determined in non-cellular in vitro assays using surrogate lung fluids may be applied to estimate overall pulmonary clearance rates. While the importance of dosimetry has long been recognized for inhalation studies, it is essential to be considered and applied as well for cell culture studies. For both in vivo (inhaled) and in vitro (cell cultures) studies, knowledge about Exposure-Dose-Response relationships is key for comparing in vitro and in vivo results on an equal dosimetric basis, and at the same time it provides an opportunity to validate in vitro assays. Ultimately, a careful attention to dosimetric details allows a scientifically justified risk extrapolation of toxicological results from animal studies to humans.

Public exposure generally includes both particles and gases of the same or different species. There is a synergy among various components in the inhaled air. There may be rapid ongoing thermodynamic processes (such as in the case of cigarette smoking), which affect the characteristics of inhaled particles and vapor and thus their fate in the respiratory tract. Mixture characterization is a necessary first step in realistic assessment of the delivered dose to the respiratory tract. A physics-based, mathematical model was developed to study the fate of inhaled mixture of droplets and vapors in the respiratory tract of humans. Droplets contain constituents of different vapor pressures. The dosimetry model accounted for the coagulation of airborne materials at high concentrations and droplet-vapor conversion of different constituents in the inhaled air while traveling through the head and lung airways. Predicted deposition of the droplets and uptake of gases were calculated on a constituent basis to accommodate component-specific risk analysis. The contributions of the droplet and vapor phases to the tissue dose depended on the physicochemical properties of the components in the mixture and lung and therefore the dose of high saturation vapor pressure were absorbed by the tissue via the vapor phase. The dose to the tissue for the low and medium vapor pressure compounds were from droplet deposition. Overall, droplets had a more significant contribution than vapors did. Model development and application for specific scenarios will be presented and discussed.
Drug-induced liver injury is a major reason of failure during premarketing and postmarketing phases of drug development. Drug responsible for more than 50% of all cases of acute liver failure worldwide, drug-induced liver injury is equally of high clinical concern. As such, up to 40% of drug-induced liver injury patients present a cholestatic liver insult pattern. Cholestasis is derived from the Greek words chole and stasis meaning bile and halting, respectively, and denotes any situation of impaired bile secretion with concomitant accumulation of noxious bile acids in the liver or systemic circulation. Drug-induced cholestasis typically, though not uniquely, starts by inhibition of one or more drug transporters directly leading to bile acid retention in the liver. This triggers a deteriorative response associated with oxidative stress, inflammation, and cell death. In parallel, an adaptive response is initiated, which is aimed at restricting bile acid synthesis and influx, while promoting bile acid efflux. In fact, these mechanisms have been embedded recently in an adverse outcome pathway construct in order to further facilitate predictive toxicology. Several additional key events in drug-induced cholestatic liver injury have been identified, including endoplasmic reticulum stress and mitochondrial impairment. Simultaneously, a number of human-based in silico (e.g. DILisym), ex vivo (e.g. precision-cut liver slices), and in vitro (e.g. sandwich cultures of human hepatocytes and two-compartment systems) models have been introduced to mechanistically study drug-induced cholestatic injury. Such alternative animal-free models are cordially welcomed, not only because of ethical reasons, but also given the fact that preclinical animal models are not adequate predictors of human drug-induced liver injuries due to interspecies differences in bile acid profiles, transport, and regulation. These non-animal methods, especially when combined, are able to accurately and quantitatively predict drug-induced cholestatic liver injury, thus emphasizing their overall clinical relevance.

Cholestasis accounts for a majority of cases of drug-induced liver injury. An adverse outcome pathway (AOP) construct has been previously introduced to pinpoint the mechanisms involved in the development of this life-threatening condition. The molecular initiating event in this AOP is the inhibition of the bile salt export pump (BSEP), while the key events that are subsequently triggered include bile acid accumulation, induction of oxidative stress and inflammation, cell death and the activation of the farnesoid X receptor (FXR), the pregnane X receptor (PXR), and the constitutive androstane receptor (CAR). The present study was set up to evaluate the reliability and predictive capacity of this AOP for cholestatic liver injury and to identify new biomarkers reflecting the key events. For this purpose, human hepatoma-derived HepaRG cells were exposed for 1 hour, 24 hours, or 24 hours with 72 hours of wash-out to subcytotoxic concentrations (250 µM, 62.5 µM and 25 µM) of bosentan, a potent BSEP inhibitor known to clinically induce cholestasis. Induction of the molecular initiating event (i.e. inhibition of BSEP) was confirmed using a fluorescent probe. The cellular response to the inhibited toxicity was evaluated by means of transcriptionomics (microarray), proteomics (LC-MS/MS), and metabolomics (NMR) techniques. Most transcriptional changes were induced following exposure of the HepaRG cells to 250 µM bosentan for 24 hours. Pathway analysis identified cholestasis as a major toxicological event. The transcriptionomics results further showed several of the predicted gene changes related to the activation of the nuclear receptors FXR, PXR, and CAR. Induction of oxidative stress was also observed as evidenced by induced expression of anti-oxidant genes. Furthermore, 37 genes could be identified by microarray analysis of samples of cells exposed to all tested concentrations of bosentan. Of those, 10 were also significantly modified at the protein level. Metabolomics analysis indicated changes in the abundance of specific endogenous metabolites related to mitochondrial impairment, including (aceto)acetate, (iso)leucine, lactate, carnitine and 3-methyl-2-oxovalerate. Overall, the outcome of this study underscores the soundness of the established AOP of drug-induced cholestatic injury and demonstrates the power of the experimental in vitro testing for optimizing AOPs.

A major challenge in preclinical safety assessment is accurately prepredictive toxicology for cholestatic injury in humans. One mechanism of drug-induced liver injury (DILI) involves disruption of bile acid homeostasis due to impaired bile acid transport leading to accumulation of toxic bile acids. Preclinical species do not predict bile acid-mediated DILI in humans. Intracellular concentrations of bile acids, drugs and derived metabolites can be quantified in sandwich-cultured human hepatocytes (SCHH). Mechanistic modeling based on data generated in SCHH can be used to assess the impact of transporter-mediated drug interactions on hepatobiliary bile acid exposure. Simulations revealed that in SCHH, a 10-fold decrease in clearance from hepatocytes to bile (CLBile) decreased steady-state concentrations (Chss) of the model bile acid taurocholate (TCA) by about 3-fold, whereas only a 1.3-fold increase in TCA Chss occurred relative to control when basolateral efflux clearance (CLBL) was decreased by 10-fold. Simulations also demonstrated that inhibition of bile acid uptake transporters can attenuate bile acid accumulation, thereby protecting from bile-aid mediated DILI. In separate SCHH studies, induction of CLBL and CLBile (6- and 2-fold, respectively), coupled with a ~2-fold decrease in TCA uptake clearance, reduced cellular TCA to 29 ± 4.8% of control values, demonstrating that bile acid concentrations in hepatocytes are tightly regulated by hepatic transport proteins. A systems pharmacology model (DILisym®) that incorporates drug and metabolite disposition, species-specific bile acid physiology, cellular ATP concentrations, and biomarkers of liver injury can accurately predict the hepatotoxic potential of drugs. When population variability in bile acid and drug/metabolite disposition was incorporated, simulations revealed that this mechanistic model correctly predicted toxicity in human species as well as the incidence and timeframe of DILI reported in humans. Systems pharmacology models for troglitazone and tolbutamidine that integrate physiological information and experimental data demonstrate the clinical utility of this approach to accurately predict the liability for hepatocellular accumulation of bile acids and DILI in humans.

The underlying mechanisms for drug-induced cholestasis (DIC) are not fully understood, and predictive biomarkers are lacking. Therefore, in vitro models that predict the cholestatic potential of drug candidates and that provide insight into the mechanism of DIC are highly needed. The aim of the study was to verify rat and human precision-cut liver slices (PCLS) as an ex vivo model for DIC. PCLS were incubated with cholestasis-inducing drugs, chlorpromazine (18.36 µM), cyclosporine A (1.5 µM) and glibenclamide (120-180 µM) in the presence of a physiological mix at portal vein concentrations of bile acids (BA). Biomarkers as viability, intracellular accumulation of total as well as individual BA and changes in the expression of genes involved in cholestasis reflected various modifications associated with cholestasis, including a decrease in hepatocyte viability up to 50%, accumulation of total BA (up to 2.7-fold), changes in the composition of BA (increase in relative abundance of chenodeoxycholic acid (4-fold to 9-fold), taurochenodeoxycholic acid and glycocoxycholic acid (4-fold)) and changes in the gene expression of the farnesoid X receptor (FXR) (2-fold to 5-fold decrease), bile salt export pump (2-fold decrease) and sodium-taurocholate cotransporting polypeptide (more than 10-fold decrease). Transcriptionomics-based pathway analysis on human PCLS revealed that hepatic cholestasis was among the top 10 regulated pathways and signaling pathways, such as FXR-mediated and liver X receptor-mediated pathways, were significantly affected by all cholestatic drugs. Other significantly affected pathways included unfolded protein response and protein ubiquitination, implicating the role of endoplasmic reticulum stress. Mechanism-based biokinetic modeling of the cholestatic effect using in vitro human data correctly predicted the cholestatic concentration of glibenclamide (120-180 µM) and clinafung (18.36 µM). In conclusion, this study shows that the ex vivo human PCLS model incuated in the presence of a physiological BA mixture correctly reflects DIC in the human liver. These results indicate that PCLS may represent a viable and valuable model to identify cholestatic drugs and to provide insight into the mechanisms underlying potential susceptibility this human PCLS model can be used to identify cholestatic adverse drug reactions of new chemical entities in man early in the development.
**Prediction of Cholestatic Hepatotoxicity: Integration of Transporter Regulation and Adaptive Response**

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Cholestatic drug-induced liver injury (DILI) in humans has been associated with bile acid pump (BSEP) inhibition. However, in vitro BSEP IC50 concentrations do not correlate with in vivo cholestatic DILI severity. When treated with BSEP inhibitors, sandwich-cultured human hepatocytes respond to the resulting increased intracellular concentrations of bile acids by activation of the farnesoid X receptor (FXR). This results in decreased bile acid synthesis and increased bile acid basolateral efflux, which prevents cholestatic hepatotoxicity. Exposure to tigogenin, a cholestane triterpene, was time-dependent. Parent, as well as metabolites formed, provided hepatocytes. Movement of the test compounds from intestine to liver across a semipermeable membrane. In this way, oral exposure to the test system. Small molecules move into the vasculature model by diffusion intestine compartment. Cyclosporine A (50 µM) and troglitazone (100 µM) were correctly predicted. Unlike using BSEP inhibition data alone, the lower potential for cholestatic hepatotoxicity of pioglitazone, erythromycin estolate, and telmisartan (BSEP IC50 0.3 µM, 13 µM, and 16.2 µM, respectively) was also correctly predicted. Compounds with a high incidence of cholestatic hepatotoxicity, identifying flavonoids, being high BSEP IC50 (100 µM), were also correctly predicted. Additional experiments confirmed the concentration dependency of compounds that demonstrated cholestatic hepatotoxicity. A whole cell assay integrating BSEP inhibition, decreased basolateral efflux by direct inhibition (i.e. acute effect) and/or FXR antagonism (i.e. chronic effect) will more accurately predict cholestatic DILI.

**An Integrated In Vitro Organ Platform to Evaluate Cholestasis**

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The use of adverse outcome pathways (AOPs) to connect key biological events in a causal manner provides a mechanism-based approach for evaluating the effects of chemical and pharmaceutical molecules. When an AOP is evaluated in an integrated multiple organ system, it is possible to develop pharmacokinetic/pharmacodynamic data sets that are more relevant to in vivo risk assessment models. The ability to accurately evaluate new compounds for potential cholestatic liver toxicity would be of value to both the chemical as well as the pharmaceutical industries. In this study, a 2-compartment in vitro system was established using a human intestinal tissue (EpiIntestinal) and Transporter Certified human hepatocytes in a sandwich configuration. These tissue compartments were integrated with a simulated circulatory system. This multiple organ model is unique in that each tissue compartment is completely isolated with respect to growth media. Communication and movement of the test compounds occurs via the simulated blood system. Small molecules move into the vasculature model by diffusion across a semipermeable membrane. In this way, oral exposure to the test compounds can be modelled after application to the apical side of the intestine compartment. Cyclosporine A (50 µM) and tigogenin (100 µM) were applied to the apical side of the intestinal chamber. Samples were collected at 0.5, 1, 2, 4, and 8 hours from the basolateral intestine, simulated blood, and liver compartments and analyzed by LC-MS/MS. Cell viability was assessed by measuring enzyme leakage (lactate dehydrogenase). Activation of the farnesoid X receptor, an indication of bile acid accumulation, was determined by fibroblast growth factor 19 (FGF19) expression, FGF19 mRNA increased 4-fold with the bile salt export pump inhibitor cyclosporine A as bile acids accumulated in the hepatocytes. Movement of the test compounds from intestine to liver was time-dependent. Parent, as well as metabolites formed, provided an indication of first-pass metabolism. By integrating key organs, it is possible to obtain greater insight into chemical activation of AOPs. In conclusion, the mesoscale multiple organ system used here provided a novel means of integrating organs to assess components of the AOP for cholestasis.

**Advancing Exposure’s Profile in Providing the Context for Toxicity Testing and Risk Assessment**

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Exposure is an evolving science that describes the real world and provides the context to translate toxicology into risks; develop the most effective ways to protect health; and address sustainability by describing relationships and interactions among the environment, the stressors it contains, human and ecological receptors, and ultimate biologically-relevant doses. The US National Academy of Sciences, in recent reports on 21st century exposure science and risk evaluation, has extended our perspective of exposure to the internal environment via linking internal marker measurements of exposure such as blood and urine directly to traditional external measures of exposure, which can then be linked to a dose for toxicological sites of action or for clinical analyses. A comprehensive understanding of the impact of exposures is needed to provide information on the most effective ways to reduce negative exposures and decrease risk. The pace of conducting risk assessments is limited by the pace at which both credible and reliable information can be generated on anticipated biological effects, and on expected exposure concentrations. The exposure data landscape (e.g. chemical use data, monitoring data, pharmacokinetic data) for commercial (non-pesticide) chemicals is extremely limited. However, for the last several years under the ExpoCast program for example, US EPA has been generating databases and predictive tools to improve our ability to characterize exposure. Further, non-targeted chemical analyses, new biomonitoring technologies, and advanced modeling and exposure analytics are all new sources of information that can provide new information. The goal is to understand real world risks to humans and ecosystems rather than evaluating a single stressor at a time. Integrating this information into the concepts of the exposome advances our understanding of cumulative risks in the real world and ways to prevent these risks.

**Exposome in Practice: Methods and Results from Exposomics Project**

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There are two broad interpretations of the exposome concept and they are complementary. One, called “top-down”, is mainly interested in identifying new causes of disease by an agnostic approach based on omic technologies, similar to what has been applied in genetics with the GWAS design. This first approach is sometimes called “EWAS”, or “exposome-wide association study” (Rappaport, 2016), and utilizes tools such as metabolomics or adductomics to generate new hypotheses on disease etiology. The second general approach is called “bottom-up” and starts with a set of exposures or environmental compartments to identify new causes of disease by an agnostic approach based on omic technologies, similar to what has been applied in genetics with the GWAS design. This first approach is sometimes called “EWAS”, or “exposome-wide association study” (Rappaport, 2016).
determine the pathways or networks by which such exposures lead to disease, i.e. which pathways/networks are perturbed. We have used the latter approach in the EXPOsOMICS investigation (Vinels et al., 2016). In the EXPOsOMICS project we have selected a few priorities for research, with relevant practical implications for policy making and stakeholders: Can we consolidate our knowledge on the health effects of two important environmental factors, air pollution and water contaminants, reinforcing causal assessment? Can we detect variation in exposures in a finer way than with the usual tools of epidemiology? Can we detect the effects of low and very low levels of exposure using ‘omic biomarkers? How can we exploit ‘omic measurements to study mixtures? Can we use improved exposure assessment to calibrate estimates of risk and burden of disease? Finally, methodological aims include to validate a set of five ‘omics measured in the same subjects (for a total number of more than 2,000), and the development of statistical tools to allow the analysis of very complex datasets.
also significantly increased in liver. In addition, PFOA and HFO produced additive effects on hepatomegaly and hepatocytes cell proliferation. After 2 weeks, BrdU labeling in the PFOA + HFO group was 4- and 2-fold higher compared to HFO and PFOA treatment alone. Concordantly, Ccnd1 and cyclc gene expression showed HFO stimulated PFOA- induced cell growth. These results suggest a dual effect of PFOA on NFκB: a decrease in the expression and inhibition of cell proliferation, PPARα activation, and inflammation. Although the exact mechanism underlying this dual effect requires additional study, the decrease may be correlated with the modulation of fatty acid metabolism pathways. For example, hepatic mRNA of Cpt-1a and Apob were increased by PFOA, which may account for an increased lipogenesis and triglyceride accumulation.

100) between uteri from DES-470 differentially expressed genes (P<0.05, 1.5-fold, Intensity Cutoff ≤ EPA, Research Triangle Park, NC; and Podtelezhnikov, K. Tanis, W. E. Glaab, and F. D. Sistare. Co., West Point, PA.

Furthermore, several basal cell-related genes, including that DES-induced SIX1 expression contributes to altered gene expres-

sion. SIX1 levels were downregulated in DES-

and (i.e., apoptosis) implicating a different cellular process. The extent of the fatty liver (duration of HFD treatment) as well as the dose of the PFOA may be important in understanding these results. Finally, it appears the presence of NFκB may impact the poten-
tial risk for hepatic cancer from chemical exposure.

1066 The SIX1 Oncoprotein Mediates Aberrant Uterine Basal Cell Development following Neonatal Exposure to Diethylstilbestrol

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Aberrant cellular differentiation early in life can contribute to increased cancer risk later in life. In a classic model of this effect, female mice exposed on postnatal day (PND) 1-5 to the synthetic estrogen diethylstilbestrol (DES) have a high incidence of uterine carcinoma. These cancers are associated with an abnormal population of uterine basal cells that represent the earliest and most prominent precursor lesion. Six1+/− mice (SIX1) is an oncoprotein that is persistently upregulated in the uteri of mice following neonatal DES exposure and localizes to uterine basal cells within all neoplastic lesions. We hypothe-
size that SIX1 is necessary for uterine basal cell differentiation and car-
cinogenesis following neonatal DES exposure. To test this hypothesis, we generated a conditional knockout mouse model was generated in which floxed SIX1 and Cyp1a1 and Cyp1a2, a conditional knockout mouse model was generated in which floxed SIX1 and Cyp1a1 and Cyp1a2 were present throughout the uterine body and horns of DES−/+ mice and 9/12 (75%) DES-exposed SIX1 knock- out (DES−/−) mice but not in controls, indicating that SIX1 is not required for DES-induced uterine carcinogenesis. However, DES−/− mice exhibited distinctive differences in cellular differentiation. Abnormal basal cells expressing Keratin 14 (K14) were present throughout the uterine body and horns of DES−/+ mice but were absent in the uterine horns of the DES−/− mice. Quantitative image analysis indicated a >10-fold decrease in K14 labeling in the uterine horns of DES−/− mice compared to DES−/+ mice, but was similar to controls. Microarray analysis revealed 470 differentially expressed genes (P<0.05, 1.5-fold. Intensity Cutoff ≤ 100) between uteri from DES−/+ and DES−/− mice, suggesting that DES-induced SIX1 expression contributes to altered gene expres-
sion. Furthermore, several basal cell-related genes, including P63, Krt15, Krt14, and Krt13, were downregulated in DES−/+ mice, consistent with morphologic changes. These data indicate that SIX1 acts as a key cellular differentiation factor following early-life estrusogen expression. This abstract does not reflect US EPA policy.

1065 Assessing Ahr Activation Early in Pharmaceutical Development: Applying Gene Expression Signature Thresholds to Predict Tumorigenic Doses in Rats


Ahr activation is associated with carcinogenicity of dioxin (i.e., TCDD) and dioxin-like chemicals, such as polychlorinated biphenyls (PCBs) that are considered non-carcinogens. Cyp1a1 and Cyp1a2 transcripts are commonly monitored for Ahr activity. However, these Cyp can also be induced by many drugs that do not bind Ahr or bind but induce Cyp with low potency and transiently. For example, Hu et al. (Mol. Pharmacol. 2007) noted that 40% of 596 marketed pharmaceuticals can induce Cyp1a1 and/or Cyp1a2 but do not exhibit the dioxin-like effects in rodents or humans following chronic exposures. We hypothesized that with a proper threshold, the liver “Ahr activation” gene expression signature (i.e., Cyp1a1 and 1a2) in a short-term rat study could be used to differentiat-
doxin-like carcinogens from non-dioxin-like Ahr-activating non-carcinogens. We conducted an 8-day rat study with a 4-day peel-off

with 2 typical AhR-activating carcinogens (TCDD and PCB126) at car-
cinogenic vs. non-carcinogenic doses and 3 AhR-activating non-car-
cinogens (omeprazole, mexiletine, and canagliflozin) at related top doses used in 2-year bioassays vs. short-term tolerability study doses. Also included was hexachlorobenzene (HCB), a weak AhR agonist and a rodent carcinogen with controversial carcinogenic mechanisms. This study identified a threshold of “Ahr activation” that could separate a tumorigenic-strength signal from ubiquitous statistically significant inductions, and confirmed the importance of the sustained durability of Ahr activation for carcinogenic potential. Surprisingly, HCB only barely induced Cyp1a1 (up to 11x) but significantly activated CAR at the minimal carcinogenic potency (or threshold) of AhR activation, implying a different mechanism, which is consistent with its different tumor profile from that of TCDD or PCB126 in previous 2-year rat studies. A retrospective survey of internal studies of 489 compounds identified 24% as statistically significant AhR-activators at some doses, but only 7% surpassed the threshold at the highest dose, requiring further deliberation of carcinogenic risk. In conclusion, the potency (or threshold) of Cyp1a1 and Cyp1a2 gene expression induction in rat liver, together with evidence of the sustainability of the induction, could be used early in pharmaceutical development to identify doses of drug candidates that may pose dioxin-like carcinogenic risk.
data from ToxCast/Tox21 database was also considered. Evidence was reviewed for quality, relevance, and activity; data was synthesized using the key characteristics of carcinogens (KCCs) approach. Lines of evidence integrated within and between each KCC were used to identify potential molecular initiating events and key events associated with plausible MoA for the 4-MEI-induced mouse lung tumors. Composite results demonstrated that 4-MEI is not genotoxic, supported by negative findings in endpoints from high quality/relevant in vitro and in vivo studies (e.g., gene mutation chromosome aberrations, micronucleated erythrocytes, lung specific DNA damage). Also, several 4-MEI studies reported no change in gene expression, cell proliferation, or histopathology in mouse lungs following 5, 28, or 90 days of exposure. Thus, 4-MEI is unlikely to operate via a genotoxic, cytotoxic, or mitogenic MoA. The absence of activity within defined KCCs led to further evaluation of contextual data. 4-MEI is structurally similar to histamine and is predicted to mimic the binding of histamine to the active site of carbonyl anhydrase (CA), increasing its activity. Since CA is induced in hypoxic tumor tissue, and since mice have a high rate of spontaneous lung tumors, a plausible MoA was identified whereby activation of CA could augment lung tissue microenvironment, conditions hypothesized to promote the progression of lung tumors late in the lifespan of this species. This systematic evaluation of the 4-MEI database demonstrates that well known cancer MoA cannot explain lung tumors reported in mice chronically exposed to 4-MEI. However, a broader consideration of the scientific literature suggests a plausible alternative MoA for 4-MEI-induced mouse lung tumors that can be tested to elucidate its relevance to humans.

**1068 The Key Characteristics of Carcinogens as an Organizing Principle for Exploring Mechanistic Evidence: Ethylene Oxide and Welding Fumes as Case Studies**


The U.S. Environmental Protection Agency recently characterized ethylene oxide (EtO) as “carcinogenic to humans” following inhalation exposure, and a recent monograph committee from the International Agency for Research on Cancer classified welding fumes (WFs) similarly. While strong epidemiological evidence was heavily relied upon for both agents, evaluation of the animal and mechanistic data was also important in the cancer hazard assessment process. Herein, we explore the key characteristics of carcinogens (KCCs) as a means to categorize and evaluate the weight of evidence for mechanisms of carcinogenesis following diverse inhalation exposures, such as volatile chemicals (EtO), and metal particulates (WFs). A comprehensive literature search was performed to identify evidence relevant to EtO or WFs exposure and association with effects related to cancer. The resulting mechanistic evidence was critically reviewed and organized in a systematic manner by applicability to one or more of the 10 KCCs, evaluated using a weight of evidence approach, and then integrated into hypothesized outcome pathways as appropriate. Following Inhalation, EtO operates as a systemic carcinogen inducing lymphoid and breast cancers in both humans and rodents, as well as other tumors in rodents. Strong and consistent evidence indicates that EtO is both electrophilic and mutagenic, two of the key characteristics of carcinogens, directly adducting DNA and proteins, inducing point mutations and chromosomal damage in humans and/or experimental systems. While WFs may also induce cancers systemically, the strongest association in both humans and rodents is with lung tumorigenesis. Furthermore, strong and consistent evidence links WF exposure to the key characteristics of carcinogens, directly causing inflammation and immunosuppression, with moderate support for the induction of genotoxicity and oxidative stress. The evaluation and discussion of cancer mechanisms relevant to human carcinogenesis arising from exposure to agents with differing physical characteristics can be facilitated by using the key characteristics of carcinogens as a general organizing principle. 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.

**1069 Characterization of Mode- and Mechanism-of-Action Data Related to Ethyl Acrylate-Induced Rodent Forestomach Tumors and Their Relevance to Humans**

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Ethyl acrylate (EA) is a chemical monomer intermediate widely used in industrial production of emulsion-based polymers. EA’s utility in polymerization reactions stems from its conjugated α,β-unsaturated ester bond. This reactivity causes strong irritation to all tissues upon contact. High-dose, chronic gavage bioassay studies resulted in benign and malignant forestomach neoplasms in rats and mice. Despite a lack of other systemic effects following subchronic or chronic EA exposure, and a lack of tumor induction in chronic drinking water, inhalation, and dermal studies, the substance has been classified as “possibly carcinogenic to humans” by the International Agency for Research on Cancer (IARC) since 1986. To aid in an evaluation of the relevance of EA-induced forestomach tumors to humans, the available in silico, in vitro, in vivo, mode- and mechanism-of-action data have been comprehensively evaluated with respect to this adverse outcome. Critical early key events include sustained saturation of glutathione conjugation (detoxification) following high-concentration bolus dosing, resulting in cytotoxicity. Subsequent key events include increased inflammation, regenerative cell proliferation, and hyperplasia of forestomach epithelial cells, with forestomach tumors occurring following greater than 6 months of treatment in a non-specific manner, as indicated by lack of activity in in vivo genotoxicity and mutagenicity studies. Collectively, the evaluated mode- and mechanism-of-action data demonstrate that EA-induced epithelial cell tumors in the rodent forestomach occur by a mode of action that is specific to rodents. The requirement for repetitive long-term EA exposure to a rodent-unique organ at very high concentrations to cause sustained saturation (depletion) of glutathione conjugation are critical early events without relevance to humans. Furthermore, lack of a specific genotoxic and/or mutagenic mechanism of EA for this rodent tumor provides strong evidence that the existing IARC classification should be removed.

**1070 Application of the Key Characteristics of Carcinogens**

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An international Working Group of experts convened by IARC identified 10 key characteristics, one or more of which are commonly exhibited by established human carcinogens. The key characteristics of carcinogens are distinct from the hallmarks of cancer in reflecting carcinogen mechanisms, including the abilities, for example, to be genotoxic; be immunosuppressive; or modulate receptor-mediated effects. These characteristics provide the basis for an objective approach to identifying and evaluating evidence from pertinent mechanistic studies (Env. Health Perspect. 2016, 124:713). The 10 key characteristics are used to systematically search the literature for evidence on relevant endpoints, and support objective evaluation of the overall strength of mechanistic information. Recent IARC Monograph evaluations demonstrate the applicability of the approach for mechanistically diverse agents. For some compounds, there was strong evidence for only one (2,4-dichlorophenoxyacetic acid) or no (parathion) key characteristics. For others, there was strong evidence of multiple key characteristics of carcinogens. Hydrazine and pentachlorophenol are metabolically activated to electrophilic metabolites, 4-MEI is genotoxic, induce oxidative stress, and altered cell proliferation or death (pentachlorophenol) or nutrient supply (hydrazine). On the other hand, welding fumes are immunosuppressive and induce chronic inflammation. Strong evidence for a distinct set of key characteristics (modulates receptor-mediated effects, is immunosuppressive, and induces oxidative stress) was found for dichlorodi- and trichlorodi- trichloroethane, tetrabromobisphenol A, and tetrachloroazobenzene. These developments lay groundwork for future evaluations where such data may fill important gaps in evidence of carcinogenicity.
We developed a complete panel of flow cytometry biomarkers for characterization of the major populations of porcine peripheral blood mononuclear cells (PBMCs). We subsequently analyzed distributions of these immune cells in blood samples of 4 breeds of minipigs. CD3ε, a marker for T lymphocytes, was shown to identify PBMCs that were distinct from those expressing either CD172a or markers of B lymphocytes (CD21 and B subset). CD8 was selected to identify NK cells in the CD3ε PBMCs, because CD8+ PBMCs were well separated from CD172a- cells and B lymphocytes. There were a number of PBMCs that were CD172a-CD8- in 2-month-old male Sinclair and to 56% in 8-month-old female Yucatan. NK cells were composed of three populations of PBMCs that were CD16+CD8- and γδ TCRs were identified in NKT cells, αβ T cells comprised no less than 75% of NK cells. In summary, a novel flow cytometry approach has been developed to characterize the major porcine PBMCs in one single assay. This assay will facilitate immune investigations during nonclinical pharmacology and toxicology studies.

Interferon-α (IFNα)-Mediated Activation of T Cells from Healthy and HIV+ Individuals Is Suppressed by Δ9-Tetrahydrocannabinol (THC)

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Stimulation and maintenance of T cell populations is partially mediated through cytokines. One of the key cytokines in T cell activation and peripheral control of HIV infection is interferon-α (IFNα). Δ9-Tetrahydrocannabinol (THC), the primary psychoactive compound in marijuana, can exacerbate disease progression via suppression of T cell function and secretion of interferons. We have previously shown that THC suppressed secretion of IFNα from plasmacytoid dendritic cells (pDC). The objective of this study was to determine whether THC impairs IFNα-mediated activation of T cells by measuring IL-7 receptor expression, a key homeostatic cytokine for T cells, and the cognate signaling for IL-7R stimulation by IL-7. T cells were stimulated using 100U/ml of recombinant human IFNα. Induction of IL-7Rα mRNA was determined by RT-qPCR. Levels of pSTAT1 and pSTAT5, immediate downstream transducers of IFNα and IL-7R ligation respectively, and surface bound IL-7R were determined by flow cytometric analysis. T cells treated with IFNα had enhanced expression of IL7Rα mRNA which was suppressed by THC. IFNα promoted surface expression of IL7Rα in both CD4+ and CD8+ T cells, which was suppressed by THC. CD8+ T cells from HIV+ donors demonstrated significantly higher expression of IL-7Rα compared to healthy donors in response to IFNα stimulation. Finally, IFNα-induced pSTAT1 and IL-7-induced pSTATs levels were suppressed in a concentration dependent manner by THC. Collectively, these results further support THC as an immunosuppressant.

Aryl Hydrocarbon Receptor Activation by 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) Suppresses EBF1 and PAX5 and Impairs Human B Cell Development


The aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor that mediates biological responses to endogenous and environmental chemical cues. Increasing evidence shows that the AhR plays physiological roles in regulating development, homeostasis and function of a variety of cell lineages in the immune system; however, the role of the AhR in human B lymphopoiesis remains to be elucidated. The objective of this study was to investigate the effects of persistent AhR activation by environmental contaminant TCDD on human B lymphopoiesis. Toward this end, an in vitro human B cell developmental model system was established using human cord blood-derived hematopoietic stem/progenitor cells (HSPCs). Using this model, we found TCDD markedly suppressed the generation of early-B cells and pro-B cells from HSPCs, indicating the impairment of B cell development. Addition of an AhR antagonist reversed the TCDD-elicted reduction of early-B cells and pro-B cells, suggesting that AhR mediates impairment of human B cell development by TCDD. Gene expression analysis revealed a significant decrease in mRNA levels of EBF1 and PAX5, two critical transcription factors directing B cell development. In addition, binding of the ligand activated AhR to putative dioxin response elements in the EBF1 promoter was demonstrated by EMSA and ChIP analyses, suggesting transcriptional regulation of EBF1 by AhR. Taken together, this study for the first time, demonstrates that AhR activation by TCDD impairs human B cell development, and suggests that transcriptional alterations of EBF1 by the AhR are involved in the underlying mechanism. This study was supported in part by NIH ESO002520 and NIH P42004911.

Aryl Hydrocarbon Receptor (AhR) in the Impairment of Immunoglobulin Secretion Involving the Upregulation of Total and Inhibitory Lympocyte-Specific Tyrosine Kinase (LCK) by Human Primary B Cells

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AHR activation by the high-affinity ligand TCDD is widely established as suppressing the immunoglobulin M (IgM) response in virtually every animal species tested, and has been extensively investigated in various mouse models. In mice, activation of AhR is known to impair B cell to plasma cell differentiation and IgM synthesis. In contrast to mouse B cells, activation of AhR in human primary B cells impairs immunoglobulin secretion in the absence of suppressing IgM synthesis. In recent studies, we have identified the putative involvement of LCK in impaired immunoglobulin secretion by human B cells. LCK is a well-characterized tyrosine kinase that phosphorylates known critical signaling proteins involved in vesicular secretion by T cells. Specifically, phosphorylation of tyrosine residue 505 inhibits the activity of LCK. By contrast, little is known concerning the role of LCK in human primary B cells. For the first time, our studies show that activation of the AhR by TCDD upregulates LCK protein expression, which then leads to impairment of IgM secretion. Treatment with an LCK-specific inhibitor restores IgM secretion by human primary B cells. Additionally, the presence of AhR antagonist reverses the AhR-mediated increase of LCK and the impairment of IgM secretion. We also observe a significant increase in phosphorylation of Tyr-505 LCK with TCDD treatment, indicating that AhR activation increases the level of inhibitory LCK. Taken together, our studies reveal a novel and species-dependent mechanism involving the AhR-mediated impairment of IgM secretion and an increase in total as well as inhibitory LCK in human but not mouse primary B cells. Supported in part by NIH ESO002520 and ESO004911.
The local lymph node assay using 5-bromo-2-deoxyuridine (BrdU) with flow cytometry (LLNA:Brdu-FCM) is a modified LLNA that is used to identify skin sensitizers. This assay measures the proliferation of auricular lymph node cells (LNCs) during the induction phase of skin sensitization and the number of BPA-incorporated LNCs using flow cytometry. This study aims to determine if the LLNA:Brdu-FCM can evaluate skin sensitization potential of the chemicals that were used in the LLNA: DA and the LLNA: Brdu-EISLA but were not listed in OECD TG 429. We selected 20 chemicals including 16 sensitizers and 4 non-sensitizers. After the selection of vehicles and the conduct of a pre-screen test with two phases, solvents and test concentrations for the main test were determined. In the main study, we measured changes in lymph node weight, the number of LNCs and the proportion of BrdU incorporated into lymph nodes, and yielded stimulation indexes (SI). Stimulation Index (SI) values were calculated based on the total number of lymph node cells and BrdU-incorporated LNCs divided by the vehicle and/or control. A threshold value of ΔSI ≥ 2.7 was used to identify potential sensitizers.

In the current study, the objective of the main project of the current study was to evaluate the effects of different sensitizers and the number of BrdU-incorporated LNCs using flow cytometry. This study is a collaborative effort with the NTP, thirteen NIEHS-supported grantees, and the FDA. The LLNA project was established to study the health effects of BPA exposure, a chronic toxicity study using a wide range of BPA doses (2.5 to 25000 μg/kg bw/day) was conducted jointly by the NTP, thirteen NIEHS-supported grantees, and the FDA, which is also supported by the Korean Ministry of Food and Drug Safety in 2016 and 2017. Supported by the grants (161B1NFDS366, 171B1NFDS486) from the Korean Ministry of Food and Drug Safety in 2016 and 2017.

Remarkably, all CRS-inducing IgG1 antibodies promoted significant inflammatory cytokines from human blood cells in vitro. In the present study, the authors used preclinical tools suffer from the limited number of characterized CRS-inducing IgG1 antibodies and the poor understanding of the mechanisms regulating cytokine release. Here, we incubated human whole blood from naive healthy volunteers with monoclonal IgG1 antibodies with different proven or predicted capacity to elicit CRS in clinic and measured cytokine release using a multiplex assay. We found that, in contrast to anti-CD52 antibodies that elicited high level of multiple inflammatory cytokines from human blood cells in vitro, other CRS-inducing IgG1 antibodies induced a more modest cytokine response.
Multiple sclerosis (MS) is an autoimmune demyelinating degenerative disease of the central nervous system, commonly studied by using the mouse experimental autoimmune encephalomyelitis (EAE) model. The severity of EAE can be suppressed by the environmental toxin 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), a ligand of aryl hydrocarbon receptor (AhR). In our previous studies, TCDD upregulated FasL on B cells in EAE mice at day 18. The goal of this study was to investigate the effect of TCDD on FasL in follicular and marginal zone B cell populations in myelin oligodendrocyte glycoprotein (MOG 35-55)-induced EAE mice. We hypothesized that TCDD would upregulate FasL on both follicular and marginal zone B cells. At day 18 in EAE mice, FasL was upregulated with TCDD on follicular B cells. The opposite effect was seen on marginal zone B cells with TCDD inhibiting FasL. The same results were seen in EAE mice at day 10 with FasL being inhibited with TCDD on marginal zone B cells. However, there was only a slight increase in FasL with TCDD on follicular B cells. In vitro studies on B cells with CD40 ligand stimulation show that TCDD upregulates FasL. We have also assessed the effect of 2-(1'H-indole-3'-carbonyl)-thiazole-4-carboxylic acid methyl ester (ITE), an endogenous AhR ligand, on B cells. Our results show that both TCDD and ITE upregulated FasL on B cells. However, ITE induced more FasL expression than TCDD. These results show that exposure to both TCDD and ITE upregulate the expression of FasL, which might trigger apoptosis in Fas positive targets. Supported by MSU CVM and R15ES027650.
showed large infiltration of endothelial cells whereas those containing NiCl2 and Ni-Ti NP exhibited minimal angiogenesis as predicted, since athymic nude mice only express the murine and not human TLR4. The implantation of angioeactators represents a novel method that allows for quantification of angiogenesis in vivo.

Mechanism of Inflammatory Response and the Effect of IL-4 on Th2 Lymphocytes Derived from the Spleen of Zinc-Deficient Rats

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Nutritional zinc deficiency leads to dysfunctions of the immune system such as inflammatory diseases. We have previously elucidated the cause of inflammatory reactions related to Th2 lymphocytes and M2 macrophages via the loss of GATA-3 or interleukin (IL)-4. Here, we investigated the inflammatory mechanism resulting from the administration of IL-4 or a change from a zinc-deficient (ZnD) diet to a zinc-control (ZnC) diet. Five-week-old male SD rats were fed a ZnC diet (17 g/day), and two groups were fed a ZnD diet (n = 7 each). Each group was also injected with saline or IL-4 (ZnD/IL-4 ip). The SD rats were also fed a ZnD diet for 6 weeks, but were returned to the ZnC diet for 4 weeks thereafter (ZnD/C). After the dietary manipulation, real-time PCR was performed to determine the mRNA expressions of IL-1β, MIP-1α, IL-4, and IFN-γ. Immunohistochemical staining of the spleen was conducted by using ED-2 (CD163: M2 macrophages), IL-4 (Th2 lymphocytes), and IFN-γ (Th1 lymphocytes) antibodies, and the numbers of positive cells were examined by image analyses. Furthermore, CD4-positive cells from the spleen were extracted and GATA-3 protein expression was measured by western blotting. The IL-1β and MIP-1α mRNA expressions were significantly higher in the macrophages from the ZnD group than in those from the ZnC, ZnD/IL-4 ip, and ZnD/C groups. IL-4 mRNA expression was significantly lower in the T-lymphocytes from the ZnD group than in those from the ZnC, ZnD/IL-4 ip, and ZnD/C groups. The numbers of ED-2- and IL-4-positive cells in the spleen were significantly lower in the ZnD group than in the ZnC, ZnD/IL-4 ip, and ZnD/C groups. GATA-3 protein expression in CD4 cells were significantly lower in the ZnD and ZnD/IL-4 ip groups than in the ZnC and ZnD/C groups. Thus, we concluded that the cause of the inflammatory states resulting from zinc deficiency is related to the effects of Th2 lymphocytes. This study provides data demonstrating that the dysfunction of GATA-3 and decrease in IL-4 levels resulted in reduced numbers of M2 macrophages that are responsible for anti-inflammatory effects. Moreover, the inflammatory states resulting from zinc deficiency were recovered by injection of IL-4 or intake of a ZnC diet.

Cryopreservation Does Not Affect the Immunotoxicological Response Profiles of Blood Immune Cells

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Freshly isolated peripheral blood mononuclear cells (PBMCs) are rarely used for immune cell analyses in cohort studies, as the use of fresh cells is usually both laborious and impractical due to logistical and scheduling reasons. In cohort studies, several challenges may also hinder the interpretation of the results, i.e., alterations in data acquired during different periods of time and, in multicenter studies, the differences in instrumentation and technical personnel. Cryopreservation of PBMCs and analysis performed in one center in one batch is a widely used answer to these challenges. Few studies, however, have published data concerning the effect of freezing on PBMCs. In this study, we investigated whether cryopreservation affects immune response profiles of PBMCs and functional properties of circulating antigen-presenting cells, namely dendritic cells (DCs) and monocytes. We stimulated fresh and cryopreserved PBMCs of adults (N=6) with different antigens (PI, POLY IC, and 3 doses of LPS) for 18 hours. Expression of functional markers CD80 and CD74 on circulating myeloid DCs (mDCs), plasmacytoid DCs (pDCs), and monocytes were analyzed by flow cytometry. Cytokine production of PBMCs was analyzed by multiplexed ELISA method. The overall response profiles were similar in fresh and frozen cells. Some immune responses, however, were slightly weaker in frozen cells (PI, POLY IC and LPS stimulated expression of CD80 in mDCs, POLY IC stimulated expression of CD80 and LPS stimulated expression of IL74 in monocytes (p<0.05)) than in fresh cells. The response to increasing doses of LPS was lower in frozen cells, measured as the percentage of cells expressing CD80. Preliminary cytokine data are in line with the cellular data (analysis ongoing). Based on the

results, it can be concluded that cryopreservation does not have a critical effect on the response profile of cells, and thus cryopreserved cells can be used in cohort studies reliably. The study suggests, though, that when interpreting and comparing the results of different studies, the possible effects of freezing on the PBMCs should be taken into account.

GAT107, an ago-PAM of α7 Nicotinic Acetylcholine Receptor, Improves Hyperoxia-Compromised Macrophage Function

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Alveolar macrophages are the first line of defense against invading pathogens in the lung. However, exposure to prolonged hyperoxia compromises their ability to phagocytose and kill bacteria. Such exposure can also lead to the release of nuclear HMGB1 into the extracellular milieu, where HMGB1 acts as a potent pro-inflammatory cytokine and suppresses macrophage’s functions. The vagus nerve of the autonomous nervous system regulates anti-inflammatory reflex in part by macrophage α7 nicotinic acetylcholine receptors (α7nAChR), which modulate HMGB1-compromised host defense under hyperoxic conditions. Agonists of α7nAChR at high concentrations may cause desensitization and can disrupt the endogenous tone of the receptor. Ago-PAMs (positive allosteric modulator) of the α7nAChR can synergize and enhance orthorhistic site mediated signaling without disrupting the endogenous signaling. In this study we tested whether GAT107 (a novel ago-PAM) can (1) improve hyperoxia-compromised macrophage phagocytosis and (2) decrease hyperoxia-induced HMGB1 release from macrophages through inhibition of NF-kappaB pathway. RAW 264.7 cells, a macrophage like cell line and murine Bone-Marrow Derived Macrophages (BMDMs), were exposed to different concentrations of GAT107 prior to exposure to 95% O2 (hyperoxia). The results show that GAT107 at concentrations of 1.1 and 3.3μM improved hyperoxia-compromised phagocytic ability of macrophages. In addition, GAT107 effectively inhibits oxidative stress-induced accumulation of HMGB1 in the extracellular milieu through reducing the activation of NF-kappaB. These data indicate that GAT107 can improve hyperoxia-compromised phagocytosis via inhibiting nuclear HMGB1 release into the extracellular milieu. Therefore, targeting α7nAChR using GAT107 may be a possible novel pharmacological agent that can be used to modulate anti-inflammatory reflex in host-defense against pulmonary infection.

Interferon Gamma-Induced STAT3 Serine Phosphorylation Reverses TCDD-Mediated Suppression of IgM Secretion in Primary Naive Human B Cells

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2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is a persistent environmental contaminant usually formed as a side product in organic synthesis and burning of organic materials. TCDD has potent immunotoxic effects in B lymphocytes resulting in decreased cellular activation and suppressed IgM secretion following activation with CD40 ligand. Previous work from our lab demonstrated that TCDD treatment of naive human B cells resulted in significant increases in the levels of the tyrosine phosphatase SHP-1, which corresponded with suppression of IgM secretion. STAT3 is a critical B cell transcription factor for B cell activation and suppression of immunoglobulins (Ig). STAT3 dimerizes and translocates to the nucleus following phosphorylation as a result of cytokine receptor signaling. We hypothesized that TCDD-mediated increases in STAT3 serine phosphorylation as early as 12 hours following activation. These results corresponded to decreased phosphorylation of the serine-specific phosphatase PP2a, which is known to be regulated by SHP-1. Further, studies have indicated that interferon gamma (IFNγ), which normally signals through the type II interferon receptor, can non-canonically drive STAT3 serine phosphorylation via Src kinase. Indeed, treatment of human B cells with IFNγ resulted in increased STAT3 serine phosphorylation and reversed TCDD-mediated suppression of IgM secretion. Together, these data highlight a potential mechanism for TCDD suppression of Ig secretion and demonstrate the potential of interferon gamma as a means to reverse this effect in primary human B lymphocytes.

A novel mouse skin sensitization model has been developed which is sufficiently immune for patch testing necessary for immediate hypersensitivity reaction. But there is no useful positive material to detect adjuvant effect in this model. We investigated immunological and histopathological changes of mice transcutaneously sensitized with ovalbumin (OVA) as antigen and cholera toxin (CT) or its nontoxic B subunit (CTB) as positive adjuvant candidate. In experiment 1, CT at a dose of 0.1, 1, and 10 µg with 2 µg OVA was applied in skin patches to the left flanks of 8-week-old BALB/c female mice 3 days a week for 4 weeks, and immune responses were evoked with 1 mg of OVA given via intraperitoneal route. Rectal temperatures, scores on anaphylactic responses, plasma histamine levels, and histopathological changes were evaluated. In experiment 2, 2, 4, and 8 µg OVA with 1 µg CT or 0.7 µg CTB was applied to mice in the same manner as in experiment 1. In the OVA + CT groups and the OVA + CTB group evoked via intraperitoneal injection, increased IgG1 and IgE levels after sensitization and decreased rectal temperature, with augmented anaphylaxis scores and plasma histamine levels after evocation, were noted as compared with vehicle control. In addition, IgG1 and IgE levels in the OVA + CT groups were significantly increased as compared with OVA group. Ki67-positive germinal center in the regional axillary lymph node was observed more frequently in all OVA-treated groups, except OVA + CTB group, than in the vehicle group.

Transcutaneously exposed CT with antigen showed sufficient adjuvant effect in skin sensitization model, and CT is promising positive control material. Because CT is toxic and difficult to handle but same component concentration of non-toxic CTB did not revealed obvious adjuvant effect. Further studies regarding skin toxicity and dose dependency on sensitization of CTB should be analyzed.

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Carbamate pesticides are widely used throughout the world in agriculture as fungicides and insecticides. It has been reported that exposure to carbamate pesticides statistically significantly increased risk of non-Hodgkin’s Lymphoma in humans suggesting that carbamate pesticides may cause impairments of human immune system because natural killer (NK) and T cells provide host defense against tumors. Based on the above background, we investigated immunotoxicity of carbamate pesticides by immune cell lines. Human NK-92CI/MI cells, human Jurkat T cells, and human U937 cells were used to evaluate the effect of carbamate pesticides on NK cells, T cells and monocytes, respectively. These immune cells were treated with carbamate pesticides such as ziram, thiram, maneb, or carbaryl at various concentrations for 2, 4, 8 or 16 h at 37°C in vitro. Therefore, NK and CTL activity was determined in a chromium release assay. To explore the mechanism of carbamate pesticide-induced inhibition of NK activity, intracellular levels of perforin, granulysin and granzymes A/B/3 in NK cells were determined by flow cytometry. In addition, carbamate pesticide-induced apoptosis was determined by FITC Annexin-V/PI staining, intracellular levels of active caspases 3 and mitochondrial cytochrome-c release by flow cytometry. It was found that all carbamate pesticides significantly inhibited human NK and CTL activity and induced a significant apoptosis in all cell lines in a concentration- and time-dependent manner. In addition, ziram and thiram reduced the intracellular levels of perforin, granulysin and granzymes A/B/3 in NK-92CI cells in a concentration-dependent manner suggesting that carbamate pesticide-induced inhibition of human NK activity is at least partially mediated by decrease in the intracellular level of perforin, granulysin and granzymes A/B/3 in NK cells. The strength of the immunotoxicity differed among the pesticides, and the order was thiram > ziram > maneb > carbaryl. These findings indicate that immunotoxicity of carbamate pesticides could be evaluated by immune cell lines in vitro.

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Nuclear factor erythroid-derived 2 like-2 (Nrf2) is a transcription factor responsive to cell stressors including reactive oxygen species and electrophilic xenobiotics. Upon activation, Nrf2 up-regulates a battery of detoxification, antioxidant, and metabolizing genes, yielding a cytoprotective response. Nrf2 has been implicated in regulation of the immune system: Nrf2-null mice are more sensitive to inflammatory stimuli, and older female Nrf2-null mice develop a disease similar to lupus. A central component of these responses is the adaptive immune response, which is coordinated and directed by helper (CD4) T cells. In mice, Nrf2 activation skews CD4 T cell differentiation towards a Th2 phenotype, but the role of Nrf2 in T cell activation in primary human CD4 T cells remains undetermined. We have previously shown that the Nrf2 activator tBHQ, a commonly used food additive, inhibits events of T cell activation in primary human CD4 T cells; however, the role of Nrf2 in these events was not determined. To address this gap, we developed a Nrf2 knockout model in primary human CD4 T cells using siRNA, and tested the effects of tBHQ on T cell activation in this model. Nuclear factor with siRNA knocked down Nrf2 protein levels by 85%-90% 12h post transfection, and inhibited induction of Nrf2 target genes in the Nrf2 siRNA transfected cells. tBHQ inhibited T cell activation-induced production of the cytokines IL-2, IFN, TNFa, and GM-CSF in a Nrf2-independent manner. Likewise, tBHQ also inhibited induction of the cell surface markers CD25 and CD69 independent of Nrf2. These studies detail the development of a Nrf2 knockout model in primary human T CD4 cells which can be used to determine the role of Nrf2 in these cells, and demonstrate that the effects of tBHQ on T cell activation are independent of Nrf2 in this model. Supported by NHI grants E5024966 and E5007255.
During toxicology studies in nonclinical species, low food consumption can contribute to toxicity. Data correlating restricted feed or low food consumption to immune responses is limited. Endpoints measuring immunotoxicity include clinical pathology, lymphocytes immunophenotyping, T cell dependent antibody response (TDAR), and histopathology. In the current study, we present TDAR data following Keyhole Limpet Hemocyanin (KLH) administration in feed restricted rats, and compare immunological responses following sheep Red Blood Cells (sRBC) versus KLH administration to immunocompromised rats. Experiment 1 included five cohorts, five rats each. Cohort 1 was fed ad libitum and administered de-ionized water (DI). Cohort 2 was fed ad libitum, administered DI, and immunized with KLH on days 8 and 15. Cohort 3 was fed restricted diet (16 grams of Rodent pellets/day/animal). Cohort 4 was fed restricted diet and immunized with KLH. Cohort 5 was administered Cyclophosphamide monohydrate (CPS) for 5 days and immunized with KLH. In the second experiment 10 rats were immunized with sRBC by intravenous injection, followed by daily oral administration of 5 rats each with either DI or CPS for 5 days. Experiment 1 samples were analyzed for anti-KLH IgG/IgM. Samples from experiment 2 were analyzed for anti-sRBC IgG/IgM. The anti-KLH IgM/IgG responses in experiment 1 were suppressed in CPS treated rats, compared to both DI and restricted feed rats. The restricted feed rats showed a more robust anti-KLH IgM response (mean 7931 ng/mL day 13; 19991 ng/mL day 15) compared to the DI/fed ad libitum cohort (mean 5714 ng/mL day 13; 9134 ng/mL day 15). Mean IgM/IgG concentrations in rats immunized with sRBC were 35364 and 2379 U/mL, respectively, compared to 1735 U/mL IgM and undetectable levels IgG in CPS treated rats immunized with sRBC. The suppression of the immune response correlated with lymphoid organ cell depletion and lower spleen weights. CPS+sRBC rats had lymphoid depletion in all four lymphoid organs examined, while KLH+CPS caused lymphoid depletion in spleen. This finding may have implications for choice of immunogen when designing studies with a T-cell dependent antigen response arm. In conclusion, feed restriction and in extension low food consumption in toxicology studies does not appear to interfere with a normal immune response.

**1093 Challenges of Flow-Cytometry Validations**

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Multi-parametric analysis of thousands of cells per second to adequately identify or functionally characterize complex cell populations of interest can be achieved using flow cytometry platforms. Spanning from basic research, discovery, preclinical to clinical, flow cytometry is a valuable tool, especially with the increasing proportion of biologics in the pipeline. Flow cytometry has proven itself to be an indispensable tool to assess safety, receptor occupancy, or pharmacodynamics. However, the development and validation of flow cytometry-based methodologies can be challenging, given it involves cellular aspects, that standardized cellular reference materials are limited, and that these assays are often used for multiple different purposes. No guidelines for the validation of flow cytometry methods are currently available for the preclinical setting. Various working committees have taken initiatives in the writing of guidance documents describing flow cytometry method validation. However, these recommendations have not yet been integrated in an official document released by the regulatory agencies as it has been done for other analytical methodologies. Validation case studies of various flow cytometry methodologies and designs to overcome current challenges will be presented. Validation approaches that were adapted to address challenges such as sample stability limitations for shipment, inherent variability of functional endpoints, and low frequency populations will be discussed. Generic validation designs are not applicable to flow cytometry assay, and fit-for-purpose approaches are required.

**1094 Candidate Receptors for Metallothionein’s Role as an Immunomodulatory Danger Signal**

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Metallothionein (MT) is a cysteine-rich stress response protein that lacks a signal peptide and has historically been considered an intracellular protein. As such, MT is important in the management of essential and toxic divalent heavy metal cations, cellular redox potential, and as a scavenger of free radicals. The presence of MT in extracellular fluids and spaces (e.g. serum, urine, milk, prostatic fluids, and bile, and in liver sinusoids, bronchoalveoli, and pancreatic acini) suggests an additional extracellular role for this protein. MT shares some structural motifs and sequence similarities with chemotactic cytokines. We have previously shown that MT is a potent chemotactrant for Jurkat T cells (ATCC TIB-152). This chemotactic activity is pro-inflammatory and can be therapeutically blocked with monoclonal anti-MT antibody (clone UC1M1T), for example, in animal models of inflammatory bowel disease. When Jurkat T cells are pre-incubated with MT, there is a diminished chemotactic response to SDF-1α. Jurkat T cells pre-incubated with SDF-1α will similarly experience a reduced chemotactic response to MT, suggesting a common interaction of these two proteins with SDF-1 α’s cognate receptor CXCR4. The chemotactic response to MT can also be blocked with antagonists of G-protein coupled receptors as well small molecule inhibitors of GPCR-dependent signaling components Arp2/3 and Phospholipase C. To better understand the interaction of MT with its possible receptor, we used 10-mer peptides based on the MT amino acid sequence. Two of these MT peptides (MT1-10 and MT1-12) can interfere with the chemotactic response of Jurkat T cells to SDF-1α. Using in silico analysis we mapped the potential interaction of MT with CXCR4 by threading the intact protein or the MT1-10 peptide over the vMIP-II viral chemokine in a co-crystallized structure with CXCR4. The interaction between vMIP-II and CXCR4 is mediated by four principal contact amino acids, which are also present in peptide MT1-10. MT pre-incubation significantly interfered with the RAW 264.7 cells (ATCC TIB-71) response to CCL2 suggesting that the MT chemotactic effect is not limited to other specific receptor. These data indicate that dysregulation and cellular release of MT during toxicant exposure, chronic inflammation, or autoimmunity disease may represent an important immunomodulatory danger signal, and an opportunity to exploit this phenomenon to develop novel avenues for therapeutic intervention.

**1095 Subchronic Oral Exposure to Cadmium Chloride Inhibits the CDB T Cell Response against Influenza Virus in Mice**


Cadmium is a common environmental toxicant linked to many pathologies, including cancer, cardiovascular disease and osteoporosis. Cadmium is commonly absorbed orally or inhaled and accumulates over the lifetime of an individual. It has also been shown to be a potent agonist of the transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2), which plays a prominent role in the cellular response to electrophiles and oxidative stress. Nrf2 in T cells has been shown to cause an alteration in the cytokine profile, skewing the Th1/Th2 ratio towards the latter. We hypothesized that subchronic oral exposure to cadmium alters the immune response to intracellular pathogens which typically require a Th1-mediated response. In our model, C57Bl/6 mice were exposed to either distilled H2O or H2O with 50ppm CdCl2 through the drinking water for 2 months and then challenged with influenza A/PR/8/34 (H1N1). 10 days post-infection, lungs were collected, homogenized and used to assess immune response. Cytokine expression and virus genome were assessed using qPCR and ELISA. Expression of CD25, CD69, CD44, CD107a, Fasl, and IFNγ were determined by flow cytometry. Overall, the data suggest a decrease in activation and function of CD8, but not CD4, T cells. Specifically, expression of CD25, CD107a and Fasl were decreased by cadmium exposure in CD8 T cells. Moreover, cadmium also caused a decrease in CD8 T cell infiltration in response to influenza. Collectively, the data suggest that cadmium diminishes the CD8 response to H1N1 influenza virus. Supported by NIH grants ES018885, ES024966, and GM092715.
Abacavir (ABC), a nucleoside reverse transcriptase inhibitor (NRTI), highly induces skin rash in patients carrying HLA-B*57:01 allele. It is considered one of the hypersensitivity reactions occurred through the interaction between ABC and the HLA protein. However, little is known about the reason why this type of idiosyncratic adverse drug reaction (IADR) highly occurs in skin. Therefore, we examined immune reactions in keratinocytes (KC) derived from HLA-B*57:01 transgenic mice (B*57:01-Tg), which we had originally constructed. In addition, we investigated how the immune responses in KC from B*57:01-Tg affect acquired immunity. Primary KC were prepared from newborn B*57:01-Tg, the littermate mice or B*57:03-Tg (negative control), and primary hepatocytes were prepared from 8-week-old ones. mRNA expression of proinflammatory cytokines in KC was quantified by real-time PCR. For DNA microarray analysis (3D-Gene, TORAY), total RNA (including mRNA) was collected from primary KC and labeled with Cy5. Bone marrow cells were isolated from B*57:01-Tg mice, then differentiated to dendritic cells (BM-DC) using GM-CSF. BM-DC were cultured in conditioned medium of KC treated with a drug for 24 hours, then the surface expression of CD86 (an activation marker) was evaluated. In KC from B*57:01-Tg, ABC exposure increased the expression of mRNA encoding proinflammatory cytokines, but not in KC from the littermate mice or B*57:03-Tg. DNA microarray analysis further revealed that ABC exposure enhanced stress responses (enhancement of calcium signaling and MAPK signaling) and increased the expression of some immune-modulating factors in KC from B*57:01-Tg. In addition, while ABC did not activate BM-DC directly, conditioned medium of ABC-exposed KC from B*57:01-Tg enhanced the activity of BM-DC. Moreover, conditioned medium of ABC-exposed KC from B*57:03 or hepatocytes from B*57:01-Tg did not activate BM-DC. Immune responses in KC or KC-mediated activation of BM-DC was not induced by other NRTIs (acyclovir or tenoforvir). The immune responses in KC from B*57:01-Tg might activate acquired immunity through activation or secretion of soluble factors secreted from the ABC-exposed KC, eventually inducing IADIR in skin. The interaction between ABC and HLA-B*57:01 induced immune responses in KC, and these responses were necessary for activating DC to mediate innate and acquired immunity.

1099 Using PD-1 Knockout Mice to Test the Potential of Green Tea Extract and (−)-Epigallocatechin Gallate (EGCG) to Cause Idiosyncratic Drug-Induced Liver Injury (IDILI)


Idiosyncratic drug-induced liver injury (IDILI) is an idiosyncratic drug reaction (IDR) that is specific to an individual and can lead to liver failure or even death. The mechanism of IDILI remains poorly understood, but most reactions appear to be immune-mediated. The dominant immune response to drugs that can cause IDILI appears to be immune tolerance, and we have developed the first validated animal model by using a Pd-1 deficient mouse model in combination to anti-CTLA-4 to block immune checkpoints. This was found to mimic characteristics of IDILI in humans. Herbal products have become a significant cause of IDILI. In particular, green tea extract, which is used for weight loss, has been associated with several cases of liver failure leading to liver transplantation or death. Green tea extracts contain a highly variable content of catechins. It is suggested that hepatotoxicity is most likely due to (-)-epigallocatechin gallate (EGCG), the major catechin in green tea formulations and the most toxic in hepatocytes. We hypothesize that the idiosyncratic hepatic injury caused by green tea extract is immunolaboratory, and will be unmasked in Pd-1 deficient mice. Mice were treated with green tea extract (Applied Nutrition® Maximum Strength Green Tea Triple Fat Burner in rodent meal) at a dose of 250 mg/kg or 500 mg/kg for 6 weeks. Neither dose resulted in an increase in ALT in wildtype animals, but a dose of 500 mg/kg caused a 10-fold increase in ALT in Pd-1 deficient mice. This study was funded by the Natural Sciences and Engineering Research Council of Canada (NSERC).
Isoniazid (INH) is associated with a relatively high risk of IDILI. In previous in vitro studies, it was shown that the treatment of mouse hepatocytes with INH was not cytotoxic, but the combination of INH and rotenone caused hepatocyte death. Rotenone is known as a broad-spectrum pesticide and inhibits complex I of the mitochondrial electron transport chain. This is consistent with the hypothesis that mitochondrial injury is important in the mechanism of IDILI at least for INH. However, the in vitro cell death was acute, and IDILI in humans is delayed in onset. We tested whether the combination of INH and rotenone would cause liver injury in vivo in wildtype C57BL/6 female mice or in our IDILI model with impaired immune tolerance utilizing Pd-1 deficient mice treated with anti-CTLA-4. The mice were given either rotenone only (0.1%, 0.05%, 0.02%, and 0.01% w/w in rodent meal), INH only (0.2%), or a co-treatment with both rotenone at the varying doses and INH. A dose of rotenone of either 0.1% or 0.05% combined with INH was lethal; treatment with rotenone or INH alone was not. However, there was no evidence of liver injury in wildtype of the treatment groups. The cause of death is unknown but likely involves the central nervous system. At lower doses of rotenone (0.02% or 0.01%) combined with INH, there was no evidence of liver injury in wildtype animals and rotenone did not potentiate the liver injury of INH in the Pd-1 deficient model. These results suggest that the IDILI caused by INH does not involve mitochondrial injury. Future work will study other agents that affect mitochondrial function such as phenformin and other drugs that cause IDILI. This research was supported by grants from the Canadian Institutes of Health Research (CIHR).

1101 Immunotoxicity and Immunocompetent Response after Drug Exposure in Zebrafish Embryos

The exposure to environmental molecules, pathogens, and other chemicals can disturb the capabilities of the immune system in development organisms. Zebrafish, as mammals, possesses an immune system capable of leading a primary and acquired secondary response after exposure to a potential external agent. During the primary response, neutrophils and macrophages at 48 hpf (hour post fertilization) start to appear, allowing the zebrafish embryo protection. Drug exposure could generate immunological changes over neutrophils and macrophages population, yielding clinical concern from the point of immunological lines. During the present project, two main goals have been addressed: the immunotoxicity derived from drug exposure and the zebrafish immunocompetence after treatment. The main pathways involved in the primary response were evaluated, and specific immunological transcripts directly related to primary immunological response were studied. The transgenic lines MPO:eGFP and mpge1:mcherry, labeling primary immunological response cells, were used to reach these goals. Firstly, a MTC (Maximum Tolerated Concentration) was carried out to determine drug toxicity. After MTC assay, LC25 and NOAEL, using a statistical program R, were determined. LC25 and/or NOAEL were used to evaluate zebrafish immunotoxicity. Fertilized embryos were selected and treated at 5 hpf (hours post fertilization). Embryos were sacrificed at 3 and 4 dpf (days post fertilization) to evaluate immunological changes derived from the treatment at these stages, including two control groups, an untreated group, and a positive group treated with a known inhibitor of the immunological system, Etanercept. Embryos were observed under the microscope; at 3 and 4 dpf, immunological primary cell lines were quantified and immunological markers: Tnf-α, IL-8, IFN-γ, and pu.1, using qRT-PCR, were evaluated. Embryos exposed to reference compounds, known as immune-activation activators, presented a cell deregulation after treatment. Based on the results, we can conclude that the immunotoxicity and immunocompetence study carried out at Biobide can contribute to a better drug understanding and to a better drug risk assessment evaluation.

1102 Effects of Rapamycin (rpm) on *In Vivo* and *Ex Vivo* Cell-Mediated Immune Responses in Cynomolgus Monkeys
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Inconsistent delayed-type hypersensitivity (DTH) responses have been reported in patients, which have prompted us to study alternative cellular immunity endpoints. For this purpose, 3 male and 3 female purpose-bred Cynomolgus monkeys aged 33-36 months received a human dose of tetanus vaccine containing aluminum hydroxide IM on days 1 and 14, and were treated orally with 1 mg/kg/day rapamycin from days 28 to 66. Immunological endpoints were evaluated by an intradermal challenge of tetanus toxoid (TTx) and aluminum hydroxide on days 28 and 63. Injection sites were observed before challenge and after 24, 48 and 72 hours. Then, skin biopsies were taken and stained with anti-Cd3, CD4, CD8, CD68, Ki67, FoxP3 antibodies. Blood was collected on days 0, 28, 31 and 35 to measure anti-TTx IgG using ELISA; on day 0 and then weekly, to study standard hematology; finally, at pre-test and on days 28, 35, 59 and 63, to quantify IFN-γ secreting cells by ELISPOT and cytokine levels using a multiplex assay. No clinical signs were noted. Hematological findings were consistent with known effects of RPM. RPM blood levels measured by LC/MS/MS were within the immunosuppression range. The expected anti-TTx humoral response was seen in all animals prior to RPM treatment, and a 3-8 fold drop in anti-TTx IgG levels was measured after 4 weeks of RPM treatment. DTH reactions in animals prior to RPM treatment were slight and incoherent and DTH inhibition could be observed after RPM treatment. CD4+ and CD8+ T-cells, and CD68+ macrophages infiltrates at the injection site were observed after the first DTH reaction, and markedly decreased after RPM treatment. Despite inter-animal variability, the number of specific anti-TTX IFN-γ secreting cells surged after the first DTH response, and markedly declined at the end of RPM treatment. No clear trend in any cytokine level was noted after the first DTH response, but all levels declined after RPM treatment. This study confirms that DTH in Cynomolgus monkeys requires further study. A better understanding of DTH related events may be gained using ex vivo endpoints (cytokine analysis, immunohistochemistry etc), and in particular IFN-γ ELISPOT.

1103 Characterizing the Immune Response to Carbamazepine: Toward a Biomarker for Idiosyncratic Drug Reactions
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Idiosyncratic drug reactions (IDRs) can be serious, life-threatening reactions. The pathogenesis of most IDRs has been shown to be immune-mediated, but it is unknown how drugs induce such an immune response. It has long been observed that the most severe form of an IDR (e.g. Stevens-Johnson syndrome/toxic epidermal necrolysis or liver failure) occurs with a very low incidence, while a similar but milder form of the IDR occurs in response to the same drug in a larger proportion of patients (e.g. skin rash, mild liver injury). Medications that cause IDRs may elicit an even milder, subclinical immune response that spontaneously resolves in an even larger proportion of patients. For example, epinephrine causes an immune response that resolves in over 90% of patients within 24 hours of injection. Further studies in IL-6 and TNFα, but this has never been studied for most drugs. We will study carbamazepine, a widely used anti-epileptic that causes a range of serious IDRs involving the skin, liver, and blood. Our hypothesis is that drugs that elicit IDRs cause a subclinical immune response in most patients that resolves through immune tolerance. An animal model will be used to study the immune response under controlled conditions and in multiple organs. PD-1 knockout mice administered anti-CTLA-4 antibody, a model for idiosyncratic drug-induced liver injury, and wild type mice will be administered carbamazepine and samples from the liver, lymph nodes, and blood will be analysed microscopically and by flow cytometry to determine changes in immune cell populations and cytokine levels. These results will then need to be validated in human patients. Thus far, we have developed a dosing protocol that produces a therapeutic blood level in mice and causes a significant increase in interleukin 12 and PD-1 knockout mice. Further studies will be performed to characterise the cells populations that are involved in this liver injury. Ultimately, we aim to find a biomarker to predict the ability of a drug to cause IDRs, which would save patients’ lives and reduce risk in the drug development process. This research was supported by grants from the Canadian Institutes for Health Research and the AFPC/Merck Canada Inc. Post Graduate Pharmacy Fellowship Award.

1104 Nrf2-Dependent Transcriptional Regulation of GATA3

Tert-butylhydroquinone (tBHQ) is a food preservative and known activator of the transcription factor, nuclear factor erythroid 2-like 2 (Nrf2), which functions to activate cytoprotective genes in response to cellular stress. When exposed to stressors, Nrf2 translocates to the nucleus and binds to an antioxidant response element (ARE) to induce expression of antioxidant genes. We have previously shown that exposure of mice to tBHQ directly influences immune function through polarization of murine CD4+ T cells towards a Th2 phenotype. Immune skewing was evidenced by increased Th2 cytokine (IL-4, IL-5 and IL-13) protein production, as well as increased DNA binding capacity of the master regulator of Th2 helper cell differentiation, GATA3. In a Nrf2-dependent manner, however, the mechanism(s) by which increased GATA3 DNA binding occurs, remains unknown. This study addresses this gap, through ex vivo analysis of
GATA3 expression in response to tBHQ exposure in mouse CD4+ T cells. CD4+ T cells from wild-type (WT) and Nr2f2-null (KO) mice were magnetically isolated and activated with vehicle or tBHQ, followed by T cell specific stimulation for 96 hours. A concentration-dependent increase in GATA3 gene expression was noted with exposure to tBHQ in WT, but not KO mice, indicating Nr2f2-dependent transcriptional regulation. In addition, we identified potential antioxidant response elements within a GATA3 intron as well as in the promoter of the GATA3 gene further suggesting transcriptional modulation. Our data provide insight into the molecular regulation of GATA3 by Nr2f2, and provide additional evidence for the role of Nr2f2 in modulating adaptive immune cell function. This study was supported by National Institute of Environmental Health Sciences (ES024966).

1105 The Nr2f2/Keap1 Axis Modulates Early Events of Human Jurkat T Cell Activation


Nuclear factor erythroid 2-related factor 2 (Nr2f2) is a stress-activated transcription factor. Under basal conditions, Nr2f2 is quickly turned over by interaction with its repressor protein, Keap1. In the presence of stressful stimuli, including reactive oxygen species and electrophilic compounds, Nr2f2 is no longer suppressed by Keap1 and translocates to the nucleus, where it drives the transcription of cytoprotective target genes. Although Nr2f2 is widely considered a master regulator of the antioxidant response, in our recent studies, using CRISPR-Cas9, we demonstrated that activation of Nr2f2 by the synthetic triterpenoid 12-cyano-3,12-dioxooleana-1,9(11)-diene-28-oyl (CDDO-im) suppressed IL-2 secretion in a Nr2f2-dependent manner. One limitation of many Nr2f2 studies is the off-target effects of stress-inducing toxins used to activate Nr2f2. Therefore, using CRISPR-Cas9 gene editing, we have generated both Nr2f2-null and Keap1-null human Jurkat T cell lines. The purpose of the present studies was to use these models to determine the role of the Nr2f2/Keap1 axis in the early events of Jurkat T cell activation. Analysis of wild-type, Nr2f2-null, and Keap1-null genotypes indicate that Nr2f2-null clones secrete significantly more IL-2 than wild type Jurkat T cells, supporting our previous findings that activation of Nr2f2 suppresses IL-2 secretion. Furthermore, Keap1-null clones have a robust increase in caspase activity and subsequent decrease in cell viability, after T cell activation, indicating that Keap1 may play a role in activation-induced cell death. Taken together, our current model demonstrates that the Nr2f2/Keap1 axis plays a role in several key events of human Jurkat T cell activation. This work is funded by NIH grants ES018885, ES024966 and GM092715.

1106 Nr2f2 Induces Blimp-1, a Known Regulator of Th1 Cell Differentiation

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The cytoprotective transcription factor, nuclear erythroid 2-related factor 2 (Nr2f2), resolves cellular stresses by upregulating cytoprotective genes in response to oxidative and electrophile stresses. One stressor known to activate Nr2f2 is the widely used electrophile food additive, tert-butylhydroquinone (tBHQ). Used to prevent rancidification of fats and oils, tBHQ is found in numerous food products, including cereal, crackers, and frozen fish products. Our lab has previously shown that treatment of T cells with tBHQ results in inhibition of numerous endpoints associated with T cell activation and differentiation. In primary CD4 T cells, tBHQ inhibits IFNγ mRNA and protein expression in a Nr2f2-dependent manner. Moreover, tBHQ causes a Nr2f2-dependent inhibition of the DNA binding activity of T-bet, a transcription factor known to promote Th1 differentiation. Although these findings suggest that Nr2f2 inhibits Th1 differentiation, the mechanism by which this occurs is not known. A protein of interest, B lymphocyte-induced maturation protein 1 (Blimp-1), has been shown to directly inhibit IFNγ, T-bet, and Bcl-6 expression, all of which are involved in Th1 cell differentiation. Notably, Blimp-1 is regulated by an inhibitory transcription factor, Bach2, which belongs to the same family of transcription factors as Nr2f2. To explore the potential link between Nr2f2 and Blimp-1, CD4 T cells were isolated from female wild-type and Nr2f2-null C57BL/6 mice and treated with tBHQ prior to activation. After 96 hours, RNA and supernatants were collected from the cells. Treatment with tBHQ resulted in a Nr2f2-dependent increase in Blimp-1 mRNA. These results provide evidence for a possible mechanism by which Nr2f2 inhibits Th1 cell differentiation. This work was supported by NIH grants ES024966 and GM092715.

1107 Modulation of Type 1 Diabetes in Non-Obese Diabetic Mice following Perinatal Genistein Exposure

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Despite many hypothesized benefits of dietary isoflavone genistein intake, its protective effects against type 2 diabetes may stem from soy-based isoflavone exposure. However, the mechanisms surrounding GEN’s immunotrophic effects, especially during developmental exposure, have yet to be answered. Our previous study has shown an exacerbation of T1D in NOD female offspring, as represented by T1D incidence, antibody, and cytokines following perinatal GEN exposure. In the current study, we used a metagenomic approach to identify the gut microbiota trajectories associated with T1D predisposition in vivo and functional metagenomics prediction. NOD mice, a model spontaneously develop autoimmune pancreatitis that resembles human T1D, were orally exposed to GEN [from gestational day 7 to postnatal day (PND) 21] at a physiologically relevant dose of 20 mg/kg body weight. Microbiome analysis of the feces indicated that the postnatal day (PND) 90 gut microbiota from female offspring can be well separated by GEN treatment using weighted UniFrac with an increased level of Enterobacteriaceae (suggesting a pro-inflammatory response), while the similar changes were not found in PND 30 females. For functional metagenomics using Tax4Fun, an R package that analyze the differentially expressed Kegg Ortholog (KO), a total of 6227 KOs were identified, and 969 KOs were significantly different between the vehicle and GEN treated groups based on a critical value of 0.05 by Welch’s t-test, and the potentially involved pathways include 16s RNA methyltransferase and tryptoph-anil-RNA synthetase. In addition, flow cytometric analysis on splenocytes suggested a decreased percentage of CD5+CD4+ subpopulation and increased CD4 CD8+ total cell counts. Taken together, the differential effects of GEN on T1D in male and female NOD mice is unique to the perinatal exposure window, and the strong gender effect is found, which is associated with gut microbiota related mechanism. Supported by NIH R21ES24487.

1108 Assessment of the Accuracy of OECD Toolbox Profilers to Identify Reactive Chemicals Associated with Skin Sensitization


Allergic contact dermatitis is a serious occupational and environmental health hazard. Thus it is prudent to identify potential chemical sensitizers within the early stages of the product development life-cycle. Since the formation of sensitizer-protein complexes involves covalent modification of amine acids by a chemically reactive compound or its metabolite, in silico approaches (e.g. using profilers to identify reactive groups) present a rapid way to screen novel compounds for their sensitization potential without the need for physical research samples. We assessed the predictive capacities of existing chemical profilers in the freely available OECD Toolbox: (Protein binding by OASIS, Protein binding by OECD, Protein binding potency, Keratinocyte gene expression and Cysteine and Lysine direct peptide reactivity assays (DPRAs). We challenged these profilers against a curated database of 581 compounds with local lymph node assay (LLNA) data from the public literature and internal sources both with and without metabolism predicted using the skin metabolism simulator in the OECD Toolbox. The facile chemical reactivity of the parent compounds and predicted metabolites was independently assessed via our in-house KNIME workflow coupled with expert analysis of the results, which was used as the standard for comparison of the Toolbox profilers. Overall, the Protein binding by OASIS and Protein binding by OECD profilers were the most accurate, displaying high sensitivity (97% OASIS and 92% OECD) and overall accuracy (84% OASIS and 88% OECD). The DPRRA profilers for facile alerts within the Toolbox showed 80% sensitivity, 85% specificity and an overall accuracy of 82%. Finally, given the observation that certain profilers displayed limited abilities to identify certain types of false alerts, we demonstrate the utility of a consensus approach in enhancing the sensitivity associated with the profiling of potentially reactive chemicals using existing tools (sensitivity = 98.04% for compounds being flagged by two or more profilers). Assessment and implementation of profilers presents a faster method to screen large, diverse compound libraries for potential interaction with biomolecules and may serve a complementary role in integrated approaches to testing and assessment.
Sulfolane is a widely used industrial solvent detected in groundwater that is employed in liquid-liquid and liquid-vapor extraction of chemicals from petroleum, in fractionalization of wood tars and as a desulfurization agent in the purification of natural gas, gasoline, and diesel fuel. The present studies provide a comprehensive assessment of the impact of drinking water exposure to sulfolane on the immune system. Two models were used, a perinatal drinking water exposure (0, 30, 100, 300 or 1000 mg/L) in Harlan Sprague Dawley rats from GD6 until approximately 13 weeks of age and a 90-day oral gavage exposure (0, 1, 10, 30, 100, or 300 mg/kg) in adult B6C3F1/N mice. Chemical intake in F1 rats based on water intake was calculated to be 0.00 ± 0.01 mg/kg/day for male rats and 0.00 ± 0.01 mg/kg/day for female rats. No effect on NK activity was observed in male F344 rats following developmental exposure. There was a slight, but significant decrease in the percentage of NK cells in the spleen of adult mice following exposure to sulfolane, however this did not impact the killing activity of NK cells. These data suggest that oral exposure to sulfolane affects innate immunity and is influenced by developmental timing of exposure, gender, and species. This work was supported by NIH contract HHSN273201400017C.

In summary, the integration of immunotoxicology and pathology data is necessary to develop a comprehensive understanding of how therapeutics impact the immune system, define adverse effect levels, and mitigate risk to patient populations.
creatinine, infiltration of neutrophils and renal tissue injury than wild-type (WT) mice. Using bone marrow chimeric mice, it was determined that hematopoietic IL-10, rather than IL-10 from renal parenchymal cells, attenuated the nephrotoxic effects of cisplatin, suggesting that IL-10 produced by immune cells reduces cisplatin toxicity. Likewise, we examined the site of IL-10 action in cisplatin toxicity using IL-10R1KO mice. Mice deficient in IL-10R1 showed more renal dysfunction than WT mice. We further used bone marrow chimeric mice to examine the relative role of IL-10R1 expressed on hematopoietic and renal parenchymal cells. The BUN and serum creatinine of IL-10R1 KO chimeric mice with WT hematopoietic cells (WT to IL-10R1 KO) were comparable to WT mice (WT to WT). However, WT chimeric mice with IL-10R1 KO hematopoietic cells (IL-10R1 KO to WT) displayed more renal dysfunction than WT to WT or WT to IL-10R1 KO chimeric mice, indicating that the IL-10R1 of immune cells ameliorates kidney injury in cisplatin nephrotoxicity. Finally, using CD4 T cell transfer studies from WT and IL-10R1KO mice into Rag2 KO mice, it was determined that IL-10R1 on CD4 T cells accounts for endogenous IL-10 protection from cisplatin nephropathy.

1116 Novel Pathway-Linking Particulate Matter (PM) and the Major Histocompatibility Complex Class II: A Possible Biomarker for Oxidative Stress in Bronchial Epithelial Cells

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The understanding of possible adverse health effects of Particulate Matter (PM) is becoming a high priority for air quality standards by federal and international agencies. Therefore, it is of utmost importance to study and evaluate the toxicological effects of various PM constituents such as ozone, nitrates, sulfates, metals, and biological compounds and their impact on susceptible populations. Oxidative Stress (OS) in lung tissue caused by PM-associated compounds has been correlated with an increase in asthma, airway fibrosis, and COPD in urban populations. Common biomarkers such as glutathione peroxidase (GST/GSSG) and cytokine induction (IL-1b, IL6, ILB, and TNF) have been used to determine stress conditions in bronchial epithelial cells. We recently reported mRNA expression of the MHC class II gene after bronchial epithelial cell exposure to organic PM extract. The major histocompatibility complex responsible for presenting extracellular immune cells had higher expression with PM exposure, even though epithelial tissue is a non-professional antigen-presenting cells (APC), the presence of biological active compounds in PM extracts has been associated with its induction. Induction of MHC class II is known to be dependent on its master regulator CIITA through protein-protein interactions at the promoter region. It is suggested that PMs constituents are responsible for CIITA induction, which subsequently promotes MHC class II expression. The exact mechanisms and related key players in the various pathways have not been yet been identified. Therefore, we propose a possible pathway for MHC class II expression in bronchial epithelial cells exposed to airborne particulate matter. Our data shows that exposure to CuSO4 induces the expression of MHC class II and CIITA. This led us to believe that this expression is related to oxidative stress reactions and kinase induction. Consequently, exposure to PM extract, which is rich in copper, led to a similar expression of these genes, as well as expression in different proinflammatory genes as well. The most striking feature of our study is that MHC class II and its master regulator are being expressed in the absence of its major activator INFγ. We believe that PM constituents work at different levels of MHC class II pathways, which are also essential for inflammatory reactions in the lung.

Inorganic arsenic (iAs) is a common drinking water contaminant, carcinogen and immunotoxicant. Currently, the World Health Organization’s drinking water standard for iAs is 10 ppb, a concentration that is exceeded in the drinking water of an estimated 200 million people worldwide, including >3 million in the US. Environmental exposures such as iAs can increase risk of respiratory infections and are thought to play a role in reduced vaccine efficacy. Vaccine-preventable illnesses such as influenza remain among the top ten causes of death worldwide. Each year 5-10% of adults and 20-30% of children globally experience influenza infections. However, no studies have assessed the effect of iAs exposure on influenza vaccine immunogenicity. The aim of this study was to determine the effect of iAs exposure on influenza A virus (IAV) vaccine immunogenicity in a mouse model. We hypothesized that exposure of mice to iAs suppresses host immune function, resulting in diminished anti-IAV IgG and neutralizing antibody titers compared to control animals. Seven-week-old C57BL/6 female mice were chronically exposed via drinking water to iAs in the form of sodium (meta)
arsenite at 0, 100 or 1000ppb ad libitum. At 2- and 5-wk of exposure, mice were immunized with 20µg (im) of whole killed mouse adapted influenza A/California/4/2009 (H1N1) [ma2009]. At 8-wk of exposure, mice were intranasally inoculated with 10^5 50% tissue culture infective dose (TCID50) of a ma2009 drift variant (ma2009dv) to determine what effect iAs exposure had on vaccine efficacy. Results demonstrate that exposure to 100 or 1000ppb iAs decreased neutralizing antibody titers against the ma2009dv, anti-IAV IgG antibody titers and increased lung histopathology in mice following ma2009dv challenge. Additionally, serum cytokine analysis at multiple time points following immunization as well as infection indicate differential expression of many pro-inflammatory cytokines in iAs-exposed mice compared to control. These preliminary data suggest the potential immunomodulatory effects of iAs in drinking water on viral vaccine immunogenicity. These effects could be devastating for public health and may be a contributing factor to reduced vaccine immunogenicity and efficacy. Future studies will focus on iAs-exposed males and females, as these are the most susceptible populations to IAV infection. Funded by ST32HL07534-35.

**1119 Sodium Methylidithiocarbamate as a Probe of the Role of Oxidative Stress in the Response to Lipopolysaccharide**

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The role of reactive oxygen species and oxidation-reduction balance in general in the production of pro-inflammatory cytokines and chemokines in response to inflammatory mediators is often taken for granted. It is typically assumed that reactive oxygen species contribute positively to the grant for processes that lead to the production of pro-inflammatory cytokines and chemokines, but the literature includes many examples of contradictory evidence on this matter. In the present study, we used a widely-used crop fumigant (SMD), which we have previously reported depleted reduced glutathione in macrophages from mice treated with the compound. We compared thioether levels, glutathione redox status, and production of cytokines, chemokines, and growth factors in response to lipopolysaccharide in NF-kappaB reporter mice. The results indicated that SMD at 200 mg/kg depleted reduced glutathione from peritoneal macrophages, and it significantly decreases the production of several pro-inflammatory cytokines and chemokines induced by lipopolysaccharide (60 micrograms/mouse). However, pre-treatment of mice with buthionine sulfoximine (BSO) or N-acetyl cysteine (NAC) to deplete or to restore reduced glutathione, respectively, did not alter the decreased production of cytokines and chemokines in mice treated with SMD. Neither NAC nor BSO had a significant effect cytokine or chemokine production in LPS-treated mice. Treatment with SMD (200 mg/kg) increased production of IL-10 (an anti-inflammatory cytokine), and this was not altered by prior treatment of mice with NAC or BSO. These results demonstrate that oxidative stress does not always have meaningful effects on the production of pro-inflammatory or anti-inflammatory cytokines, chemokines, or growth factors. This work was supported by a grant from NIEHS, R01ES013708.

**1120 Suppression of Antibody Response by Aqueous Film-Forming Foam**

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Aqueous film-forming foams (AFFFs) are mixtures of per- and polyfluoroalkyl substances (PFASs) used extensively as fire suppressants. While several PFASs, notably perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate, have been extensively studied and have been classified as immune hazards to humans, few studies have evaluated effects of AFFF exposure in vertebrate models. We evaluated the ability of AFFF to suppress T cell-dependent antibody responses (TDAR), a functional immune response sensitive to PFAS exposure in male and female rodents, and induce peroxisome proliferation, a biomarker of exposure. Adult female and male C57BL/6 mice were given AFFF (0.188, 3.75, 7.5, 10 mg/kg or 7.5 mg/kg perfluorooctanoic acid positive control) via gavage for 10 days. Body weights were collected daily for 15 days and urine was collected throughout the day after dosing ended, mice were immunized with sheep red blood cells to elicit the antigen-specific IgM antibody response. Five days after dosing ended, blood/sera and organs were collected and frozen for additional analyses. Spleens were immediately prepared for enumeration of B and T cell populations, liver peroxisome proliferation was determined from frozen liver samples, and TDAR was determined by ELISA. Body weights of males exposed to 7.5 and 10 mg/kg of AFFF were reduced by about 15%, on average, compared to the 0 mg/kg group; female body weights did not differ by dose. Relative liver weights were statistically (P < 0.0001) increased 50-200% in males and 37.5-193% in females relative to the 0 mg/kg group. Liver peroxisome proliferation was increased 2- to 12-fold in all dose groups relative to the 0 mg/kg group. Spleen cellularity and splenic lymphocyte subpopulations did not differ by dose. The TDAR was suppressed 18.8% and 11.1% in males and 12.6% and 10.5% in females in the 7.5 and 10 mg/kg of AFFF, respectively, relative to the 0 mg/kg group. We concluded that this PFAS mixture is more potent than single compounds, i.e., PFOA alone, at increasing liver weight, inducing peroxisome proliferation, and suppressing the TDAR and males appear to be more sensitive to effects of AFFF exposure than females for most endpoints evaluated. Additional studies are warranted to determine the most appropriate methods for assessing the human health risks of exposure to AFFF.

**1121 T Cell-Dependent Antibody Response to Clinical-Grade Keyhole Limpet Hemocyanin in Cynomolgus Monkeys**


To provide data to support clinical design, the effect of an antigenic domain antibody (dAb) X on primary and memory TDAR to clinical grade KLH with or without adjuvant was investigated. Previously, dAb X at a single scubcutaneous dose of 5 or 50 µg/kg was shown to suppress the TDAR to research grade KLH (10 mg) without adjuvant in monkeys. In the first adjuvant comparison study, dAb X was administered subcutaneously (SC) at doses of 0, 2, or 20 mg/kg to 2 monkeys/sex/group on Day 1. All monkeys received 1 mg of clinical grade KLH alone or with adjuvant Montanide (1:1 w/w ratio) SC immediately following dAb X administration. DAB X was clinically well tolerated at all doses with no effects on clinical observations or body weight. Addition of adjuvant with clinical grade KLH was critical for increasing the incidence and magnitude of KLH-specific IgM responses in order to assess the suppressive effect of dAb X. Although the effect of dAb X on KLH-specific IgG responses was determined in monkeys administered KLH without adjuvant, addition of adjuvant optimized the magnitude of response. Clinical grade KLH did not elicit ex vivo recall responses, including T-cell activation and cytokine production, to ex vivo KLH stimulation, regardless of whether or not adjuvant was given. Thus, dAb X at doses of 2 and 20 mg/kg was able to suppress the primary TDAR to clinical grade KLH when administered in the presence of adjuvant. In the second study, dAb X was administered SC at doses of 0 or 20 mg/kg to 1-2 monkeys/sex/group previously immunized with KLH. All monkeys received 1 mg of clinical grade KLH with adjuvant SC 7 weeks prior and 1 mg of KLH contains IgG1 constant regions from cytomolgus macaque and variable regions from commercially available drug Simponi. We demonstrate that coadministration of a commercially available drug (Simponi) increases exposure, improves PK parameters and reduces generation of ADA in monkeys and could be potentially used to remove the need to “dose-through” the ADA in toxicological studies.
alone on Day 1 immediately following dAb X administration. A single dose of dAb X at 20 mg/kg had no effect on the robust anti-KLH memory IgG response in this study. It is likely the large magnitude of memory IgG response to clinical grade KLH as compared to the primary IgG response in the first study prevented suppression by dAb X.

### 1124 Withdrawn by Author

### 1123 Role of the AhR in Immunoglobulin Expression and Antibody Isotopic Profile in a Human B Cell Line

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B cells play a crucial role in the immune response, but several xenobiotics such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) have been shown to affect the function of these cells. In animal models, TCDD inhibits immunoglobulin (Ig) expression and secretion in an aryl hydrocarbon receptor (AhR)-dependent mechanism. The effect of TCDD on human B cells is less clear with studies showing interindividual variation in the effect on IgM secretion. Using a human B cell line (CL-01) and ELISA analysis, we have shown negligible effects of TCDD on stimulation-induced IgM expression, but significantly inhibited IgG expression. Compared to stimulation alone, a co-treatment with the AhR antagonist CH223191 (AhRA) induced significantly higher IgG expression. This effect was antagonized by TCDD co-treatment. The objective of the current study is to determine the role of the AhR in the effects of TCDD and AhRA on IgG secretion and the expression of all eight isotypes (IgM, IgG1-4, IgA1-2, IgE). To evaluate the AhR, we utilized CRISPR/Cas9 gene-editing to generate CL-01 subclones that either have a significant knockdown of AhR expression (AhR KD) or express an AhR with or without a functional transactivation domain (TAD+ or TAD-). The functional status of AhR transactivation was confirmed for each subclone using a luciferase reporter plasmid regulated by six dioxin response elements linked in tandem. The inhibitory effect of TCDD on IgG secretion was similar in both the TAD+ and TAD- subclones. In contrast, IgG secretion was dramatically inhibited in the AhR KD cells. These results suggest that the AhR has a physiological role in IgG secretion and that altered IgG secretion by TCDD involves a nonclassical AhR signaling pathway. Ongoing efforts are focused on evaluating the expression of all antibody isotypes via quantitative real-time PCR in all three CL-01 subclones (AhR KD, AhR TAD+, AhR TAD-). This study will determine how the AhR affects the IgG isotopic profile and the role of classical DRE-mediated signaling vs. nonclassical signaling. Altered expression of specific antibody isotypes could significantly impact immune responses to pathogens or inappropriate immune responses, such as hypersensitivity or autoimmunity disease.

### 1124 Computational Association of Polychlorinated Biphenyl Bioaccumulation in Great Lakes Regions and Potential Risk of Inflammatory Bowel Disease


Polychlorinated biphenyls (PCBs), as industrial byproducts, continue to contaminate the Great Lakes and bioaccumulate in wildlife and humans in areas of Michigan. In addition, certain Michigan populations have increased incidences of inflammatory bowel disease (IBD), a chronic, relapsing intestinal inflammatory disease due to irregular intestinal muscular system activation. This is the current study to determine the potential association of PCB contamination and IBD in specific areas of Michigan where PCB river contamination continues to be problematic. Using a computational systems disease-read across approach incorporating comparative inflammatory-related diseases prevalent in the study region including colorectal cancer (CC), and the two principal types of IBD, inflammatory ulcerative colitis (UC) and Crohn’s Disease (CD), disease-gene networks were developed and common nodes among the disorders were analyzed. Detailed disease incidence maps were developed for the region and genetic susceptibilities through inflammatory cytokine expression was evaluated for both CC and IBD with a focus on local heritage. Of major interest was a SNP of the transporter ABCB1 which is crucial for cellular efflux of chemicals. Potential PCB and ABCB1 interaction was confirmed with a computational chemical/protein docking model. In addition, in this area Mycobacterium avium subspecies paratuberculosis (MAP) infection has been linked to CD. Studies in animals have shown a decrease in MAP IgG after PCB exposure thereby decreasing immunity to IBD. Based on the comparative networks and association analyses, a molecular initiating event for potential PCB-associated-IBD was proposed: PCB induces ABCB1 disruption, which would lead to bioaccumulation of xenobiotics and inflammatory mediators within intestinal cells, exacerbated by MAP infection and resulting in sustained inflammation.

### 1125 Ethanol-Induced Gastric Ulcer: Molecular Ulcerogenic Mechanism

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Ethanol is one of the numerous gastrointestinal toxicants to which humans are consciously and deliberately exposed daily. Ethanol-induced gastric ulcer is one of the widely studied experimental ulcers among other ulcer models. The fulcrum for this established fact is due both to pathological semblance of the model with most of the symptoms observed in gastric ulcers and most importantly in alcoholics and easy reproducibility of the method. For instance, many bioactive compounds in different plants have been known to show gastric anti-ulcer potencies against ethanol-induced gastric ulcer. This may be attributed to the ability of these agents to interfere with one or more of the cascade of steps involved in the pathogenesis of gastric ulcer as they have been variably reported to be cytoprotective or gastroprotective. However, despite the plethora of the gastric ulcer method in literature, very little is known about the mechanism involved in its pathogenesis. A thorough literature search through medline was made between year 1997 and 2017 for this purpose and was used as the basis for this review. The review holistically analyse the mechanism, at the molecular level, involved in ethanol induced gastric ulcer model in relation to the healing.

### 1126 In Vitro Cytotoxicity and Inflammatory Responses Elicited by Different Types of Rock Dust: Similarities and Material-Specific Differences

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Rock dusting in underground coal mines helps to prevent the propagation of coal dust explosions. The commonly used rock dust refers to limestone or marble dusts which may be utilized as is or treated to prevent clumping under high humidity conditions. Although potential occupational exposures to rock dust are real, safety or toxicity information on such exposures are largely lacking. The aim of this study was to evaluate the cytotoxicity, cellular damage and inflammatory responses of respirable untreated limestone (UL) and marble (UM) rock dust as well as to examine whether the treatment of dust affects the outcome. Human lung epithelial cells (AS49) were exposed for 24 and 72 h to various concentrations (0 - 1mg/ml) of four rock dust samples. The results showed dose- and time-dependent cytotoxicity and cell damage with the least effect upon exposure to treated limestone (TL). The extent of inflammatory responses evaluated by the number of cytokines produced and released, increased with the concentration of tested materials. Clustering analysis of the inflammatory cytokines/chemokines revealed an overall stronger effect of marble compared to limestone samples. Furthermore, untreated rock dust induced a greater inflammatory response as compared to treated samples in both cases. Similar to the cytotoxic and cell damage results, treated limestone revealed the lowest inflammatory response compared to other samples. Overall, our results unveiled treatment related differences as well as material dependent changes in biological responses.

### 1127 Defining Interstitial Macrophage Populations in Lungs and Changes Based on Nanomaterials

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Nanoparticles are increasingly utilized in fields such as medicine, agriculture, and industry. Carbon nanotubes have important uses in many of these fields, so understanding their potential for adverse effects is vital. Macrophages are a key component of the immune system and are known to separate into functional subsets that generate distinct responses in the body. Pulmonary macrophages, key regulators of immune responses to respiratory exposures, have yet fully to be sep-
Lightfoot3, J. J. Guers4, S. F. Vatner4, L. McGuinness2, L. J. Kerkohf2, and S. C. Campbell2. 1

Healthy aging, including protection against diabetes, obesity, and cardiovascular disease, we propose to conduct a head-to-head comparison of microbial communities (species and strains) between AC5KO and wild-type (WT) mice to reveal whether there are differences that promote longevity and healthful aging (protect against disease states). Male and female AC5KO and WT mice were fed a normal diet and randomly assigned to exercise or sedentary groups. After 6 weeks, animals were sacrificed, and duodenum/ileum and colon tissues were fixed for immunohistochemistry for intestinal inflammatory marker cyclooxygenase-2 (COX-2). DNA extraction, PCR amplification, and data analysis tools (MiniON) were used to compare the microbial communities of the different groups of mice. Following in vitro polarization of AMs, we detected increased levels of YM1/YM2 in the M2a polarized AMs, we detected increased levels of YM1/YM2 in the M2a polarized cells compared to the controls. We were also able to find marker differences in subsets of pulmonary macrophages following the seven-day exposure. Using Flow Cytometry and in vitro polarization of AMs, we could conclude that macrophages were expressing predicted markers of polarization. The data collected suggests that subsets within the interstitial macrophages do exist and further research is needed to classify them. Being able to separate the macrophages from the seven-day exposure into interstitial and inflammatory interstitial demonstrates differences between interstitial macrophages in the lungs, which needs to be studied further.

1129 Evaluating Intestinal Inflammation and Microbiota in the Healthy Aging AC5KO Phenotype

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Healthy aging, including protection against diabetes, obesity, cardiovascular stress, and enhanced exercise tolerance, have been observed in our adenyl cyclase type 5 knock out (AC5KO) mouse model. This is a critical observation, because the aging population would not enjoy extra years if they were accompanied by chronic conditions. Since the gut microbiota has been shown to be an important determinant of age-associated pathological states such as inflammation, diabetes, obesity, and cardiovascular disease, we propose to conduct a head-to-head comparison of microbial communities (species and strains) between AC5KO and wild-type (WT) mice to reveal whether there are differences that promote longevity and healthful aging (protect against disease states). Male and female AC5KO and WT mice were fed a normal diet and randomly assigned to exercise or sedentary groups. After 6 weeks, animals were sacrificed, and duodenum/ileum and colon tissues were fixed for immunohistochemistry for intestinal inflammatory marker cyclooxygenase-2 (COX-2). DNA extraction, PCR amplification, and data analysis tools (MiniON) were used to compare the microbial communities of the different groups of mice. Following in vitro polarization of AMs, we detected increased levels of YM1/YM2 in the M2a polarized cells compared to the controls. We were also able to find marker differences in subsets of pulmonary macrophages following the seven-day exposure. Using Flow Cytometry and in vitro polarization of AMs, we could conclude that macrophages were expressing predicted markers of polarization. The data collected suggests that subsets within the interstitial macrophages do exist and further research is needed to classify them. Being able to separate the macrophages from the seven-day exposure into interstitial and inflammatory interstitial demonstrates differences between interstitial macrophages in the lungs, which needs to be studied further.

1130 Determining the Bio-Distribution and Efficacy of Limonene in Lymph Cannedul, Immunosuppressed Rats with Colitis


Limonene is a naturally occurring terpene that is an effective solvent for cleaning applications and generally regarded as safe (GRAS) by the US Food and Drug Administration. However, the clinical use of limonene as an anti-inflammatory agent is currently being investigated, specifically for inflammatory bowel disease. While using immunosuppressants aids in reducing inflammatory responses, specific treatment for preventing intestinal fibrosis is still unavailable. The use of a lymph cannulated rat colitis model under various immunologic conditions can aid in understanding the pharmacokinetics and efficacy of limonene. Colitis was induced in healthy Sprague-Dawley (SD) rats and athymic nude rats (RNU) by adding L. casei Dextran Sulfate Sodium (DSS) to the drinking water for a period of 7-10 successive days to induce colitis. All rats were then orally dosed Limonene daily for 7-30 days according to their testing groups: (1) 100 mg/kg; (2) 10 mg/kg; (3) 1 mg/kg; (4) control (vehicle only). A reproducible method of lymph collection was established by surgically cannulating a sampling catheter within the thoracic duct into the cysterna chyli of all the rats. Serum and lymph fluid were collected for biochemical analysis and pathological evaluation with tissues collected for histopathology. Induction of colitis by DSS was confirmed based on clinical and histopathologic signs of ulcerative colitis in each testing group. Differences in tumor necrosis factor (TNF) expression and degree of GI inflammation was evident between SD and RNU rats in the various dose groups. Bioanalysis (LC/MS) of serum and lymph fluids was used to determine systemic exposure of Limonene in groups 1 and 2 for both RNU and SD rats. Group 1 revealed a marked reduction in GI inflammation compared to the other groups not treated with either an increase in their unique microbial species or new species present that are a result of exercise training.

1131 Inflammation-Mediated Pathway in Association with Organochlorine Pesticide Levels in the Etiology of Epithelial Ovarian Cancer

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Epithelial ovarian cancer (EOC) is also one of the hormone dependent cancers and begins with the transformation of cells that comprises the ovaries including surface epithelial cells, germ cells and the sex cord or stromal cells. It has been suggested that endocrine disruption, inflammation, exposure to xenobiotic and subsequent oxidative stress may antagonize ovarian cancer and contribute to its pathogenesis. Pesticides exposure has been found to be associated with a range of
human health problems like immune suppression, cancer, etc. Exposure to environmental chemicals and their improper metabolism is one of the factors causing cytokine imbalance and may trigger inflammatory responses. Hence, the present was designed to assess the risk of EOC by analyzing blood OCPs level, gene environment interaction between OCPs levels and mRNA expression of inflammatory genes (IL-6, TNF-α, NFkB and COX-2) in EOC (cases, n=60) and controls n=60. Quantification of OCP levels was done by Perkin Elmer Gas Chromatograph (GC) equipped with 63Ni selective Electron Capture Detector. mRNA expression of IL-6, TNF-α, NFkB and COX-2 were checked by real time qPCR. Levels of β-HCH, endosulfan-I, endosulfan-II, pp’-DDT, pp’-DDE and heptachlor were found significantly high in cases of epithelial ovarian cancer as compared to control. A significant high mRNA expression of IL-6, TNF-α, NFkB and COX-2 was also observed. While performing the regression model testing, a significant increase in CA-125 level was observed when high mRNA expression of IL-6, TNF-α, NFkB and COX-2 are present with high pesticide levels. Results indicate the plausible role of OCPs and inflammatory pathway genes with the pathogenesis of Epithelial ovarian cancer among north Indian population.

1132 Measuring Traumatic Brain Injury in Rat Models Using Tissue Clearing and 3D Histology

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Traumatic brain injury (TBI) is debilitating trauma with myriad clinical presentations that can persist for years after the initial injury. Early stratification of the injury and treatment of secondary injury are critical to optimize recovery. A cascade of inflammatory responses in the brain following the initial injury contributes to the severity and progression of the damage over time. The calcium-binding protein S100b has been extensively examined for its role in TBI as a biomarker of acute astrogial injury and in addition to a damage-associated molecular pattern. In this experiment, we examined levels of S100b in key areas of rat brains following TBI and in controls using whole-mount immunolabeling paired with reversible, solvent-based tissue clearing, enabling the spatial profiling of S100b as a part of the inflammatory cascade resultant from TBI. Observation of the biomarker S100b using confocal microcopy and 3D reconstruction in relation to astrogial injury in the hippocampus, a critical region of the brain whose increased aggregate of S100b is implicated in several conditions resulting from TBI, allows for the extraction of a significant amount of data from each sample and better visualization when compared to the limitations of traditional 2D histology. Profiling of S100b in 3D will provide an unparalleled, well-rounded understanding of a critical alarmin’s pathway in brain injury. A greater understanding of the role S100b plays in TBI will contribute to the development of more effective therapeutic strategies to mitigate long-term injury from TBI.

1133 Neurotoxicity in Mice Exposed to Diesel Exhaust Particles: Inflammation and Impaired Autophagy

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Epidemiological and toxicological studies have shown that particulate matter (PM) may induce degenerative diseases on the central nervous system (CNS). However, the underlying mechanisms remain unclear. Therefore, an experiment was conducted to explore the CNS toxicity induced by PM. In the first experiment, 8-week-old male C57BL/6J mice were exposed to diesel exhaust particles (DEPs) by intratracheal instillation (IT) twice within two weeks. DEPs of 300µg (high-exposure group), DEPs of 100µg (low-exposure group), or PBS (control group) were administered. Morris water maze (MWM) test was conducted to evaluate the capacity of spatial learning and memory. Pre-inflammatory cytokines, including IL-6, TNF-α, NFkB and COX-2, were measured using western blot analysis. The escape latency in the high-exposure group was significantly longer than the control group on the fourth day (35.4 vs. 14.2 seconds, P < 0.05, Kruskal-Wallis test). Pre-inflammatory cytokines were also increased in the high-exposure group, including TNF-α, IL-1β in the cerebellum (CE), IL-1β in the SP, the medulla (ME), and the cortex (CO); and IL-1β in the ME and the CO. Further study on autophagy expression showed that LC3-II/L-I ratio decreased in cerebral cortex on the third post-exposure day (p<0.05, Wilcoxon rank sum test). However, beclin-1 was not different between exposure and control groups. Our results showed that acute exposure to DEPs caused poor spatial learning together with increased inflammation in the brain. Further, acute exposure to DEPs may impair autophagy in the cerebral cortex. Our study provides information on the possible mechanisms underlying the PM-related degenerative CNS diseases.

1134 In Silico Cardiomyocyte Action Potential Modeling Using GLP Manual Patch Clamp Inhibition Profiles from CiPA Ion Channels

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The Comprehensive in vitro Proarrhythmia Assay (CiPA) calls for multi-ion channel inhibition profiling using high-throughput screening (HTS) and in silico modeling of cardiomyocyte action potential. This study aimed to apply in silico modeling in ion channel inhibition profiling generated with manual patch clamp under conditions applied for GLP studies. IC50 values for CiPA-related cardiac ion channels (Nav-1.5, Kv4.3, CaV-1.2, hERG, KLOQT1, and Kir2.1) were tested with cisapride (1 µM), terfenadine (1 µM), amiodarone (1 µM) and verapamil (10 µM). HEP 293 cells with stable expression were used in manual whole-cell configuration. The O’Hara-Rudy and ten Tusscher models were applied to manual patch clamp inhibition profiles, and the durations of the cardiac action potentials at 90% repolarization (APD90) were calculated during stable pacing of single cells at 60 bpm. In silico O’Hara-Rudy modeling displayed the greatest level of modulation and, from in vitro inhibition profiles obtained from hERG (cisapride 93%, terfenadine 91%, amiodarone 61%, verapamil 92%), CaV-1.2 (cisapride 67%, terfenadine 65%, amiodarone 46%, and verapamil 35%), and NaV-1.5 (cisapride 40%, terfenadine 33%, amiodarone 30%, verapamil 52%) channels. In silico modeling using manual patch clamp data quantitatively predicted increased action potential duration and QT prolongation. A HTS method was used for CiPA-related in silico modeling. Our results suggest that manual patch clamp inhibition profiles obtained using GLP study conditions can be applied to in silico modeling and quantitatively predicts cardiomyocyte APD changes and consequently QT prolongation. The O’Hara-Rudy model, using the 6 cardiac ion channel currents and their respective IC50 values for CiPA-related cardiac ion channels, was used to model the O’Hara-Rudy model with the ultimate goal to estimate ECG effects. The use of truncated panel (i.e., IC50 or IC50, IC50 only) extensively high the high contribution of IC50 in APD estimation with the O’Hara-Rudy model. When using the ten Tusscher model, APD was predicted to increase with a truncated panel using IC50, IC50 only, only indicating the balance of the ion channel balance in this model. Overall, the ten Tusscher model under-performed the O’Hara-Rudy model for pharmacological estimations of drug effects on APD with a full or truncated ion channel panel.

1135 Organ-on-Chip Technology Recapitulates Thrombosis Induced by an Anti-CD154 Therapeutic Monoclonal Antibody

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Blocking of CD40L-mediated signaling represents a validated therapeutic strategy for treatment of several autoimmune diseases; however, development of therapies against this target was stalled for several years because of unexpected thrombotic and cardiovascular events during clinical development of the anti-CD40L mAb HuScB. These side effects were not detected during preclinical testing. Platelet activation assays have been used to test the hypothesis that thrombosis was caused by binding of HuScB1 or FcγRIIb receptors on platelets. To provide additional confidence in the safety of new anti-CD40L mAbs that are designed not to bind FcγRIIa, a micovessel chip was developed that could capture human-relevant endpoints for detection of coagulopathy, providing a patient-specific platform for safety testing. The micovessel chip includes a vascular channel lined by human endothelial cells and perfused with human whole blood at a physiologically relevant shear rate. Treatment with clinical-relevant concentrations of huScB and CD40L, resulted in endothelial injury and platelet activation, platelet aggregation, fibrin clot formation, and increased secretion of thrombin anti-thrombin (TAT) complex. Conversely, these endpoints were atten-
Cardiac safety is one of the leading causes of late-stage compound attrition in the pharmaceutical industry and accounts for 28% of the safety-related withdrawals of FDA-approved drugs from the market. Current cardiac safety preclinical evaluations are heavily focused on approximately 3-7 main ion channels involved in maintaining the cardiac action potential; however, over 70 different types of ion channels are expressed in the heart and participate in the overall cardiac safety-related withdrawals of FDA-approved drugs from the market. These safety testing methods overemphasize electrophysiologic assessment of cardiotoxicity and fail to evaluate cardiomyopathy and other forms of structural cardiotoxicity. Metabolic perturbations are one of the primary mechanisms underlying the cardiotoxicity elicited by pharmaceuticals. Stemina has developed a novel, marker-based assay for evaluating the cardiotoxicity potential of compounds based on changes in the metabolism and viability of human-induced pluripotent stem cell (hPSC)-derived cardiomyocytes. In this study, we exposed hPSC-derived cardiomyocytes to a training set of 57 compounds and a test set of 12 compounds. The cardiotoxic compounds were broken into three categories: structural, functional, and general (compounds that cause both). Metabolomic analysis of spent media identified a set of predictive biomarkers. The biomarker-based model classified the test set with 86% accuracy and the training set with 86% accuracy, based on comparing the concentration where metabolism was perturbed to the therapeutic Cmax. This assay is an attractive new model that can identify both structural and functional cardiotoxic compounds that could be used in conjunction with CIPA and other endpoints to provide a more comprehensive evaluation of a compound’s cardiotoxicity potential.
**1140 Characterization of hiPSC-Derived Cardiomyocytes Formatted into a Physiologically-Relevant High Throughput Screening Platform**


Current approaches on the study of acute cardiac toxicity and safety have been transformed with the availability of human-induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CM). Cardiotoxicity plays an important role in the failure of therapeutic agents in late stages of clinical trials, as well as in the removal of approved drugs from the market. The Comprehensive in vitro Proarrhythmia Assay (CIPA) constitutes a novel screening proposal intended to replace current regulatory strategies that have failed to predict the acute cardiotoxic effects of developing drugs. Through the CIPA initiative, researchers from diverse organizations such as the FDA, academic institutions, and pharmaceutical companies are evaluating hiPSC-CMs as an integral tool for the preclinical assessment of novel therapeutic compounds. Noteworthy key challenges under consideration for the hiPSC-CM system are sub-cellular morphology, sub-cellular structural organization, and overall electrophysiological maturity. As an example, hiPSC-CMs frequently display undefined or disarrayed sarcomeric organization when plated in standard cell cultureware. We have developed a novel high-density screening platform for hiPSC-CMs that is micro-engineered to emulate correct cardiac muscle fiber organization. This platform allows for passive self-alignment of hiPSC-CMs, leading to improved sarcomeric organization, as seen by readily identifiable, correctly patterned myofibrils along the cell body. Concomitantly, directionality of contraction of hiPSC-CM preparations were observed to be increased in this platform. We also observed increased gene expression of ryr2, atr2a2, and α1b, key components of cardiomyocyte calcium handling pathways, which are crucial for cardiac physiology. The expression levels of cardiac ion channel genes were assessed (such as cacna1c, scnn1a, and cngala1), as well as cardiac cell junction components gja1, gja5, and dsp, also showed an increase. Furthermore, a comprehensive analysis of calcium flux in hiPSC-CMs indicated that hiPSC-CM alignment in this platform influences cardiomyocyte physiology, which can be of great importance for the evaluation of compound cardiotoxicity. Altogether we describe a novel hiPSC-derived cardiomyocyte platform with greater physiological relevance that is pre-formatted to high-throughput screening.

**1141 Non-Rodent Isolated Mature Ventricular Cardiomyocytes for Multi-Ion Channel Inhibition during Proarrhythmia Screening**

H. Huang1, M. Accardi2, R. Forster3, F. Duguay4, and S. Authier1,2.

Multiple ion channel inhibition screening on stable expression cell lines is classically used to assess the effects of compounds on early- and late-action potential properties, largely in the assay development stage. To assess therapeutic potential of novel pharmacological tools in a more physiologically-relevant way, we introduced a model with integrated physiological multi-ion channel expression, primary cardiomyocytes may be clinically relevant. The purpose of this study was to evaluate the effects of ion channel blockers on primary cardiomyocytes and compare their torsadogenic potential among three animal models. HEK 293 cells with stable expression were used in manual whole-cell configuration. IC50 values for ion channels (Na v1.5, K v4.3, Ca v1.2, hERG, K vLQT1, and Kir2.1) were tested with various ion channel blockers TTX (IC50 = 5.4 µM in HEK293) at 5 µM. Multiple ion channel inhibition screening on stable expression cell lines may be considered as a tool in proarrhythmic risk assessment.

**1142 Chronic Antiretroviral Treatment Disrupts Mitochondrial Homeostasis and Induces Mitochondrial Genome Instability to Promote Premature Endothelial Senescence**

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Highly active antiretroviral therapy (HAART) has led to a dramatic reduction in morbidity among the human immunodeficiency virus type 1 (HIV-1) infected patients. However, cardiovascular complications have become one of the most prevalent causes of death among HIV-1 infected patients. Nuclear reverse transcriptase inhibitors (NRTI) are the backbone of HAART. The combination of emtricitabine (FTC) and tenofovir disoproxil fumarate (TDF) is considered a potent co-formulation commonly used in current HAART. NRTI was shown to induce a mitophagy-associated endothelial toxicity. In addition, single-dose NRTI treatment induced endothelial dysfunction and increased reactive oxygen species production after 24 h, which was partially rescued by overexpression of the mitochondrial antioxidant enzyme manganese superoxide dismutase. These findings suggest that mitochondrial oxidative stress is involved in the pathogenesis of NRTI-induced endothelial dysfunction. Mitochondrial dysfunction, including a compromised mitochondrial biogenesis, has a causal role in endothelial senescence that can exacerbate cardiovascular disease development. In this study, we evaluated mitochondrial function and integrity in human aortic endothelial cell after chronic FTC (10 µM) and/or TDF (10 µM) treatment for 2-12 passages. High-resolution respirometry showed that mitochondrial respiration was increased in chronically treated cells, suggesting mitochondrial uncoupling. β-galactosidase staining in NRTI-treated cells demonstrated a higher level of senescence. Western blotting indicated a lower expression of Parkin, a mitophagy modulator, in cells treated chronically with NRTI compared to control cells. Using quantitative PCR, mitochondrial DNA (mtDNA) copy number per cell decreased by 20% after treatment, while mtDNA content decreased in senescent NRTI-treated cells compared to equivalent-passage control cells. Our work suggests that long-term use of NRTI impairs mitophagy and depletes mtDNA, resulting in premature endothelial senescence.

**1143 Assessment of Drug Effects on Cardiomyocyte Function: Comprehensive In Vitro Proarrhythmia Assay (CIPA) Results**

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The ongoing comprehensive in vitro pro-arrhythmia assay (CIPA) initiative aims to improve drug safety testing and help more beneficial chemical entities reach the market. The project consists of three main parts, which include 1) electrophysiological investigation of drug effects on human ventricular ion channels, 2) characterization of the in silico cardiotoxicity model predictions, 3) assessment of discrepancies and gaps in fully integrated biological systems (human-induced pluripotent stem cell-derived cardiac myocytes (iPSC-CMs)) and the human ECG). To assess and validate drug effects on hiPSC-CMs in a combined measurement of cardiac excitation and contraction, a subset of compounds which are part of the Phase II CIPA study were tested. A prospective comparison study using combined extracellular field potential (EFP) and impedance technology was conducted across 4 independent laboratories testing 12 reference compounds in 4 independent commercially available hiPSC-CMs. Our data demonstrate that NRTI channel blockers, such as Tatalol and Dofetilide, prolonged field potential duration (FPD) at low concentration and induced arrhythmias as measured by field potential (FP) recording and impedance (IMP) recordings at higher concentrations. The cross-cell comparison displayed different minimal effective concentrations regarding FPD prolongation and cessation of beating. On the contrary, Diltiazem, a calcium channel inhibitor, weakened cell contractile activity and shortened FPD. Multichannel inhibitors, such Quinidine, increased FPD, weakened the contraction force, and induced arrhythmia. Furthermore, validation on low-risk proarrhythmia compounds such as Tamperil and Verapamil and intermediate-risk compounds such as Cisapride and Cholpomazine will be presented. Comparison of the compound effects across the 4 different sites showed the consistent trend of the effect. Taken together, commercially available hiPSC-CMs, in conjunction with combined measurements of ion channel activity and contracility, is a reliable approach for risk assessment of proarrhythmic compounds.
Adult cardiac stem cells (CSC) and progenitor cells (CPC) represent a population of cells in the heart critical for its regeneration and function over a lifetime. The impact of chemicals on adult human CSC/CPC differentiation and function is unknown. Research was conducted to develop an improved assay to assess the impact of chemicals on CPC differentiation and function [3]. CPCs (Cellular Dynamics International), as well as adult-like cardiomyocytes (CM) from murine heart were present in the Biowire™ II tissues. Isoproterenol, a β2-adrenergic receptor agonist, elicited a 7- to 10-fold increase in contractile force. The Biowire™ II tissues displayed adult-like electrical, calcium handling, and contractile function of the Biowire™ II tissues, as well as mature neurovasculature with elevated numbers of perivascular supporting cells. The models were used to screen a library of 38 chemicals with a range of predicted adverse effects on vascular and neural tissue function. Chemicals that facilitated significant changes in total endothelial network area and area overlap between EC and either PC or AC were categorized as “hits” after fluorescent image analysis. The chemicals were assigned low, medium, high, and critical priorities based on the repeatability and magnitude of their effects on EC, PC, and AC behavior over multiple replicates. The highest-priority chemicals were further characterized through multiple measurements of fluorescent images, including EC network area, EC network protrusions, PC/AC area, and PC/AC overlap with EC networks. Dose-response curves generated from these data have demonstrated expected behavior based on the initial chemical screen scores. These were consistent with similar measurements found in the EPA ToxCast™ database. These models promise to identify critical cell types that are affected by chemical exposure, and facilitate the discovery of adverse outcome pathways that compromise the integrity of the neurovasculature.

Heart Failure (HF) can be induced by a range of factors, including genetics and acquired risk factors. It can also be an unwanted consequence of HF drug discovery and cardiac safety testing. The ability of CPC to differentiate into functional CM. This adverse effect may impact the heart in terms of susceptibility to disease and longevity. This abstract does not represent US EPA policy.

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Ginseng and its major active ingredients, ginsenosides, are widely consumed for various health problems including cancer without a special precaution. Previously, we demonstrated that Rg3, one of the ginsenosides with a potent anticancer activity, may cause contractile dysfunction, and structural damage on vascular smooth muscle through apoptotic cell death. However, the mechanism underlying Rg3-induced vascular toxicity was not fully elucidated. Here we demonstrated that the vascular toxicity of Rg3 occurs through actin disruption and Bmf-initiated mitochondrial apoptotic pathway, the mechanism which may contribute to its anticancer effects. In rat primary vascular smooth muscle cells, Rg3 induced apoptosis and contractile dysfunction as determined by caspase-3 activation, TUNEL staining, and insufficient myosin light chain phosphorylation. Rg3 induced actin degradation with Rho A inactivation, which was followed by mitochondrial translocation of Bmf and dissociation of mitochondrial membrane potential (Δψ). Pre-treatment of jasplakinolide, an inhibitor of actin polymerization, significantly blocked Rg3-induced q dissipation and apoptosis. Notably, Rg3 induced actin disruption in hepatoma cell line, HepG2, which could be reversed by jasplakinolide, reflecting that the same mechanism may contribute to the anticancer effects of Rg3 at least in part. Collectively, these results demonstrated that Rg3 may induce vascular toxicity as well as anticancer effects through disrupting actin, which may alert the potential toxicity associated with inadvertent use of ginseng and its products.

1100 Myricetin as an Effective Protectant against Anthracycline-Induced Cardiotoxicity

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Toxic side effects of anticancer drugs are increasingly generating concerns for the US FDA, patients, and researchers. One of the most critical side effects is cardiotoxicity caused by anthracyclines, a class of efficacious chemotherapy drugs that has been used for over 50 years as part of combination therapies to treat various types of cancer. Approaches to predict anthracycline-induced cardiotoxicity have included limiting lifetime cumulative doses, using less cardiotoxic analogs, and optimizing delivery strategies, which are at best, only partial solutions. A potentially more promising approach for reducing cardiotoxicity has been to supplement anthracycline treatment with cardioprotectants. The only clinically approved cardioprotectant is dexrazoxane, which is approved for use only in advanced or metastatic breast cancer patients. Novel cardioprotectants with improved efficacy and safety profiles not only have the potential to significantly reduce drug-induced cardiotoxicity risk, but could also enable higher anthracycline dosing and expanded usage for patients who are excluded from anthracycline therapy due to pre-existing cardiac issues. To this end, we have applied a data-driven algorithmic approach to identifying compounds that could mitigate anthracycline-induced cardiotoxicity and tested the compounds in human iPSC-derived cardiomyocytes. Using this approach, we identified members of the flavonoid class of polyphenolic compounds as having varying degrees of cardioprotection capabilities. Specifically, we identified myricetin as one of the most effective cardioprotectants that mitigated anthracycline-induced cardiotoxicity without interfering with its anticancer activity. Treatment of myricetin provided protection against doxorubicin-induced cardiomyopathy both in human iPSC-derived cardiomyocytes and a mouse model. Ejection fraction and fractional shortening were rescued with the co-dosing of myricetin with doxorubicin in mice. Mechanism of action of myricetin is likely to be in synergy with doxorubicin, reversing expression of genes and pathways that are impaired by doxorubicin in the cardiomyocytes such as hypoxia, oxidative stress, and mitochondrial health. We show that myricetin likely exerts its protective capability in a novel way by binding to TOP2B, in contrast to dexrazoxane, which decreases the protein level.

1149 Adult Human Stem Cell-Derived Cardiomyocytes: An Alternative Model for Evaluating Chemical and Environmental Pollutant Cardiotoxicity

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Heart disease is increasing globally, with a significant percentage of the increase being attributed to chemical and pollution exposures. Currently, no alternative or in vitro testing models exist to rapidly and accurately determine the cardiac effects of chemicals and/or pollutants. This research addresses this need by employing stem cell technology to evaluate the cardiotoxicity of chemicals and pollutants. Adult human stem cell-derived cardiomyocytes (SC-CM) were exposed to either saline or DMSO vehicle, extracts derived from residual oil fly ash (ROFA) or diesel exhaust particles (DEP) at 1-20 µg/ml, suspension of Ag 10 nm nanoparticles (NP, 3 or 6 µg/ml), perfluoroalkyl substances (PFNA, PFOS, PFBS, PFHxS, 25 - 100 µM), or tricosan (TCS, 2.5 - 10 µM) and monitored for viability, beat rate (BR), beating amplitude (BAMP), and beat rate irregularity (BRI) at 0.5, 24, and 48 hr of exposure using the xCELLigence RTCA Cardiac platform (ACEA Biosciences). All SC-CMs cultures were challenged with isoproterenol (ISO) after 48 hr of exposure. No chemical or pollutant examined affected SC-CM viability. ROFA and DEP extracts had no effect on SC-CM BR, BAMP, or BRI. ROFA and DEP extracts inhibited ISO stimulation of SC-CM BR and BAMP, respectively, in a dose-response manner. Ag NP induced a sustained 15% and 30% decrease in SC-CM BR and BAMP at 3 µg/ml, respectively. PFNA exposure induced an immediate and significant dose-dependent change in SC-CM BR and increase in BRI up to 24 hr of exposure while inhibiting ISO stimulation of SC-CM in a dose-dependent manner. PFOS induced an immediate and significant dose-dependent response in SC-CM BRI over the 48-hr exposure period. TCS exposure induced an immediate increase in SC-CM BR and decrease in BAMP over 48 hr of exposure. TCS inhibited ISO stimulation of SC-CM in a dose-dependent manner. These data demonstrate that adult human SC-derived CMs are a viable scalable alternative for in vitro testing model to assess a variety of chemicals and environmental pollutants for cardiotoxicity at low concentrations. This model evaluates critical cardiac physiological endpoints that are easily translatable to animal and human studies. This abstract does not represent US EPA policy.

PCB126 Induces Monocyte/Macrophage Polarization and Inflammation

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Polychlorinated biphenyls (PCBs) are persistent organic pollutants that can contribute to a range of adverse health effects, including inflammatory diseases such as atherosclerosis, which involves macrophages. Although many cells are involved in the initiation and progression of atherosclerosis, macrophages play a key role in the overall inflammatory response and lipid deposition. We hypothesize that dioxin-like PCBs can contribute to monocyte/macrophage polarization and macrophage inflammation. To test this hypothesis, monocytes (THP-1) were differentiated to macrophages, and subsequently exposed to PCB 126. Inflammatory cytokines and genes associated with oxidative stress (e.g., Nrf2 signaling) were studied. Exposure to PCB 126 increased the expression of inflammatory cytokines, such as TNF-α, IL-1β and IL-6, suggesting polarization to the M1 phenotype. In addition, the monocyte chemoattractant protein-1 ( MCP-1) was expressed in PCB-activated macrophages, suggesting induction of chemokines, which regulate immune cell recruitment and migration and infiltration of monocytes/macrophages into vascular tissues. Furthermore, nuclear factor (erythroid-derived 2)-like 2 (Nrf2) and downstream genes, such as glutathione S-transferase (GST) and NAD(P)H quinone oxidoreductase 1 (NQO1), were induced following PCB exposure, indicating induction of xenobiotic-linked defensive mechanisms. Our data demonstrate the involvement of PCB 126 in monocyte/macrophage polarization and inflammation, suggesting another important role of dioxin-like PCBs in the pathology of atherosclerosis. These data have translational implications, suggesting that a compromised cardiovascular system may be able to modulate vulnerability to environmental insults. Supported in part by NIH/NIEHS grant P42ES007380.
Phenotypic Profiling in Human-Based Phenotypic Assays Supports an AOP for CV Toxicity

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Data mining of a large reference database containing drugs, experimental chemicals, and other agents profiled in a human primary cell-based systems has been used to discover novel associations and mechanisms of toxicity associated with certain adverse events. These include acute toxicity, organ toxicity, liver toxicity, skin rash, skin sensitization, and thrombosis-related side effects. Using this approach, an in vitro signature for cardiovascular toxicity comprised of increased cell surface levels of serum amyloid A (SAA) measured in a coronary artery smooth muscle cell-based model of vascular inflammation (BiMAP CASM3C system) was found and was associated with MEF, HDAC, GR/MR, IL-6, pathway, and SIRT1. Since SAA is a clinical biomarker associated with risk of cardiovascular disease in humans, to further understand the regulation of SAA, we performed data mining in the same assay system to identify those agents that reduce the level of SAA. Agents were selected if treatment with that agent for 24 hours at two or more concentrations decrease the cell surface level of SAA without causing overt cytotoxicity. Fewer than 1% of agents in the database were found to decrease level of SAA relative to vehicle control at two or more tested concentrations and with an effect size of ≥ 20% (33/3,800). Notable agents that met these criteria include GLP-1, an endogenous peptide developed as a drug used for treatment of diabetes, roflumilast, a PDE IV inhibitor used for the treatment of chronic obstructive pulmonary disorder, the BCR-Abl inhibitor and oncology drug, imatinib, and a mimetic of ApoA-1, the major lipoprotein of HDL. These represent agents that have been shown to have cardiovascular protective effects in clinical or in vivo studies (some within their class). These results, showing that decreased cell surface levels of SAA in this human primary cell-based phenotypic model are associated with beneficial cardiovascular outcomes, along with the previous finding that increased levels of SAA are associated with cardiovascular toxicity, support the construction of an adverse outcome pathway for cardiovascular toxicity.

1153 Contractility Assessment in Conscious Rats and Guinea Pigs: Comparison from Whole Heart to Whole Body

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Since the inception of ICH S7 B, no drug has been removed from the market for the development of a fatal ventricular polymorphic arrhythmia, Torsades de Pointes. However, drug-induced cardiotoxicity remains a leading cause of drug attrition early in the drug development process, for changes in myocardial contractility needs models that are translatable to the clinical outcome that require minimal amounts of test article. The objective of the study was to evaluate the effects of of ICR isolated guinea pigs (50 mg/kg, SQ) was assessed per the NIST ACM standard as repeatability standard deviation. Rate automated interval detection. Interval measurement repeatability was assessed by average reduction in rSD of 27.4% on the treatment change and drug-related changes in T-wave morphology as assessed by average reduction in rSD of 27.5ms for Lead II and at 30.0 and 33.9ms for sM lead, respectively. Our results demonstrate that a single Lead II ECG is adequate for assessing QT interval prolongation, but may produce different and more variable results for assessment of JTp interval relative to the sM lead derived from multiple ECG lead measurements.

Speckle Tracking with Semi-Automated Strain for Cardiac Contractility Assessments in Dogs, Minipigs, and Non-Human Primates

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M-Mode presents significant limitations owing to the unidimensional rectilinear approach with high risks for variations due to changes in ultrasound probe angle. Speckle tracking imaging enables semi-automated contractility evaluation of the entire ventricular circumference with segmental ventricular analysis as well as global integrated contractility assessment. The goal of this study was to demonstrate the performance of this methodology in dogs, minipigs, and non-human primates in the context of drug safety assessment. Echocardiography with speckle tracking imaging was performed on Beagle dogs, Göttingen minipigs, and cynomolgus monkeys at baseline and after pimobendan and itraconazole administration. Dogs and minipigs were evaluated with the right parasternal short- and long-axis views in right lateral decubitus. The transducer (Siemens 4v1c probe) was used for 2D and 3D video image acquisition. Electrocardiogram was taken for systole and diastole for calculation of heart rate, displacement, and stress (peak and time to peak). In dogs, endocardiac circumference strain per ventricular segment for the middle anterior, middle inferolateral, and middle inferoseptal segment were the most stable with the lowest standard deviations at baseline (SD<11%). To assess the evolution of measures with time, the first and last acquisition of multiple sessions were compared, and results showed a non-significant trend toward lower variability at the first capture. Respectively, pimobendan and itraconazole were associated with increased and decreased circumferential endocardiac strain rate at the expected Tmax. As expected, the average of repeat measures showed lower variability than single-cycle measures. Interestingly, standard deviation values were animal dependent, with some animals presenting consistently lower or higher variability than others. The linear proportional correlation for paired standard deviation values between two observers was R2=0.948, supporting a strong animal dependence for variability. In conclusion, the comparison with strain measurements could be considered to assess global or segmental effects on ventricular contractility in non-rodent species, and the average of repeated measures can be used to increase assay sensitivity.

Comparison of QT and JTp Derived from Spatial Magnitude vs. Single-Lead ECG in Beagle Dogs

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The US FDA has recently laid the groundwork for the potential use of J to T-peak (JTP) interval as a biomarker of cardiac arrhythmia liability in human subjects. Johannesen et. al. demonstrated that global JTP is superiour to a number of other biomarkers. As a result of FDA interest in this area, interest in preclinical evaluation of JTP has grown. Preclinical studies often acquire a single-lead ECG, whereas FDA measurements of JTP were derived from a vectorcardiogram (e.g., spatial magnitude) computed using multiple leads. This study compares JTP from a single lead with JTP derived from a spatial-magnitude (sM) ECG. Four beagle dogs were instrumented with 3-lead Holter monitors to acquire continuous surface ECG recordings for three consecutive days. A 24-hour baseline recording was obtained on day 1. On day 2, dogs were given dofetilide (40 mg/kg orally). On day 3, dogs were administered atropine (0.044 mg/kg SQ) followed by dofetilide (40 mg/kg orally) 30 minutes later. The published transform (Hamilin 1960) was used to derive the sM ECG from the 3-lead Holter recordings. The Lead II and sM ECGs were automatically analyzed using the AE-1010 Rhythm Express software (VivaQuant, St. Paul, MN) without manual intervention or editing of the results. The software has been shown to remove up to 95% of in-band noise without distorting ECG morphology and facilitate accurate automated interval detection. Interval measurement repeatability was assessed per the NIST ACM standard as repeatability standard deviation (rSD). JTP and TpTe measured from sM ECG are more robust to changes in posture and drug-related changes in heart rate as demonstrated by average reduction in rSD of 27.5% on the treatment days. Maximum prolongation of QTc and JTP was detected at 22.7 and 25.7ms for Lead II and at 30.0 and 33.9ms for sM lead, respectively. Our results demonstrate that a single Lead II ECG is adequate for assessing QT interval prolongation, but may produce different and more variable results for assessment of Jtp interval relative to the sM lead derived from multiple ECG lead measurements.

Proposed QT-Interval Correction Method for the Influence of Changes in Core Body Temperature Based on Circadian Variations in Conscious Non-Restrained Cynomolgus Monkeys


QT interval lengthening may be related to the inhibition of the hERG channel, decrease in heart rate, or decrease in core body temperature (BT). To discriminate between these causes, QT correction formulas are available for drug-induced changes in BT in several animal species, but no correction formula has been proposed for conscious non-restrained cynomolgus monkeys. This may be especially important for drugs acting on the central nervous system and known to induce at least transient changes in BT in standalone safety pharmacology investigations or
integrated safety pharmacology studies within regulatory toxicology studies in monkeys. In the present study, physiological changes in QT interval duration and BT during dark and light periods were continuously recorded over 26 hours by telemetry in 22 freely moving telemetered monkeys. The relationships between the duration of QT, QTcB (Bazett) or QTcF (Fridericia’s formula) and BT were evaluated. BT ranged from 29.4°C to 31.4°C, with a mean of 30.4°C. For QT, QTcB, and QTcF intervals were 219 ± 25, 250 ± 18, and 293 ± 20 ms, respectively. Plotting QT, QTcB, and QTcF individual values against BT produced linear regressions described as Y = 27X + 125, Y = 15X + 911, and Y = 21X + 1075, respectively. A body temperature value of 38°C for conscious telemetry data was assumed to be normal in monkeys. It was used to correct the duration of QTcB and QTcF intervals for changes in BT: QTcBcT = QTcB - 158 (BT) and QTcFcT = QTcF - 178 (BT). When plotting QTcBcT and QTcFcT individual values against BT, horizontal regression lines were obtained, Y = 0.076X ± 341, Y = 0.349X ± 277, respectively. These results suggest that physiological changes in animals are induced by changes in BT during QTc interval duration. Dark and light periods may be used to estimate QT correction for the influence of drug-induced changes in BT. The correction method should be based on potential effect on heart rate. To confirm the accuracy of these formulas, additional investigations using a larger range of changes in BT are needed. This method is associated with an independent QT correction for BT. Validation of these correction formulas with appropriate reference pharmacological tools in conscious non-restrained cynomolgus monkeys is also required.

**1157 Protective Effect of Dimethyl Fumarate on Diabetic Cardiomyopathy Possibly via Nrf2 Pathway in Type 1 Diabetes Mice**

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Oxidative stress and inflammation play key roles in the development of diabetic cardiomyopathy (DCM). We have extensively shown the protective effect by inducing endogenous nuclear factor erythroid 2-related factor 2 (Nrf2) pathway against DCM. Dimethyl fumarate (DMF), an FDA-approved medicine for relapsing multiple sclerosis, has manifested its antioxidant and anti-inflammatory function mostly in the neurotic system. Here we repurpose the use of DMF for testing whether it possesses protective effects on heart disease, and whether it is associated with Nrf2 activation. Method: Type 1 diabetes mouse model was induced using multiple low doses of streptozotocin. DMF or vehicle treatment was given to the mice for 3 months. Blood glucose and body weight were measured regularly throughout 3 months. Cardiac functions were detected by echocardiography. After the animals were sacrificed, the heart weight and tibia length were measured. Cardiac tissues were used for western blot analysis (to quantify target proteins such as 3NT, 4HNE, TNP-α, PAI-1, TGF-β, and CTGF). Real-time PCR (on the RNA transcription levels of genes such as ho-1, nqo-1, cat, and sod-1) and Sirius Red staining were performed to semi-quantitatively calculate collagen accumulation. We demonstrated that diabetic mice showed evident diabetes-induced cardiac dysfunction and structural damage compared to the control group, whereas DMF significantly reduced oxidative stress, inflammatory reactions, and remodeling changes in the heart of diabetic mice, resulting in a prevention of cardiac dysfunction. Furthermore, DMF increased activation of Nrf2 and its downstream phase II antioxidant detoxifying genes. These findings suggest that DMF may be potentially applied in clinics for DCM prevention and treatment, most likely through activation of the Nrf2 antioxidant pathway.

**1158 The Effects of Inhaled Traffic-Generated Pollutant Exposure on Angiotensin II Receptor Expression and Blood-Brain Barrier Integrity in Wildtype Mice on Either a High- or Low-Fat Diet**

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Exposure to traffic-generated air pollution has been shown to be a risk factor in cerebrovascular disorders, including stroke, which may be associated with decreased blood-brain barrier (BBB) integrity and permeability. We have previously reported subchronic inhalation exposure to a mixture of diesel and gasoline engine emissions (MVE) promotes BBB disruption in C57BL6 mice on a high-fat diet. While the mechanisms involved have not been fully elucidated, one signaling pathway that has been reported to alter BBB integrity is angiotensin (Ang) II signaling mediated via the Ang II type 1 (AT1) receptors. Thus, we tested the hypothesis that inhalation MVE exposure mediates increased Ang II production and AT1 receptor expression in the cerebral microvasculature of C57BL6 mice, which is exacerbated by consumption of a high-fat diet. 3-month-old male C57BL6 mice on a high-fat (HF, 21% fat) or low-fat (LF, standard chow) diet were randomly assigned to be exposed by whole-body inhalation to either filtered air (FA) or MVE: 70 µg PM/m³ diesel exhaust + 30 µg PM/m³ gasoline exhaust for 6 h/rd for 30d. Treatment with the sodium fluorescein (Na-F) showed a 3-fold increase in Na-F transport from the systemic circulation into the brain parenchyma in MVE+HF animals, compared to MVE+LF or FA+LF controls, indicating increased BBB permeability. This alteration in BBB permeability was associated with a significant increase in plasma Ang II (2-fold) and induced expression of AT1 receptors. Induced expression of AT1 receptors (~20%) in the cerebral microvasculature of MVE+HF C57BL6 mice. Additionally, tight junction protein claudin-5 expression significantly reduced in the cerebral microvasculature of MVE+HF mice, as determined by immunofluorescence. In an in vitro BBB model, when treated with plasma from the study animals, the TEER increased (~30%) in transendothelial electrical resistance (TEER) measurements and TJ protein expression in the endothelial cells treated with plasma from MVE+HF animals, compared to FA+HF controls. Such findings indicate that inhalation exposure to traffic-generated pollutants, combined with a high-fat diet, results in altered BBB integrity, which may be mediated, in part, through Ang II - AT1 signaling.

**1159 Electrophysiological Assessment of Cardiac Conduction in Chronic Diabetic Minipigs**

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Abundant evidence shows that patients with type 1 diabetes are at high risk for several cardiovascular disorders: coronary heart disease, stroke, peripheral arterial disease, cardiomyopathy, and congestive heart failure. Our objective was to assess potential cardiac electro-cardiovascular changes linked to chronic insulin-dependent diabetes in the Yucatan miniature swine. Type 1 diabetes was induced in Yucatan minature swine, and animals were maintained on a daily insulin regimen for 3 to 6 years (total N = 22). Diabetic animals were divided into different groups based on duration of diabetes (Group 1: normal; Groups 2, 3, and 4: diabetes duration of 3-4, 4-5, 5-6 and >6 years, respectively). Cardiac electrophysiological parameters were acquired in conscious diabetic and normal control animals using a standard lead II configuration. Routine measurements of electrocardiograms, including HR (bpm), RR, PR, QRS, QT, and QTc, were done (all in msec). A heart rate correction for the QT interval (QTc) was calculated using the Fridericia method [QTc=QT/(cubed root of RR)]. Mean heart rate was increased for the diabetic Groups 2, 4, and 5 compared to the mean heart rate for normal animals (52, 54 and 54 versus 75 bpm, respectively). The mean PR interval was increased in all diabetic animals compared to normal animals, and the effect increased with the duration of diabetes (132 versus 138, 140, and 172 msec for Groups 1, 2, 3, and 4, respectively). The mean QRS interval was increased for all of the diabetic animals compared to normal animals (47 versus 56, 66, and 64 msec for Groups 1, 2, 3, 4, and 5, respectively). There were no pronounced QTc abnormalities in this study when comparing diabetic to the normal animals, although one animal in Group 3 did have a QTc prolongation of 43 msec. In addition, one animal in Group 5 had a prolonged PR segment (224 msec) associated with frequent ventricular escape complex. This abnormality in rhythm would possibly go along with the duration and severity of the diabetes. In conclusion, chronic diabetes in Yucatan miniature swine manifests with progressive effects on HR, QRS, and QT duration. This indicates that the diabetic minipig could provide a good model to test preventative approaches for progressive cardiac therapies in diabetes, using electrocardiography segments as markers of early heart damage.

**1160 Inhibition of p53 Prevents Diabetic Cardiomyopathy by Preventing Early-Stage Apoptosis and Cell Senescence, Reducing Glycolysis, and Impaired Angiogenesis**

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Elevated tumor suppressor p53 expression has been associated with heart diseases, including the diabetic heart. However, its precise role in the pathogenesis of diabetic cardiomyopathy (DCM) remains unclear. We hypothesized that the development of DCM is attributed to upregulated p53-mediated both early cardiac cell death and persistent cell...
Mitochondrial morphology was assessed with transmission electron microscopy, and mitochondria isolated from freshly obtained left ventricular tissue were used to measure respiration rate with an XF96 Extracellular Flux Analyzer and susceptibility to mitochondrial permeability transition pore (mPTP) opening in a swelling assay. To determine whether antioxidant treatment could alter any of the effects of radiation, rats were treated with gamma-tocotrienol 24 hours before irradiation. Two weeks after local heart exposure to both a single dose and fractionated radiation, cardiac mitochondrial showed alterations in shape, function, and an increased susceptibility to mPTP opening. When local heart irradiation was combined with daily suntinib for 2 weeks, mitochondrial alterations were more severe than after each treatment alone. While gamma-tocotrienol pretreatment reduced the effects of radiation on cardiac mitochondria, long-term changes in cardiac function and collagen deposition were not altered. In conclusion, local heart irradiation and TKI treatment may worsen each other’s effects on cardiac mitochondrial morphology and function. The role of mitochondrial alterations in cardiac toxicity of cancer treatment is not yet fully understood.

Sex is one of the risk factors in development of cardiotoxicity induced by an anticancer drug, doxorubicin (DOX). In a mouse model developed in our laboratory, adult male hearts showed a greater susceptibility to DOX toxicity compared to females as cardiac lesions were observed only in male mice at 18-27 mg/kg cumulative doses. The left atrium was more vulnerable to DOX toxicity than the right atrium or ventricular myocardium in male mice. DOX toxicity was more pronounced in male mice than in female mice, whereas kidney and liver toxicity were similar in both sexes. Our study suggests that sex is a significant factor in determining the susceptibility to DOX toxicity and underscores the need for sex-specific approaches in the development and optimization of anticancer treatments.

Although epidemiological, human, and animal data have conclusively linked air pollution exposure to adverse cardiovascular outcomes, the severity of the response depends on a number of intrinsic (diet, underlying disease, etc.) and extrinsic (e.g., co-stressors) factors. As such, cardiovascular function is maintained by adequate levels of certain essential micronutrients like vitamin D. Unfortunately, vitamin D deficiency (VDD) has become highly prevalent in the United States, as well as in the world, even affecting otherwise healthy individuals. We previously showed that VDD worsens cardiac arrhythmias and causes autonomic imbalance in mice exposed to acrolein, which is a ubiquitous gaseous air pollutant. A number of studies suggest that vitamin D exerts its effects in the body through a putative anti-aging protein called klotho, which is reduced in VDD. Thus, the purpose of this study was to determine whether klotho treatment would ameliorate the acrolein-induced effects due to VDD. We hypothesized that klotho would decrease arrhythmogenesis and autonomic imbalance during acrolein exposure. Three-week old mice were placed on a VDD or normal diet (ND) for 19 weeks and treated with 1 mg/ml klotho (intraperitoneally) every other day for a month starting at 18 weeks of age. Mice were implanted with radiotelemeters for the measurement of heart rate (HR), electrocardiogram, and heart rate variability (HRV) and allowed 7-10 days to recover from surgery. Mice were...
exposed to filtered air and then acrolein for 3 hours on each separate days. HR and HRV were significantly decreased during acrolein exposure in VDD mice when compared to ND; this response was blocked by klotho treatment. Although arrhythmias were observed in VDD mice treated with klotho during exposure, there was no difference from ND. Acrolein exposure also caused a significant decrease in tidal volume and increase in ventilatory timing (i.e., airway irritation) in VDD mice, which was blocked by klotho. In conclusion, VDD appears to modify the cardiopulmonary response to air pollution through a mechanism involving klotho. Although additional studies are needed to verify these findings, these data suggest klotho treatment could potentially lessen the effects of air pollution in people with VDD. This abstract does not reflect US EPA policy.

**1165 Organophosphate (OP) Toxicity Model in Female Mice: Cardiac Treatment Paradigms**

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Gulf War Illness (GWI) is a long-lasting, multi-symptom disorder with features characteristic of a “sickness” behavior. It is strongly tied to the military, particularly to male cohort. However, there are important questions as to how females respond to GWI and how they might be treated. We have developed a chemical toxin protocol that uses DFP (diisopropyl fluoroophosphate) to elicit a syndrome with features similar to GWI. The aim is to use this model in female mice to study the cardiovascular profile using ECHO and to test the role of Tumor Necrosis Factor alpha (TNF-α) using a biological inhibitor (Enbrel). Protocol: Female mice were divided into four groups: control (C), ovariec-tomized (OVX), DFP (OVX+DFP), and OVX+DFP+Enbrel (OVX+DFP+E). The rationale for use of OVX animals is to provide a situation in which cycling of the sex steroids is absent, similar to the male condition. GWI was induced by combined treatment with corticosterone (200 mg/l) followed by DFP exposure (1.5 mg/kg, s.c.). Enbrel (10 mg/Kg/p.o) therapy was administered 2 weeks after DFP. DFP exposure increased end systolic area (ESA). Enbrel produced amelioration of the toxic change produced by DFP (C: 6.00±0.30; OVX: 7.85±0.32; DFP: 11.62±0.70; DFP+E: 6.73±0.39). Systolic function, evaluated by ejec-tion fraction (EF), was reduced in all DFP groups after exposure and remained reduced in the last evaluation in DFP group but not DFP+E (C: 85.21±1.47; OVX: 80.49±1.49; DFP: 58.02±2.66; DFP+E: 84.28±1.70). Fraction Shortening (FS) and Cardiac Output (CO) were reduced after DFP exposure; however, after therapeutic the DFP+E group shows enhanced shortening (C: 25.39±0.76; OVX: 22.77±1.09; DFP: 16.1±1.05; DFP+E: 25.02±1.32, and CO increased (C: 17.71±0.99; OVX: 18.32±1.03; DFP: 13.06±3.34; DFP+E: 20.1±1.14). Systolic function recovery and fractional recovery of EF. Finally, myocardial performance index (MPI) was significantly increased in DFP exposure mice and re-established in Enbrel group after drug ther-apeutic trial (C: 0.35±2.008; OVX: 0.334±0.008; DFP+Enbrel: 0.419±0.01; DFP+E: 0.315±0.009). Results demonstrate that DFP exerts a negative impact on cardiac function in female mice, while Enbrel prevents most of the dys- functions produced by DFP. This suggests that a therapeutic trial could be a feasible strategy for treatment of veterans with GWI.

**1166 Bisphenol A and Phthalates Escape from Medical Devices and Induce the NLRP3 Inflammasome in Murine Cardiac Infarcts and Suture Wounds**

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Most medical devices, including perfusion pumps, catheters, and IVs, have plastic parts. Yet it is unclear how much of the incorporated bisphenol or phthalates escape into patients or whether any exposure modulation contributes to wound healing. To define exposure, we quantified the metabolites of 6 bisphenols and 10 phthalates in 9 men before, and immediately after, cardiac surgery. Exposure levels were measured using a biological inhibitor (Enbrel). Protocol: Female mice were divided into four groups: control (C), ovariec-tomized (OVX), DFP (OVX+DFP), and OVX+DFP+Enbrel (OVX+DFP+E). The rationale for use of OVX animals is to provide a situation in which cycling of the sex steroids is absent, similar to the male condition. GWI was induced by combined treatment with corticosterone (200 mg/l) followed by DFP exposure (1.5 mg/kg, s.c.). Enbrel (10 mg/Kg/p.o) therapy was administered 2 weeks after DFP. DFP exposure increased end systolic area (ESA). Enbrel produced amelioration of the toxic change produced by DFP (C: 6.00±0.30; OVX: 7.85±0.32; DFP: 11.62±0.70; DFP+E: 6.73±0.39). Systolic function, evaluated by ejec-tion fraction (EF), was reduced in all DFP groups after exposure and remained reduced in the last evaluation in DFP group but not DFP+E (C: 85.21±1.47; OVX: 80.49±1.49; DFP: 58.02±2.66; DFP+E: 84.28±1.70). Fraction Shortening (FS) and Cardiac Output (CO) were reduced after DFP exposure; however, after therapeutic the DFP+E group shows enhanced shortening (C: 25.39±0.76; OVX: 22.77±1.09; DFP: 16.1±1.05; DFP+E: 25.02±1.32, and CO increased (C: 17.71±0.99; OVX: 18.32±1.03; DFP: 13.06±3.34; DFP+E: 20.1±1.14). Systolic function recovery and fractional recovery of EF. Finally, myocardial performance index (MPI) was significantly increased in DFP exposure mice and re-established in Enbrel group after drug ther-apeutic trial (C: 0.35±2.008; OVX: 0.334±0.008; DFP+Enbrel: 0.419±0.01; DFP+E: 0.315±0.009). Results demonstrate that DFP exerts a negative impact on cardiac function in female mice, while Enbrel prevents most of the dys- functions produced by DFP. This suggests that a therapeutic trial could be a feasible strategy for treatment of veterans with GWI.

**1167 An Automated Heart Rate Detection Platform in Wildtype Zebrafish for Cardiotoxicity Screening of Fine Particulate Matter Air Pollution**


Exposure to air pollution-derived particulate matter (PM) causes adverse cardiovascular health outcomes, with increasing evidence implicating soluble components of PM; however, the enormous number of unique PM samples from different air sheds far exceeds the capacity of conven-tional in vivo approaches to characterize potential health impacts. The zebrafish (Danio rerio) is a vertebrate model widely used to evaluate heart function in vivo given the high degree of functional conservation with humans. The purpose of this study was to develop a rapid cardiotoxicity screen for PM. Here, we present an assay that relies on measure-ment of a simple health metric, i.e., heart rate (HR), acquired quickly from hundreds of zebrafish embryos without the use of drug anesthetics, restraint, fluorescent labeling, or confocal microscopy. Short videos acquired using bright-field imaging of 2-day-old wild-type zebrafish were processed using an original algorithm and scripts that derived HR from heart contraction patterns. To examine the utility of this approach in the cardiotoxicity testing of PM, we compared the effects of treatment (5.5 h) with organic extracts of PM collected from compressor-generated diesel exhaust (C-DEP), low sulfur diesel (B0), soy biodiesel (B100), and a 1:1 blend of B0 and B100 (B50). Epinephrine, a drug known to increase HR, caused dose-dependent increases in HR (peak increase = 14%, 177 beats per minute (bpm) vs. vehicle control (155 bpm)); whereas cloni-dine, a drug known to decrease HR, and all PM extracts caused decreases in HR (peak decreases of 10% for clonidine (120 bpm), 20% for C-DEP and B0 (125 bpm), and 8% for B100 (143 bpm), vs. vehicle (155 bpm, p < 0.001 for all). Preliminary screen for potential drug candidates of the PM samples indi-cated C-DEP = B0 > B100. Analysis of B50 is in progress. Taken together, this approach may help expedite relative cardiotoxicity determinations of PM, drug candidates, and other chemicals and mixtures This abstract does not reflect US EPA policy.

**1168 PON1 Status and HDL Characteristics in Patients with Cardiovascular Diseases**

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Serum paraoxonase 1 (PON1) is a high-density lipoprotein-associated enzyme capable of hydrolyzing a wide spectrum of substrates including oxidized lipids. Some evidence indicates that the genotype and phe-notype of PON1, as well as HDL concentrations and HDL subclasses, could associate with development of cardiovascular diseases. The aim of this study was to evaluate between HDL concentration and lipid profile in patients with cardiovascular diseases (CVD) or cardiovascular risk factors (CRF), or who were healthy. A case-con-trol study was conducted in 69 volunteers from an institution of health in Nayarat, Mexico. The study was approved by the local Institutional Ethics Committee. A written informed consent was obtained from each patient in compliance with good clinical practices, and the research
according to the principles in the Declaration of Helsinki. Blood samples were obtained to evaluate lipid profile, HDL subclasses by SDS-PAGE, and PON1 concentration by ELISA. T-Cholesterol, LDL-C, and ApoB levels were higher in the healthy group compared to the CVD and CRF groups. Among the groups, the CVD group had the lowest PON1 concentration (3.21, 2.76-3.94 µg/mL). Respect to HDL subclasses, HDL2a and HDL3a were found in higher proportions in CVD and CRF groups vs. the healthy group, whereas HDL3b and HDL3c were found in lower proportions. The healthy group had HDLs with the smallest average diameter (8.53 ± 0.07 nm). A logistic regression model found that if PON1 increases one unit, it decreases 1.86 the odds (p=0.00) to fit in the cardiovascular disease group. On the other hand, if the diameter increases one unit, it increases 17.89 the odds (p=0.00) to fit in the cardiovascular disease group. Lower proportions of small HDL (3c) and PON1 concentration were found in CVD group. Our data suggest that the study of PON1 status and HDL characteristics could be assessed to predict susceptibility for development of CVD.

**1169** Effect of Ethanol on IL-6-Mediated Effect on Human Angiotensinogen Using Human Hepatocytes: An In Vitro Model

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Human angiotensinogen (hAGT) is an acute-phase reactant type II (APR II) protein modulated by interleukin-6 (IL-6). Angiotensinogen is the source of angiotensin II that is a vasoactive peptide. AGT plays a significant role in blood pressure regulation, and increased levels of AGT are found among hypertensives. AGT participates in fibrinotic transformation of liver after hepatocellular carcinoma. Among chronic alcoholic patients, the immune system is downregulated while levels of circulating cytokines, like IL-1β, IL-6, and IL-8, are increased. In a cell-based model using HepG2 and Huh7 cells, the effects of alcohol on IL-6 mediated effects on secreted and cellular AGT levels were determined from 24 hrs to 72 hrs. It was observed that IL-6 and alcohol (50 and 100 µM) both increased the levels of secreted AGT from both HepG2 and Huh7 cells from 24-72 hrs. The cellular levels of AGT were also increased concomitantly to the secretions after IL-6 and alcohol treatment. The effects of alcohol and IL-6 combination treatment were additive in both cells. It was observed that HepG2 was more sensitive to treatment with IL-6 and alcohol as compared to Huh7 cells.

**1170** Qualification of a Thrombin-Anthrombin Complex Quantification Assay for the Detection of Hypercoagulable Conditions in Rats

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The purpose of this study was to qualify an assay for the measurement of thrombin-antithrombin (TAT) complexes for the detection of hypercoagulable/pro-thrombotic conditions in rats due to a continuous low-grade activation of coagulation factors. Such conditions are difficult to identify in preclinical studies, as continuously evaluated coagulation times are not sensitive indicators. In hypercoagulable conditions, the low-grade activation of the coagulation factors leads to the formation of thrombin, which is inhibited by antithrombin present in blood, inducing the formation of TAT complexes. Quantification of such complexes is considered a sensitive and specific indicator of thrombin activation in human medicine and indicative of hypercoagulability. TAT complexes were measured using a qualified ELISA method (Rat TAT, Elasbiene) in a rat model study of inflammation. Rats were administered once intraperitoneally with a low dose of lipopolysaccharides (LPS). Injection of LPS triggers proinflammatory cytokines and upregulates the expression of tissue factor, and leads to the activation of the coagulation system. Range of response was 0.50 to 16.00 ng/mL in plasma EDTA. Selectivity: % Recovery was within ± 25% for 4 out of 5 lots tested. Long-term TAT stability in plasma was proven for 43 days at -20°C. Short-term stability: 6 hours at ambient room temperature or 4°C. Freeze-thaw stability: 2 freeze-thaw cycles at -20°C. Level of TAT obtained were higher (~50%) with EDTA versus citrated plasma. In an in vivo study, a 2.5-fold TAT increase was observed following initiation of coagulation cascade after addition of neoplastic and calcium. During the in vivo study, rat blood samples were collected from the jugular vein in EDTA tubes, prior to LPS administration and 3, 8, 24, and 48 hrs post-dose. Increases in TAT concentration were observed at 3 hrs, peaked at 8 hrs (up to 6 folds), and gradually decreased at 24 and 48 hrs post LPS administration. Such changes indicated an excessive generation of thrombin and hypercoagulability, which gradually reversed. Based on the results observed, the quantification of TAT complexes is an interesting marker for the evaluation of hypercoagulable conditions in rats.

**1171** PBDEs Exaggerate Adrenal Epinephrine Content in Salt-Loaded Rats Showing Hypertension: Involvement of Sympathetic and Renin-Angiotensin-Aldosterone Systems

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Polybrominated diphenyl ethers (PBDEs) are organobromine compounds that are used as flame retardants in a wide array of products, including electronics and home furnishings. Our earlier studies revealed that developmental exposure to the penta-PBDE mixture, DE-71, exaggerates blood pressure responses to Na+ loading with sensitivity to the autonomic ganglion blocker (GB), pentolinium tartrate (19.2 mg/kg, s.c.), and renin-angiotensin-aldosterone system (RAAS) blockers captopril (CAP; 15 mg/L, drinking water, 2 weeks) and spironolactone (SPIRONO; 40-50 mg/Kg, oral gavage, 5-7 days) (Shah et al, 2011; Spurgin et al 2014), suggesting sympathetic nervous system (SNS) and RAAS involvement. Since PBDEs can enhance catecholamine release from adrenal chromaffin cells (Dingemans et al, 2007), we examined the activity of the sympathoadrenal axis (SAS) in hypertensive and normotensive rats perinatally exposed to DE-71 or corn oil vehicle infused cefetra toxins given to dam mothers (30.6 mg/kg/day; GD6-PND21). At PND50-70, adult male offspring were treated with hypertonic NaCl (3.5%; i.p.; Hyper) or normotonic NaCl (0.9%; i.p.; Norm). PBDE hyper rats were injected with Oil, Hyper, PBDE Hyper + Oil, PBDE Hyper + CAP, PBDE Hyper + SPIRONO. Adrenal content of catecholamine (CA) was measured via trihydroxyindole method and fluorometric detection at pH 7 (Kelner et al, 1985). Epinephrine (Epi) was measured at pH 2. Results showed a significant increase in both total CA and Epi in response to hypertensive treatment, but the effect on Epi was greater in PBDE-exposed rats (p<0.05 - .01, n=6-10). The elevated adrenal Epi content in PBDE Hyper was prevented by pretreatment with GB, CAP, and SPIRONO (p<0.001, n=6-10), suggesting involvement of SNS and RAAS. These blockers also reduced basal Epi content. Using ELISA, plasma levels of hyperosmotic-stimulated Epi were similar in PBDE and Oil rats, and only trend to GB (p<0.05, n=4-8). These results indicate that PBDE exaggerates the rise in adrenal Epi content under hypertensive stress. Our findings show that developmental exposure to PBDEs increases SAS, and RAAS activity under sodium loading may lead to hypertensive responses. Supported by UCMEUS (KS) and COR from UC Riverside (MCC).

**1172** Evaluation of Hypertension in Schoolchildren Exposed to Inorganic Fluoride in Chihuahua, Mexico

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Inorganic fluoride (F) is an abundant element in nature, its main source of human exposure being drinking water. Epidemiological and experimental studies have shown that fluoride exposure is related to adverse cardiovascular effects such as hypertension, with limited reports in children. We evaluated the relationship of fluoride exposure with the presence of hypertension in children. A cross-sectional study in 366 schoolchildren at 5-12 years old were selected from two schools, located in two communities of Chihuahua, Mexico, with 0.32±0.5 mg/L and 1.38±1.1 mg/L in drinking water. Concentration of fluoride in water and urine were measured by fluoride ion-selective electrode. Fasting blood glucose and lipid levels were evaluated using a chemical autoanalyzer. Resting blood pressure was measured in triplicate using a mercury sphygmomanometer in a sitting position. Hypertension and prehypertension were defined using national guidelines. Children that showed systolic and diastolic blood pressure greater than 95% and between 90th and 95th percentiles, respectively, for age, sex, and height. The mean urinary fluoride levels were 2.5±1.2 mg/L (0.67-6.75 mg/L). The prevalence of obesity (15.3%) was lower than overweight (17.9%). A total of 9.6% (n=35) of children had prehypertension, and a further 10.7% (n=39) of children had hypertension; both were related with body mass index (OR = 9.96, 95% CI = 4.70-21.10) and plasma atherogenic index (OR = 2.28, 95% CI = 1.37-3.80), but unre-
lated to urinary fluoride concentration (OR=0.90, 95% CI=0.70-1.17). Our results indicate that the prevalence of hypertension in these children is not related to fluoride exposure. Our finding also emphasizes the importance of the prevention of obesity and overweight. Supported by CONACYT-Mexico, Children’s Environmental Health Network #280296.

1173 Dioxin-Like PCB 126 Increases Systemic Inflammation and Accelerates Atherosclerosis in Lean LDLR Receptor-Deficient Mice

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Exposure to dioxins and related persistent organic pollutants likely contributes to cardiovascular disease (CVD) risk through multiple mechanisms, including modulation of chemokine expression. Epidemiological studies have shown that leaner individuals may be more susceptible to the detrimental effects of lipophilic toxics because they lack large adipose tissue depots that can accumulate and sequester these pollutants. This phenomenon complicates efforts to study mechanisms of pollutant-associated atherosclerosis in experimental animal models where high-fat feeding and adipose expansion limit the bioavailability of lipophilic pollutants. Here, we investigated whether a model dioxin-like pollutant, PCB 126, could increase inflammation and accelerate atherosclerosis in Ldlr-/- mice fed a low-fat atherogenic diet. We fed Ldlr-/- mice the Clinton/Cybulsky diet (10% kcal fat, 0.15% cholesterol) and sacrificed mice at 8, 10, or 12 weeks post PCB (2 doses of 1 μmol/kg) or vehicle gavage. To characterize this novel model, we examined the effects of PCB 126 on markers of systemic inflammation, hematological indices, fatty livers, and atherosclerotic lesion size. Mice exposed to PCB 126 exhibited significantly increased plasma inflammasome cytokine levels, increased circulating biomarkers of CVD, altered platelet and red blood cell counts, increased accumulation of hepatic fatty acids, and accelerated atherosclerotic lesion formation in the aortic root. PCB 126 also increased circulating neutrophils, monocytes, and macrophages as determined by flow cytometry analysis. Exposure to dioxin-like PCB 126 increases inflammation and accelerates atherosclerosis in mice. This low-fat atherogenic diet provides a useful tool to study the mechanisms linking exposure to lipophilic pollutants to increased risk of CVD.

1174 Differential Responses in Maternofetal Vasculature after Engineered Nanoparticle Inhalation


The continued development of engineered nanomaterials (ENM) across a broad range of fields has given rise to concerns over the potential mechanisms, including inflammation. Exposure to titanium dioxide nanoparticles (nano-TiO2) has been shown to impair fetal development and impact the health of surviving offspring. Given the inherent heterogeneity of the maternofetal vasculature, the purpose of this investigation was to assess the impact of a single ENM exposure at 3 time points during gestation on vascular reactivity at 4 distinct anatomic levels. Timed-pregnant Sprague-Dawley rats were exposed to nano-sized titanium dioxide (TiO2) aerosols (10-15 μg/m3) for 4 hours, 4 times/week, from gestational day 4, 12, or 17. Rats were euthanized on GD 20. Pregnancy parameters (fetal number, implantation sites, fetal and placental weights) were recorded. Wire myography (DMT-Liga) was used to evaluate active tension generation in the thoracic aorta, uterine artery, umbilical vein, and fetal thoracic aorta in response to chemical stimuli to assess endothelium-dependent (EDR) [methacholine (10-6-10-4 M)], -independent (IER) [spasmic/NONOate (SP, 10-6-10-4 M), and vascular smooth muscle (VSM) (phenylephrine (10-6-10-4 M)] reactivity. A single pulmonary exposure to nTiO2 early in gestation (GD 4) resulted in a greater number of reabsorption sites (2.3±1.9 vs. 0.6±0.2) and significant increase in placental weight (p=0.001) with no differences in litter size or pup weight. Pulmonary exposure to nTiO2 significantly impaired EDR in the thoracic aorta by 78.5±1.1% compared to control. EDR of the uterine artery, umbilical vein, and fetal aorta after exposure were not significantly different. Following ENM exposure, EDR was impaired in the thoracic aorta (75.6±23.0%, SPR 10-4M) and fetal aorta (160.8±38.3%) compared to controls, while significance was not noted for other vessels. There were no significant differences in VSM responses. Together, these results indicate a susceptibility of critical maternofetal vasculature to a single pulmonary exposure of nTiO2 during gestation. Additionally, gestational age at time of exposure influences fetal development, thereby identifying windows of fetal susceptibility to ENM. Supported by NIH R00-ES024783; P30-ES05022; R25-ES020721; ASPET-SURF.

1175 Effects of Orally-Administered Entresto on Systemic and Left Ventricular Pressures in Telemetered Normotensive St. Kitts Green Monkeys

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The St. Kitts green monkey (Chlorocebus sabaeus) is a primate species with close physiological and genetic homology to humans. It is gaining attention as a highly clinically translatable species for drug development, specifically in discovery and safety pharmacology testing. In the current study, we investigated the effects of orally administered Entresto (sacubatril/valsartan) on systemic and left ventricular pressures in telemetered normotensive St. Kitts green monkeys to evaluate respective pharmacodynamics and characterize the employed telemetry system. Following pole and collar training for 7 days, 3 monkeys with intubating Stellar TSE PPBTA-XL catheters were dosed via nasogastric intubation with either Entresto or vehicle for 7 days using a crossover design. Baseline pressures were relatively stable, although one monkey presented as hypertensive (systolic BP >150 mmHg). Entresto reduced systemic blood pressures by ~15-18 mmHg with minimal effects on heart rate (HR). Baseline left ventricular pressures (LVP), although relatively consistent through time, were outside of physiologically relevant ranges, reflecting calibration drifts in LVP signals. Entresto administration resulted in relative drops in LV systolic, diastolic, and mean pressures. This experiment highlights the potential utility of employing radio telemetry in St. Kitts green monkeys to evaluate cardiovascular pharmacodynamics and safety pharmacology testing with novel telemetry with other modalities, including ultrasound and circulating serum biomarkers, allows for the comprehensive evaluation of test article-induced cardiovascular effects in the highly clinically translatable St. Kitts green monkey.

1176 Group II Innate Lymphoid Cells and the Microvascular Response to Pulmonary Titanium Dioxide Nanoparticle Exposure


The reproductive and cardiovascular effects of pulmonary exposure to engineered nanomaterials (ENM) are poorly understood. Inflammation remains the most frequently explored mechanism. However, the key mediators remain to be uncovered. The purpose of this study was to determine the uterine inflammatory and vascular effects of pulmonary exposure to titanium dioxide nanoparticles (nano-TiO2). We hypothesized that pulmonary nano-TiO2 exposure initiates a Th2 inflammatory response mediated by Group II innate lymphoid cells (ILC2), which is associated with an impairment in uterine vascular reactivity. Female, virgin Sprague-Dawley rats (8-12 weeks) were exposed to 100 μg of nano-TiO2 via intratracheal instillation 24 hours prior to vascular assessments. Blood samples were obtained at 0, 1, 2, and 4 hours post-exposure for multiplex cytokine analysis. ILC2 numbers present in lungs were determined via flow cytometry 4 hours post-exposure, and bronchoalveolar lavage was performed for cytokine analysis. ILC2 cells were isolated by magnetic bead separation, and phospho-NF-kB levels were measured by ELISA. Immunohistochemistry of lung tissue for interleukin (IL)-33, a marker for epithelial cell damage, was also performed. Wire myography was used to evaluate uterine artery active tension generation, while pressure myography was used to assess vascular reactivity of radial and basal arterioles. Uterine vasculature was treated with cumulative concentrations (1×10-6-1×10-4 M) of phenylephrine, acetylcholine, and sodium nitroprusside. Four hours after nano-TiO2 exposure, a 2-4 fold increase in IL-18, 4, 5, and 13, and TNF-α was observed, indicative of an innate Th2 inflammatory response. ILC2 were significantly increased in lungs from exposed animals (1.7±0.2%) compared to controls (0.3±0.6%). Phosphorylation of NF-kB in isolated ILC2 was also increased by nano-TiO2 exposure (129±14% of controls), and pre-liminary data show a marked increase in lung IL-33. Radial and basal arteriolar endothelium-dependent reactivity was impaired by (27±12%), while endothelium-independent dilation (7±4%) and alpha-adrenergic sensitivity (8±2%) were not affected. These results suggest that pulmonary nano-TiO2 exposure may trigger an innate systemic Th2 inflammatory effect within 4 hours by stimulating lung resident ILC2. Supported by NIH R01-ES015022 (TRN), NSF-1003907 (TRN, ABA).
Neonatal rats are valuable models for the evaluation of cardiovascular development and associated toxicities. However, despite evidence for immature vascular α-adrenoceptors and a cardiac dominance of fast myosin isoforms in neonatal rats, few data exists on the normal cardiovascular state of this transitioning system. This study characterized systemic/left-ventricular hemodynamics, vascular responsiveness, and functional indices in rats ranging from the neonate period (10–14 days, n = 34) to young adulthood (>8 weeks of age, n = 7). Neonatal and young adult Sprague-Dawley rats were anesthetized with isoflurane (1.5–2%) and instrumented for aortic and left-ventricular pressures via carotid artery catheterization; in a subset of animals, 2D-guided M-mode echocardiography was also performed. Hemodynamic data were obtained before and after pharmacological vascular challenges with sodium nitroprusside (SNP, 25 μg/kg IV) and/or phenylephrine (PE, 10 μg/kg IV) in order to assess both cardiovascular state and responsiveness. When compared to adult physiology (at a matched-anesthetic plane), neonatal rats had marked cardiovascular differences, characterized by low arterial pressures (MAP: 44±1 vs. 100±3 mmHg in adults) and normal heart rates (HR: 372±4 vs. 366.8 bpm in adults). Neonates also had lower peak rates of LV pressure development (dP/dt max: 4058±123 vs. 9478±231 mmHg/s in adults), but faster-estimated velocities of shortening (CI: 152±3 vs. 144±5 1/s in adults) and preserved ejection fraction (EF 78±0.5% vs. 81±0.4% in adults). Moreover, the α-AR pressor capacity was incompletely developed in neonates, demonstrated by blunted responses to PE (ΔMAP: +12.2% vs. +37.2% in adults); however, NO-mediated vaso- relaxation was intact, as SNP resulted in an acute hypotension (ΔMAP: -25.2% vs. -30.2% in adults), albeit reflexive tachycardia was blunted (ΔHR: +21% vs. +12.2% in adults). These data confirm and document a maturing cardiovascular system in neonatal rats, characterized in vivo by hypotension, immature vascular α-AR responsiveness, and blunted cardiac baroreflexes, as well as by preserved systolic function and increased left-ventricular shortening velocity. These observations are consistent with the known neonatal ex vivo vascular adrenoceptor profile as well as with the predominance of the fast, but load-intolerant, alpha-myosin isoform in neonatal hearts.

**Exposure to Environmentally Persistent Free Radicals Leads to Decreased Vascular Responsiveness**

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Environmentally persistent free radicals (EPFRs) are formed during the combustion of waste at Superfund sites when pollutants are chemisorbed to redox-active transition metals. In vitro studies have demonstrated that cells exposed to EPFRs produce increased IL-6, TNF-α, and ROS, indicating inflammation, which is an initiator of vascular dysfunction. In addition, in vivo data demonstrate that adult C57BL/6 mice exposed to 1.5 mg/m³ of the EPFR DCB230 for either 4 hours or for 10 consecutive days exhibit significantly reduced NO and increased ET-1, suggesting endothelial dysfunction. However, analysis of BALF and lung tissue for inflammation was unremarkable, indicating the vasculature as a direct target of injury after EPFR exposure. Therefore, we hypothesized that at an intermediate time point, DCB230 exposure will lead to systemic inflammation, resulting in a decrease in vascular responsiveness. Adult C57BL/6 mice were subjected to whole-body inflation of 1.5 mg/m³ DCB230 for 4 hours a day for 3 consecutive days prior to flow cytometry and vessel reactivity analysis. DCB230-exposed mice demonstrated significantly reduced monocytes in circulation and increased lymphocytes versus the air-only control group. Analysis of aortic segments indicated a significant reduction in maximum relaxation in DCB230 versus control mice. Together these data demonstrate that EPFRs lead to alteration of the peripheral inflammatory cell milieu and reduced vascular responsiveness, which may ultimately culminate in the development or exacerbation of cardiovascular disease.

**Intravenously Injected Polyethylene Glycol (PEG)-Coated Liposomes Result in Microvascular Dysfunction**

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Polyethylene glycol (PEG) is a coating often used to improve biodistribution and safety of small molecules and liposomes for drug delivery. Despite PEG being commonly used, the microvascular impacts are largely unknown. Therefore, we hypothesize that PEG-coated liposomes will negatively impact microvascular function following intravenous (IV) injection. Balb/C mice were IV injected with either PEG-coated or non-PEG-coated liposomes (SmM), and mesenteric arterioles were harvested three days post exposure. Endothelium-dependent and -independent reactivity was assessed with acetylcholine (ACH, 10⁻⁴ M), and sPentine NONOate (SPR, 10⁻⁴ M) via pressure myography. Vascular smooth muscle vasoreactivity was assessed with phenylephrine (PE, 10⁻⁶ M). Following assessments, superoxide and hydrogen peroxide were scavenged in the presence of the superoxide dismutase mimetic 2,2,6,6-tetramethylpiperidine-N-oxyl (TEMPOL, 10⁻⁴ M) and catalase (50 U/mL). The contribution of nitric oxide (NO) was evaluated by inhibiting nitric oxide synthase with N°-monomethyl-L-arginine (L-NMMA, 10⁻⁴ M). Following PEG-coated liposome delivery, endothelium-dependent dilatation was significantly attenuated (-3.9 ± 24% vs. control 69 ± 4%), and this impairment was not present following non-PEG-coated liposome delivery (55 ± 10% vs. control 69 ± 4%). Furthermore, there was no significant impairment in endothelium-independent dilation for the two liposome groups (PEG, 2 ± 6% vs. non-PE, 70 ± 6%). Taken together, these results indicate that PEG-coated liposomes induce microvascular dysfunction that is present up to three days following injection, and these impairments may be due to changes in oxidative stress and/or endothelial cell signaling. Furthermore, this is the first report of adverse microvascular effects associated with PEG-coated liposomes. Since liposomes are being increasingly used as a therapeutic delivery method, establishing these negative microvascular effects is essential since they may contribute to adverse cardiovascular responses.

**Intranasal Administration of Amiodarone Causes Pulmonary Fibrosis and Triggers Recruitment of Immature Macrophages to the Lung in C57BL/6 Mice**


Amiodarone (AD) is an antiarrhythmic drug that causes pulmonary toxicity in 10-17% of patients. Pulmonary fibrosis associated with AD is characterized by the presence of foamy macrophages and hyperplasia of type II alveolar epithelial cells. The precise role of the innate immune system in the development of AD-induced pulmonary toxicity is unknown, and this represents the focus of the present studies. AD administration (16 mg/kg intranasally every 5 d) to 8-12-week-old male and female C57BL/6 mice resulted in the development of pulmonary fibrosis 25 d post exposure. This was characterized by collagen deposition around the airways and in the lung parenchyma, as assessed by Masson’s Trichrome staining, which was associated with an accumulation of macrophages in the lung in histologic sections. To analyze the phenotype of these cells, we used techniques in flow cytometry; two macrophage populations were analyzed. The population that was scavenged by bronchoalveolar lavage, and tissue-associated macrophages selected from a collagenase-mediated whole-lung digest by magnetic selection of F4/80+ cells. We found that the ratio of F4/80+SiglecF-CD11C++CD11B+ to F4/80+SiglecF-CD11C++CD11B- populations of tissue-associated macrophages increased from 1.12 in sham animals to 1.77 and 1.90 in PBS and AD-exposed animals, respectively. In contrast, there were no changes in alveolar macrophages. Immunohistochemistry demonstrated that inflammatory (CD11B-) macrophages were mainly present in areas of collagen staining in lung tissue. The increased ratios of F4/80+SiglecF-CD11C++CD11B+ to F4/80+SiglecF-CD11C++CD11B- populations may be due to changes in oxidative stress and/or endothelial cell signaling. Furthermore, this is the first report of adverse microvascular effects associated with PEG-coated liposomes. Since liposomes are being increasingly used as a therapeutic delivery method, establishing these negative microvascular effects is essential since they may contribute to adverse cardiovascular responses.
Vanadium is a transition metal that can be found in respirable particulate matter in occupational and environmental settings. Here, we investigate the effects of vanadium administered via pulmonary exposure on respiratory function, bronchial ring contraction, and inflammatory response. C57BL/6 mice were treated with 0, 0.1, 1, 10, 30, or 90 µg of VOSO₄ dissolved in saline by pharyngeal aspiration. A forced oscillation pulmonary function system (Flexivent, Scireq Inc.) was used to assess airway resistance and compliance 24 hours post-exposure. This included administering increased doses of methacholine (0–50 mg/ml) by inhalation to induce bronchoconstriction, to characterize lung damage. Additionally, a comparison study between VOSO₄ and VO₂O₄ was conducted to examine inflammatory responses in the lung with 0, 0.1, 1, or 10 µg VOSO₄ or VO₂O₄ by pharyngeal aspiration. Bronchoalveolar lavage fluid (BALF) and serum samples were collected 24 hours post-exposure. BALF samples were stained via basophilic staining, followed by counting the number of macrophages and neutrophils, and a Bradford protein assay was used to measure protein concentration. Bronchial rings were isolated from naïve mice and treated ex vivo with VOSO₄ to examine the impact on contraction to methacholine using a myograph. Pulmonary function data showed negligible differences among exposure groups, although dose-related differences in methacholine-induced bronchial rings were evident. Both VOSO₄ and VO₂O₄ did not affect macrophage percent, but a slight increase in neutrophils was seen with high-dose VO₂O₄ treatment. Bronchial rings treated with VOSO₄ were more sensitive to methacholine-induced contraction than naïve rings. Soluble VOSO₄, even at high levels, did not produce substantial impacts on pulmonary function, although age-related differences in methacholine-induced reactivity were observed. C57BL/6 mice were treated with 0, 0.1, 1, 10, 30, or 90 µg of VOSO₄ or VO₂O₄, and these exposure groups were compared using age-matched naïve control animals. Exposure to 2,4-DB acid or to MCBP acid caused laryngeal effects at doses of 0.02 mg/L and above. These effects recovered partially following 1 week of recovery. These studies add to the weight of evidence characterizing laryngeal squamous metaplasia in the rat by providing additional information on the degree of recovery that may be anticipated after shorter recovery periods. Given that herbicide use patterns often require several weeks between applications, such information is relevant for determining the appropriate use of rodent toxicity data in a human health risk assessment context. This poster will present the results of these studies and discuss their contribution to the overall weight of evidence on interpretation of the adversity and human health significance of laryngeal squamous metaplasia in rodent inhalation studies.
Potassium titanate whisker (PTW) is one of the man-made mineral fibers and used for an alternative to asbestos. There is a report that a certain type of PTW was positive for mesotheliomagenesis in intraperitoneal injection study. As the particles of PTW are respirable in size, inhalation studies are essential for the assessment of its respiratory toxicity. A rat study has been reported with a low lung burden for a mass concentration of the aerosol, suggesting the presence of a large proportion of non-respirable agglomerates in the aerosol. In order to establish an inhalation exposure condition of PTW for a precise hazard assessment with better dosimetry, we conducted a mouse short-term whole-body inhalation study of a commercially available PTW in Japan, using our dispersion method and inhalation system designated as Taquann Method and Taquann Direct-Injection Whole Body Inhalation System (J Tox. Sci. 2013). In our study, the mass concentration was 4.1 mg/m³, and relative concentration was 7.575 mg/mL measured by condensation particle counter (Model 3776, TSI). MMAD on aerosol in the chamber was 1.348 nm measured by MOUDI (Model 125, Kanomax). The fiber length of PTW aerosol was 4.3±3.7 micrometer (max. 23.8), and the fiber diameter was 338±171 nm (max. 990), observed by scanning electron microscopy (VE-9800, Keyence). Male C57BL/6 mice, 12 weeks old, were exposed for two hours per day for five consecutive days. Mice in control group inhaled clean air in the same manner. The lung burden of mice immediately after the last exposure (Day 0) was 15 micrograms per animal, and the length of PTW recovered from the lung was virtually identical to the last exposure (Day 0) was 15 micrograms per animal, and the length of PTW recovered from the lung was virtually identical to the last exposure. Pulmonary densities of B (CD45R+), T (CD3+) and 2) assess the impact of the dietary ω-3 polyunsaturated fatty acid docosahexaenoic acid (DHA) on cSiO₂ triggering of ectopic lymphoendothelial structures (ELS) in the lungs of mice genetically prone to develop lupus. ELS are tertiary lymphoid organs, associated with autoimmunity, that develop in areas of chronic inflammation and are characterized by the formation of organized B cell and T cell aggregates and germinal centers with follicular dendritic cell networks. The goal of this study was to 1) determine the kinetics of cSiO₂-induced ELS development in the lungs of lupus-prone mice and 2) assess the impact of the dietary ω-3 polyunsaturated fatty acid docosahexaenoic acid (DHA) on cSiO₂ triggering of ectopic lymphoendothelial structures (ELS). Cohorts of 6-week-old lupus-prone female NZBWF1 mice were nasally instilled with 1 mg cSiO₂, or saline vehicle alone, once per wk, for 4 consecutive wks. Animals were sacrificed 1, 5, 9, or 13 wks after the last cSiO₂ instillation. Pulmonary densities of B (CD45R+, T (CD3+) and follicular dendritic (CD21+/CD35+) cells were immunohistochemically and morphometrically determined. Lymphoid cell densities significantly increased over time in the lungs of cSiO₂-treated mice. DHA supplementation markedly attenuated cSiO₂-triggered B and T cell, but not follicular dendritic cell, infiltration over the entire 13-wk post-exposure period. These results suggest that the development of ELS in the lung may be an early indicator of cSiO₂-induced autoimmunity and that dietary DHA may effectively prevent development of these aberrant structures. Research funded in part by NIH grant ES027353, Lupus Foundation of America grant 362470, and the Dr. Robert and Carol Diebel Family Endowment.

From an occupational standpoint, exposure to silica can have devastating consequences. An estimated 2.3 million workers in the US are exposed to dust containing crystalline silica, annually. In addition, of the 140 million people over the age of 20 employed in the US, 30% are obese. If and how diet-induced obesity modifies silica-induced pulmonary toxicity is unknown. Therefore, the objective of this study was to determine the effect of diet-induced obesity, if any, on silica-induced pulmonary toxicity. Rats (Fischer 344, male) were fed either a regular-fat diet (RFD; 18% kcal as fat) or a high-fat diet (HFD; 60% kcal as fat) and exposed by whole-body inhalation to either air or crystalline silica (15 mg/m³, 6 hours/day, 5 days). At designated post-exposure time intervals (1, 3, 6, and 9 months), pulmonary toxicity was determined. Toxicity parameters including lactate dehydrogenase (LDH) activity, oxidant production, cell counts (including infiltrating neutrophils and alveolar macrophages), inflammatory cytokine levels (IL-1β, IL-10, TNF-α, MCP-1, and MIP-2), and lung histopathology were assessed. Body weights and serum triglyceride levels, indicators of diet-induced obesity, in the HFD rats were higher compared to those in RFD rats. The results showed that silica particles were seen in lung sections from the exposed animals. Silica inhalation resulted in pulmonary toxicity, which progressed across all post-exposure time points, as evidenced by enhanced neutrophil infiltration, increased LDH activity, oxidant production, and increased inflammatory cytokine levels. The incidence and severity of silica-induced lung pathology was similar between the two diet groups up to 6 months post-exposure. However, by 9 months post-exposure, silica-induced pathology tended to be slightly more severe in animals fed a RFD compared to those fed a HFD. In summary, our results indicated that certain pulmonary toxicity parameters induced by silica inhalation were modified by diet-induced obesity in rats.

Humans exposed to asbestos and/or asbestiform fibers are at high risk of developing many lung diseases, including asbestosis, lung cancer, and malignant mesothelioma. However, the disease-causing potential and related processes associated with various asbestos/asbestiform fiber exposures in triggering the different (non-)carcinogenic outcomes is still largely unknown. In this study, we investigated whether exposure to different asbestos/asbestiform fibers leads to differences in inflammatory responses and gene expression profiles at acute/sub-acute phases that can be related to pathological outcomes observed at extended time points of post-exposure. We exposed mice to asbestos (crocidolite, tremolite asbestos), asbestiform fibers (erionite), and a low-pathogenicity mineral fiber (wollastonite) using oropharyngeal aspiration. We observed some shared inflammatory and tissue damage responses, albeit to different extents, at day 1 and 7 post-exposure. In addition, exposure to different fibers also exhibited distinct changes in the regulation and release of a number of inflammatory mediators. Further, a detailed comparison of gene regulation changes in the lungs on day 7 post-exposure also suggested differential biological responses that were consistent with histopathological changes at day 7 and 56 post-exposure. Taken together, these results suggest clear differences in the magnitude of various pulmonary responses and gene regulation that were consistent with pathological alterations upon exposure to the four asbestos/asbestiform fibers studied. Further mechanistic studies focusing on long-term endpoints are critical for understanding how early biological responses are associated with pulmonary exposures to asbestos/asbestiform materials and their ability in triggering different carcinogenic outcomes, i.e., lung cancer versus mesothelioma.
Evaluation of the Dose-Response and Fate in the Lung and Pleura of Chrysotile Containing Brake Dust Compared to Chrysotile or Crocidolite Asbestos in a 28-Day Inhalation Toxicology Study

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This study was designed to provide an understanding of the biokinetics and potential toxicology in the lung and pleura following inhalation of brake dust (from brakes manufactured with chrysotile) in a 28-day repeated multi-dose inhalation toxicity study (6 h/d, 5 d/wk, 4 wk) followed by 28-days of exposure-free recovery and served as a range-finding study for a subsequent ongoing 90-day repeated-dose inhalation toxicology study with lifetime recovery. Comparative fiber control groups were included of a similar grade of commercial chrysotile as used in the brakes and a commercial crocidolite asbestos sample. The aerodynamic fiber distribution of the chrysotile and crocidolite asbestos was similar (Fibers L = 20 µm/cm²; Chry-LD 42, Chry-HD 62; Croc-LD 36, Croc-HD 55; WHO f/cm³ Chry-LD 192, Chry-HD 219; Croc-LD 211, Croc-HD 255). The total number of particles in the aerosol of the brake dust groups was similar to that in the chrysotile groups (Part/cm³: 211, Croc-HD 255). A macrophage dose response to the brake dust groups was observed with no fiber-related effects. In the fiber control groups, the study differentiated between similar exposures to chrysotile and crocidolite asbestos. The chrysotile exposure resulted in a dose-dependent particle-laden macrophage response characterized by Wagner Grade 1 to 3. In contrast, following crocidolite exposure there was a dose-response accumulation of fiber-laden macrophages and interstitial fibrosis during exposure (Wagner score Grades 3 to 4), which increased in incidence following exposure (Wagner score Grade 4). Confocal microscopy images obtained from chestwalls deep frozen at sacrifice revealed no difference between the air control, brake dust, and chrysotile high-dose exposure groups and no difference in the visceral or parietal pleura thickness at 28 or 56 days. The crocidolite exposure resulted in more than double the thickness of the visceral pleura and parietal pleura, with associated extensive inflammatory response and collagen development observed, including adhesions between the visceral and parietal surfaces. These results provided a basis for the design of the 90-day study. Study funded by Honeywell International Inc.

Endocannabinoids Attenuate Staphylococcal Enterotoxin B (SEB)-Mediated Acute Lung Injury through Regulation of microRNA That Target the Induction of Myeloid-Derived Suppressor Cells and T Regulatory Cells

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Acute Lung Injury (ALI) is still a significant cause for morbidity and mortality in the human population, with a high incidence of over 200,000 cases/year in the US. In this study, we used a single dose of Staphylococcal enterotoxin B (SEB) (50µg) intranasally to induce ALI. The inhalation of SEB, a category B agent as defined by the CDC, leads to robust activation of T cells and a cytokine storm that causes significant damage to the lungs. In the current study, we induced SEB-mediated acute lung injury in C57BL/6 mice and investigated if treatment with an endocannabinoid (AEA) would attenuate ALI. Our data demonstrated that a dose of (40mg/kg) of AEA significantly improved the lung function tests in mice with SEB-induced ALI when compared to vehicle controls, as determined by plethysmography. Analysis of mononuclear cells in lung of SEB-AEA mice showed significant increase in myeloid-derived suppressor cells (MDCSs) that were CD11b+Gr1+ and anti-inflammatory. Our results also demonstrated induction of T regulatory cells that were CD4+FOXP3+. Microarray analysis of miRNA in lung-infiltrating cells in SEB+AEA group revealed that miR-23a-3p and miR34a-5p were downregulated when compared to controls. These miRNA targeted TGF-β1, TGF-β2, and FOXP3, as shown by transfection of splenocytes activated by SEB with mock, mimic, or inhibitor of both miR 23a-3p and miR34a-5p separately, which resulted in specific alterations in the target genes. The expression of all miRNAs and target genes involved were validated by RT-PCR. Together, our data demonstrated that endocannabinoids can attenuate ALI and inflammation mediated by SEB through modulation in the expression of miRNA which induce immunosuppressive MDCSs and Tregs. Supported by NIH grants P01AT003961, R01AT006888, R01AI29788, R01AI123947, R01MH94755, P20GM103641 to PN and MN, and HCED Iraq to MS.

Role of microRNA in Tetrahydrocannabinol (THC)-Mediated Amelioration of SEB-Induced Acute Lung Injury

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Staphylococcal enterotoxin B (SEB) produced by Staphylococcus aureus may cause food poisoning, acute respiratory distress syndrome, and multiple organ failure, and could be fatal. Tetrahydrocannabinol (THC) is the main ingredient found in marijuana cannabis, which has psychotropic effects and is used to treat anorexia and nausea, as well as used as an analgesic. In the current study, we investigated the therapeutic potential of THC on immune cell apoptosis and inhibition of nuclear factor kappa B (NF-κB). To this end, acute lung injury was induced by a dual dose of SEB in C57/HeJ mice, which were treated with vehicle or THC. SEB caused expansion of a large number of T cells and massive release of inflammatory cytokines such as TNF-α. Furthermore, stimulation through its receptor led to activation of the MAP kinase (MAPK) complex, which causes activation of general stress response factor NF-κB. Apoptosis, a process of programmed cell death, was detected by the TUNEL & DIO6C staining. Moreover, we had identified that THC caused cell cycle arrest in G2 and apoptosis. In order to determine the epigenetic mechanisms of THC-induced effects, we performed high-throughput microRNA microarrays with lung-infiltrating mononuclear cells from vehicle and THC-treated mice. Ingenuity Pathway Analysis software was used to analyze the microarray dataset. The analysis showed that THC treatment led to immunosuppression through several mechanisms, including downregulation of miR-185 that may be responsible for increased expression of Runx, NKRA5, IL10, and Foxp3, while upregulation of miR-21 led to decreased NF-κB and Stat-3. Furthermore, validation of the expression of miR-185 and miR-21 by RT-PCR using lung mononuclear cells confirmed our data from high-throughput analysis. Together, THC plays a major role through epigenetic mechanisms that modulate immunological pathways that suppress SEB-induced acute lung injury. Supported by P01AT003961, R01AT006888, R01MH94755, P20GM103641, R01AI129788, R01AI123947, and M01ESR for AKM.

Infiltrating Monocyte-Derived Macrophages Contribute to the Development of Radiation-Induced Pulmonary Fibrosis


The lung is a radiation (RT)-sensitive organ, and the development of chronic inflammation that progresses into pneumonitis and pulmonary fibrosis (PF) is often observed in exposed individuals. RT induces an injury response involving the recruitment of immune cells to execute inflammatory and wound healing programs. However, RT can alter the lung microenvironment, dysregulating immune responses and preventing the return to homeostasis. This leads to chronic inflammation, pneumonitis, and PF. The extent to which recruitment of inflammatory monocytes, which are bone marrow derived and express CCR2, contribute to RT-induced PF has not been fully investigated, and we hypothesized that RT-induced PF is reliant on this population. To test this, CCR2-/- mice were exposed to 12.5 Gy thoracic RT. PF development was evaluated, and alterations in pulmonary macrophage subpopulations were assessed by flow in immune cells enriched from lung digests collected at 12 - 18 wk following RT. In CCR2-/- recipient mice that received C57Bl/6j bone marrow (WT > CCR2-/-) and WT syngeneic mice, fibrotic foci were observed in the periphery of the lungs by 12 and 16 wk following exposure, respectively. In contrast, in mice that received bone marrow lacking CCR2 (CCR2-/- > WT and CCR2-/- > syngenic mice), no PF was observed for at least 22 wk post-exposure and correlated with decreased numbers of infiltrating and interstitial macrophages compared to controls, and reduced proportions of proinflammatory Ly6C+ macrophages, observed at 12 - 18 wk following exposure, suggesting CCR2+ macrophages contribute to radiation-induced pulmonary fibrosis. Reduced proportions of CD206+ lung macrophages, indicative of an alternatively activated phenotype, were present at this time in CCR2+/WT recipient chimeric mice, regardless of AEA mouse bone marrow type, suggesting this resident phenotype is influenced by CCR2. In all chimeras, RT decreased the percentage of F4/80+ cells, expressed on mature macrophages and monocytes, suggesting the presence of less mature macrophages following RT exposure. These results suggest infiltrating monocytes influence resident macrophage phenotype and play a role in the development of RT-induced PF.
Comparing the RMVs against the Alexander equation during restraint gave values of 13.3 breathes/min, 132 mL/min, and 1.73 L/min at Day 16. 5 min prior to dosing. The values from 2 to 21 hrs post-dose markedly increased to 40.9 breathes/min, 124 mL/min, and RR, TV, and RMV from 16.1 breathes/min, 130 mL/min, and 2.04 L/min. The average TV for Day 8 was 193 mL/min, decreased to 142 on Day 16. The average RR on Day 8 was 25.4 breathes/min, which decreased to 20.1 after Day 10 and 14 showed a general decrease in RR. The RR, TV, and RMV values, which may mask potential effects in duration. The aim was to minimize stress-related elevations in respiratory minute volume (RMV) values, which may mask potential effects of administered test material. Pre-dose measurements of tidal volume (TV), respiratory minute volume (RMV), and activity, are potential strategies for the treatment or prevention of DNA damage, as well as mitigation of ROS production and activity, are potential strategies for the treatment or prevention of these persistent toxic, lethal effects of pulmonary radiation injury.

The minipig is increasingly being used as a species of choice for toxicological and pharmacological studies due to improved animal welfare considerations and increased ethical scrutiny of using non-human primates. However, this is not the case for inhalation studies. Prior to the inhalation dose values with pre-dosing values (-1 to -0.67 hr), the values at Days 10 and 14 showed a general decrease in RR. The RR, TV, and RMV values for the females were higher than the males. Comparing the inhalation dose values with pre-dosing values (-1 to -0.67 hr), the inhalation restraint procedure gave a marked average decrease in RR, TV, and RMV from 16.1 breathes/min, 130 mL/min, and 2.04 mL/min. These values markedly increased to 40.9 breathes/min, 124 mL/min, and 6.11 mL/min 5 min prior to dosing. The values from 2 to 21 hrs post-dose gave values of 13.3 breathes/min, 132 mL/min, and 1.73 mL/min at Day 16. Comparing the RMVs against the Alexander equation during restraint procedures found that this dataset produced RMV values that were up to 70% higher than predicted based on the bodyweight, suggesting that this equation overestimates the RMV for minipigs and RMV values should be ascertained prior to every study. However, it must be noted that no equivalent data were used in that equation’s derivation. In conclusion, the RR, TV, and RMV decreased with the number of acclimatized exposures (4%) but were considerably higher than observed pre- and post-exposure. The RMV of minipig is 70% lower than predicted by the Alexander et al. RMV equation, an RMV determination should be undertaken prior to every study, and a species-specific RMV equation is necessary.
In 2016, the American Conference of Governmental Industrial Hygienists lowered the 8-hr threshold limit value-time-weighted average (TLV-TWA) for toluene disocyanate (TDI) from 5 ppb to 1 ppb, and the 15-minute short-term exposure limit (STEL) from 20 ppb to 5 ppb, to protect against respiratory effects. However, the human evidence indicates that maintenance of 8-hr average TLV-TWA concentrations less than or equal to 5 ppb and peaks less than 20 ppb (i.e., the previous 8-hr TLV-TWA and 15-minute STEL) is protective of occupational asthma (OA) in most workers, and is also protective of lung function decrements and other respiratory effects. Some of the available studies that suggest occupational asthma cases at TWA concentrations less than 5 ppb were likely affected by very high peak exposures, well above 20 ppb. Advances in industrial hygiene measures have reduced peak exposures and the incidence of upset conditions such as spills and accidents, so these high peak exposures are unlikely to occur in modern TDI manufacturing facilities. The animal literature supports the human evidence and indicates that TDI-induced asthma is a threshold phenomenon. The evidence does not indicate that the lower TDI TLVs will result in a lower incidence of respiratory effects, including OA.

### 1197 Critical Evaluation of Thresholds for Respiratory Effects of Toluene Disocyanate


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Prolonged exposure to hyperoxia (>95% O2) has been shown to contribute to the accumulation of HMGB1 in the airways of patients receiving oxygen therapy. Excessive production of intracellular reactive oxygen species (ROS) during prolonged hyperoxia exposure can compromise innate immunity, predisposing patients receiving ventilation to the development of ventilator-associated pneumonia (VAP). Nuclear factor (erythroid-derived 2)-like 2 (Nrf2) is a transcription factor that regulates the expression of endogenous antioxidant proteins. The present study investigates whether sulforaphane (SUL) and resveratrol (RES), Nrf2 inducers, can ameliorate hyperoxia-compromised macrophage functions. RAW 264.7 cells, a murine macrophage-like cell line, and bone marrow-derived macrophages (BMDM) were exposed to hyperoxia for 24 hours in the presence or absence of different concentrations of SUL or RES. SUL treatment ameliorated the increase of intracellular levels of reactive oxygen species (ROS) as well as hyperoxia-induced compromised phagocytic function. In addition, SUL was found to rescue HMGB1-compromised macrophage phagocytosis. Furthermore, both SUL and RES were found to inhibit HMGB1 release, activate Nrf2, and increase levels of the endogenous antioxidant compound HO-1. This increase in macrophage phagocytic function in the presence of Nrf2-inducing compounds suggests that enhanced antioxidant capacity through the activation of the Nrf2 pathway can improve innate immunity, providing a therapeutic approach to the treatment of VAP.

### 1198 Evaluation of the Toxicity of Sodium Dodecyl Sulfate (SDS) in the MucilAir Human Airway Model In Vitro

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MucilAir™ is an in vitro airway model with morphology and functions mirroring the trachea-bronchial epithelium. MucilAir™ units comprise cells derived from human airway biopsies cultured at the air interface on permeable membranes by Epithelix. This model is increasingly used in asthma research and early development of inhaled drugs.

To investigate the toxicity of sodium dodecyl sulfate (SDS) in the MucilAir model, cells derived from human airway biopsies were cultured at the air interface in vitro. SDS was added to the culture medium at different concentrations (0-10 mM) for 24 h (37°C; humidified 5% CO2 atmosphere). Monolayer integrity was assessed using metabolic competence (resazurin reduction), membrane integrity (lactate dehydrogenase release), and other parameters such as trans-epithelial electrical resistance (TEER) and lactate content. After 24 h, histology demonstrated that pseudo-stratified epithelial morphology did not recover, recovery of basic cellular functions was limited, and cytokine release was increased. These results indicate that SDS is a potent toxin in the MucilAir model, providing a therapeutic approach to the treatment of VAP.

### 1199 Effects of Cationic Disinfectants on Surface Activity of Pulmonary Surfactant Monolayers


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Institute for Environmental Studies, Tsukuba, Japan; and 3St. Marianna University School of Medicine, Kawasaki, Japan. Quaternary ammonium compounds (QAC), such as benzalkonium chloride (BAC) and cetetylpyridinium chloride (CPC), constitute a group of cationic surfactants, widely used as disinfectants, preservatives, and detergents, for personal hygiene and medical use. It has been reported that inhalation of aerosols of BAC or CPC increases pulmonary inflammation in rats. Therefore, BAC or CPC deposited in the alveolar region could alter pulmonary function directly when humans inhale those aerosols. We investigated the effects of BAC, CPC, and pyridinium chloride (PC, as a control) on surface activity of the pulmonary surfactant monolayer using both Surfacten (Mitsubishi Tanabe Pharma), a modified bovine lung surfactant, and 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), a major component of the lung surfactant. One mM DPPC or Surfacten dissolved in chloroform was spread dropwise on a subphase in the trough filled with distilled water containing designated concentration of BAC, CPC, or PC. To examine the effect of BAC, CPC, or PC on the surfactant, we compared the features of surface pressure/roughness area (n-A) isotherm by the Langmuir-Wilhelmy method and atomic force microscopy (AFM) topographic images of lipid monolayer transferred onto the mica disc. The n-A isotherms showed that addition of BAC or CPC to the subphase expanded the isotherm at lower pressures to much greater areas, suggesting a reduced packing density of monolayer. The collapse pressure diminished with increasing concentration of CPC in subphase. Conversely, addition of PC (control) into subphase affected neither DPPC nor Surfacten monolayer isotherm. The compressibility modulus (Cs) of each n-A isotherm was also measured. The higher Cs value reflects the higher rigidity of the model membrane. The maximum Cs value of both DPPC and Surfacten monolayers decreased with increasing concentration of BAC or CPC in the subphase. Topographic images indicated that the presence of BAC or CPC in the subphase resulted in smaller condensed lipid domains compared to those on the subphase of distilled water or water containing PC. These results suggest that the deposition of BAC or CPC aerosols on the pulmonary surface may be related to dysfunction of surfactant activity in the lung.

### 1200 Nrf2 Inducers Ameliorate Hyperoxia-Compromised Macrophage Functions


To further investigate the role of Nrf2 in hyperoxia-compromised macrophage functions, we tested the effect of sulforaphane (SUL) and resveratrol (RES) on macrophage viability, apoptosis, and cytokine release in a hyperoxia model. RAW 264.7 cells, a murine macrophage-like cell line, and bone marrow-derived macrophages (BMDM) were exposed to hyperoxia for 24 hours in the presence or absence of different concentrations of SUL or RES. SUL treatment ameliorated the increase of intracellular levels of reactive oxygen species (ROS) as well as hyperoxia-induced compromised phagocytic function. In addition, SUL was found to rescue HMGB1-compromised macrophage phagocytosis. Furthermore, both SUL and RES were found to inhibit HMGB1 release, activate Nrf2, and increase levels of the endogenous antioxidant compound HO-1. This increase in macrophage phagocytic function in the presence of Nrf2-inducing compounds suggests that enhanced antioxidant capacity through the activation of the Nrf2 pathway can improve innate immunity, providing a therapeutic approach to the treatment of VAP.
1201 Two Novel Models for Study of Particle-Induced Alveolar Macrophage Toxicity: ASC-Transfected Raw 264.7 Cells and Max Planck Institute Cells
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Both human epidemiological and mouse experimental studies reveal that lung exposure to crystalline silica triggers autoimmunity. Silicosis-induced pathogenesis has been linked to NLRP3 inflammasome activation in alveolar macrophages (AM), which drives caspase-1 activation, extracellular release of mature IL-1beta, and pyroptotic cell death, thereby activating and recruiting additional immune cells to the lung. In vitro elucidation of early silica-triggered molecular events is limited by low numbers of AM that can be obtained from a single mouse (≈10^5). To address this, we compared two new in vitro AM models relative to silica-induced inflammasome activation. In the first model, RAW 264.7 murine macrophage cells were stably transfected with the ASC protein (adaptor protein essential for inflammasome assembly). Following priming with LPS for 2 h, RAW 264.7 + ASC cells were highly sensitive to silica-induced cell death and showed over a ten-fold increase in the release of IL-1beta as compared to non-transfected RAW 264.7 cells. In the second model, Max Planck Institute (MPI) cells were used. MPI cells are self-renewing macrophages, derived by culturing fetal mouse livers in GM-CSF-supplemented medium and are phenotypically similar to murine AM. Both cytokotoxicity and IL-1beta release were evident in LPS-primed silica-treated MPI cells. Taken together, the responses of silica-treated RAW 264.7 + ASC cells were reflective of responses seen in vivo upon exposure to inhaled silica. Both types of cells might be suitable in vitro alternatives to primary AM for characterizing the up- and downstream events associated with silica- triggered activation of the NLRP3 inflammasome in AM. Supported by NIH grant ES027353, the Institute for Integrative Toxicology NIEHS Training Grant T32ES070255.

1202 Long-Term Culture of Nasal, Tracheal, Bronchial, and Small-Airways Human Airway Epithelia at Interconnected and Dynamic Liquid Flow Conditions
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We herein report the first interconnection of four fully differentiated epithelia reconstituted from primary human cells from different anatomical origins, namely the nose, the trachea, and the bronchial and small airways (SmallAir™). The system is composed of a culture plate allowing 3D models grown in transwells to be (i) interconnected via the basal compartment through meso-fluidics (0.3 μl/min of a common culture medium) and (ii) maintained at the Air-Liquid Interface. Stability in terms of morphology and function of the four fully differentiated human airway epithelia was evaluated. Endpoint measurement included longitudinal tissue integrity assessment (TEER); cilia activity (Cilia Beating Frequency), and histological evaluation (%Eucalcian blue staining). Quantitative immune-histological analysis didn’t reveal major differences in cell proliferation (Ki67) or ciliation (FoxJ1) between interconnected and static conditions. The study concluded that minor differences are observed for all tested endpoints after 6 weeks of culture at interconnected and dynamic liquid flow conditions; therefore, this model allows testing the toxicity of the chemical compounds simultaneously on several anatomical regions of the respiratory tract, as well as the interplay of different organs/tissues in vitro.

1203 Respiratory Irritants Cause Reversible Up-Regulation of Pro-Inflammatory Cytokines on Human Nasal Mucosa Reconstituted In Vitro
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Respiratory irritants are considered as substances of higher risk, at the same level as carcinogens, mutagens, and toxic chemicals for reproduction. Respiratory irritation is defined as a non-corrosive effect inducing a reversible local inflammation on the mucosa. However, until now there is no validated in vitro cell model for identifying the respiratory chemical irritants. The aim of this study is to develop an in vitro cellular assay for identification of respiratory chemical irritants based on human 3D nasal airway epithelium (MucilAir™). Epithelia were reconstituted with primary human nasal cell pooled from 14 donors. MucilAir™ is not only morphologically and functionally differentiated; it can also remain at a homeostatic state for more than one year, allowing repeated-dose and long-term toxicity testing. 11 chemical compounds, belonging to 3 classes (irritants “H35”, highly toxic “H350”, and highly toxic chemicals through inhalation), were tested. As testing strategy, 30 μl of chemical solution were applied on the apical surface of the epithelium. In order to monitor recovery, endpoints were recorded 1, 2, 5, and 7 days post-exposure. Effect on tissue integrity was monitored by Trans-Epithelial Electrical Resistance (TEER), cell viability through LDH release, high-molecular-weight, effect on cilia motion by monitoring Cilia Beating Frequency, and morphological changes through visual inspection. Proinflammatory cytokines IL-8 and IL-6 were used as biomarkers for discriminating these molecules. Interestingly, at subtoxic doses, only the respiratory irritants upregulated reversibly the secretion of IL-8 and IL-6 upon acute challenge. As conclusion, this standardized human nasal epithelium model MucilAir™ is a promising in vitro platform for identifying the respiratory irritants, and IL-8 seems to be a reliable biomarker.

1204 Low-Molecular Weight PAH-Induced Early Signaling in Lung Epithelial Cells Involves Eicosanoids
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Low-molecular weight (LMW) polycyclic aromatic hydrocarbons (PAHs) are more prevalent in the environment and occupational settings, as well as secondhand smoke (SHS), when compared to their high-molecular-weight counterparts, such as benz[a]pyrene (B[a]P). Previously, we demonstrated that SHS-prevalent LMW PAHs activate p38-MAPK-dependent dysregulation of gap junctional intercellular communication (GJIC), which may be involved in inflammatory lung diseases. However, there is little known about the early mechanistic events leading to inflammation, specifically those mediated through lipid signaling and eicosanoids. Secondhand smoke is a complex mixture, and to model this feature in vitro we examined the effects of a binary mixture of 1-methylnaphthacene (1-MeA) and fluoranthene (Fltn) in C10 cells, a mouse, non-tumorigenic alveolar type II cell line for the ability to dysregulate GJIC (scalpel-loaded/dye transfer assay) via activated phospholipase C’s (pPLA2)) and induce cyclooxygenase-2 (COX-2), downstream prostaglandins, and pro-inflammatory mediators via immunoblotting, focused metabolomics approaches, and quantitative RT-PCR from 30 min-8 hr. Specific inhibition of pPLA2 led to significant reversal of dysregulated GJIC in response to the binary PAH mixture after only 30 min. COX-2 mRNA and protein was also significantly induced in response to these PAHs following activation of the lipases as early as 2 hr. The subsequent downstream effects were significant prostaglandin production (PGE2, and PGE1) and pro-inflammatory cytokine mRNA induction, including IL1β, IL6, CCL2, and Kc. These effects were all prior to PAH metabolism, determined via GC/MS analysis. Our results represent a mechanistic pathway of inflammatory lung disease from exposure to a more relevant real-world binary mixture of LMW PAHs through the early induction of lipid-mediated GJIC dysregulation and downstream production of eicosanoids and pro-inflammatory cytokines. Funded by R15 ES 024893-01 (AKB) and the Flight Attendant Medical Research Institute (FAMRI) CIA 130022 (AKB).

1205 In Vitro Model of the Hepatic Contribution to Lung Epithelial Cell Toxicity Induced by Ethylbenzene, Styrene, and Naphthalene
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Ethylbenzene (EB), styrene (ST), and naphthalene (NA) cause toxicity in the lung, but this is thought to require metabolic activation by cytochrome P450 monooxygenases (P450s) in either the liver or the lung. To date, we are developing an in vitro approach to determine whether hepatic bioactivation of these aromatic hydrocarbons can contribute to lung toxicity. We hypothesize that hepatic enzymes are capable of escalating the lung epithelial cell damage caused by EB, ST, and NA. Airway epithelial cells from either female mouse trachea (MuEC) or the human bronchiolar epithelial cell line, HBE1, were grown and differentiated at the air-liquid interface in transwell inserts. Cells were exposed to 5μM or 10μM EB, 5μM or 10μM ST, or 5μM to 3mM NA, in media containing female mouse liver microsomes. Liver microsomes were boiled to inactivate P450s for negative control experiments. Lung epithelial cell toxicity was
assessed by measuring cell permeability and cell density. MuEC cells exposed to 10μM EB with liver microsomes present resulted in a 50% decrease in cell density. No significant MuEC cell death occurred with 10μM EB and boiled liver microsomes. The 5μM EB exposure did not result in significant cell death for MuEC cells. MuEC exposed to ST or NA at 5μM and 10μM resulted in no significant cell death with or without active liver microsomes. For a higher dose of NA, 20μM, MuEC cell death was observed, but the extents were similar in the active and boiled microsome groups (~50% decrease in cell density). No significant cell death was detected in HBE1 cells exposed to 5μM to 3μM NA without active liver microsomes present. With the active microsomes present during exposure to 3 and 0.3μM of NA, a significant (20%) drop in HBE1 cell density was detected. Liver microsome-generated EB metabolites are more potent than liver microsome-generated NA or ST metabolites in causing toxicity in the mouse trachea epithelial cells. MuEC, which are primary mouse trachea cells, had apparently greater intrinsic ability to generate toxic NA metabolites, and were more susceptible to NA toxicity, compared to the HBE1 cells. Supported by R01 ES020867, P30 ES023513, NIOSH 2U54OH007550, and T32 HL007013.
to examine effects of DEP exposure on cell types beyond the epithelium. To this test, we developed a transwell-based organotypic airway model where bronchial epithelial cells are grown on a collagen matrix on the apical side of a porous membrane, and lung fibroblasts are grown in the basolateral compartment. We conducted exposures by adding DEP to the confluent epithelial cell layer and assayed changes in the expression of interleukin-8 (IL-8) and heme oxygenase-1 (HMOX1), markers of pro-inflammatory and oxidative stress, respectively, in both cell types. Exposure of epithelial cells in the organotypic model resulted in a 25- and 5-fold induction of HMOX1 and IL-8, respectively, which was greater than the 15- and 4-fold inductions observed in matched exposures lacking fibroblasts. Further, despite a lack of direct exposure, HMOX1 and IL-8 were induced by 3.300- and 3-fold, respectively, in fibroblasts in the basolateral compartment. Our findings indicate that toxic exposures can have effects outside of the directly exposed cells, suggesting this organotypic airway model can elucidate aspects of exposure response not well-addressed by traditional monoculture models. Utilizing this model in future studies could facilitate understanding the role that fibroblasts play in local and systemic adverse health effects of toxic exposures.

1210 Effects of Acute Exposure to an Environmental Electrophile on Human Platelet Bioenergetics

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Exposure to air pollution is a global public health problem associated with cardiovascular morbidity and mortality. Exposure to particulate matter (PM) has been reported to activate circulating platelets in vulnerable populations (patients with type 2 diabetes or coronary heart disease) within hours of exposure. 1,2-naphthoquinone (1,2-NQ) is an oxidative environmental stressor found associated with diesel exhaust particles. Given the dependence of platelet function on cellular bioenergetics, we investigated the effect of exposure to 1,2-NQ oxygen consumption rates (OCR) in human platelets using extracellular flux technology. Human platelets (HP) were isolated from venous blood collected from healthy male subjects using IRB-approved protocols. HP (30 million per well, n=3-4 per treatment group) were isolated from a blood sample collected on the day of the assay and subjected to a mitochondrial stress test using an extracellular flux analyzer (Seahorse platform) in order to assess indices of mitochondrial respiration inferred from changes in oxygen consumption rates (OCR) in response to specific inhibitors. Treatment of HP with a concentration of 1,2-NQ as low as 3 µM decreased the mitochondrial basal respiration, maximal respiration, and spare respiratory capacity in HP, while a dose of 10 µM of 1,2 NQ increased non-mitochondrial respiration, possibly associated with redox cycling, compared to vehicle-treated controls. These findings provide insights into the mechanisms through which exposure to environmental contaminants can affect cellular bioenergetics and suggest potential effects of air pollution exposure on hemostasis and cardiovascular health. This abstract of a proposed presentation does not necessarily reflect US EPA policy.

1211 Air Pollution-Mediated Alterations in CYP Enzyme Expression Dependent Upon Age and Diet in the Brains of C57B16 Wild-Type Mice

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Inhalation exposure to traffic-generated air pollutants has been reported to have deleterious effects to the central nervous system (CNS), including blood-brain barrier (BBB) disruption and neuroinflammatory, and neurodegenerative diseases. Cytochrome P450 enzymes (CYPs) are one of the main classes of biotransforming enzymes present in the body, which mediate reactions of thousands of endogenous and exogenous substrates. Altered expression of certain CYP enzymes are associated with neurodegenerative disorders. We chose to investigate the effects of inhaled traffic-generated air pollutants on the expression of these enzymes in the CNS on the BBB. Young (2 mo) or aged (18 mo) male C57B16 wild-type mice were placed on either a “Western” high-fat (21% fat by content) or low-fat diet, and subsequently exposed to either 300 µg/m³ of mixed exhaust (MVE: 250 µg/m³ PM diesel engine + 50 µg/m³ PM gasoline engine emissions) or filtered air (FA, controls) for 6 hr/day, 7 days/wk, for 50 days. Brain tissue was collected at the end of the exposure period, and prepared for either real-time RT-qPCR or double immunofluorescence, to detect variation in CYP enzyme expression in the cerebrum and/or BBB. MVE exposure resulted in significant increases in CYP2D (~1.5-fold), CYP2E1 (~2-fold) enzymes, with trending increases in CYP1B1 enzyme expression in the CNS of aged mice on a low-fat diet, while the expression of these same enzymes had the opposite trends in expression (decreased with MVE exposure) in the CNS of young C57B16 mice on a low-fat diet. There was no statistical change observed in expression of CYP1A1 in either young or old mice. In the high-fat fed C57Bl6 mice, we observed a significant decrease in CYP1B1, with trending decreases in CYP2D, CYP2E1, and CYP3A1 mRNA in the aged mice. Inverse (increasing) trends in expression in CYP2D, CYP2E1, and CYP3A1 were observed in the young mice on a high-fat diet, compared to FA controls. Thus, our preliminary findings suggest that inhalation exposure to traffic-generated pollutants alters cerebral expression of CYP enzymes in the young vs. aged differently (typically inversely), which is further complicated with concurrent consumption of a high-fat diet. Research funded by NIHES R00ES0126586 and UNT RIG Grant GA9306 (AKL).

1212 Examining the Association of Environmental Degradation and Poor Cardiovascular and/or Respiratory Health Outcomes in a Disadvantaged Community

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The objective of the study was to examine whether residential proximity to multiple environmental air pollution sources [two TRI industries (Industry 1 and Industry 2) and freeway] and individual-level risk factors (age, gender, and body mass index [BMI]) were associated with the poor cardiovascular and/or respiratory health outcomes of residents in the Southeast Community of Newport News, Virginia. Surveys were employed to collect demographies and health outcomes data of the Southeast Community residents. Geo-referencing was used to plot the locations of residents on a map for assessing the proximity to the pollution sources. Lakes Environmental Screen View™, which is a user-friendly interface for the US EPA SCREEN3, was used to predict the ground-level concentrations of pollutants released into the air from TRI reporting facilities. Exposure to air pollutants from the industries was defined by the distance between each geocoded residential address and industries. The distance of residence from the industry and freeway were grouped into two categories: <1 mile and ≥1 mile based on the observed increased predicted concentrations of pollutants measured, we considered a valuab...
2.51-fold increases in CB exposure group and recovery group, compared with the control. The comet assay showed that nanoscale CB particles could induce DNA damage significantly in lung cells of rats. The levels of interleukin-6, interleukin-8, interleukin-17, and tumor necrosis factor alpha in lung tissue but not in serum were significantly increased in CB exposure group compared with the control. The cell counts of white blood cell, monocytes, and neutrophils had 1.72-, 3.13-, and 2.73-fold increases in CB exposure group compared with the control. The percentage of cluster of differentiation (CD) 4+ lymphocytes and the rates of CD4+/CD8+ in CB exposure group were statistically increased, compared with the control. The stimulation indexes of the peripheral blood cell, monocytes, and neutrophils had 1.72-, 3.13-, and 2.73-fold increases after CB treatment compared with the control. CD4+/CD8+ in CB exposure group were statistically increased, compared with the control. The peripheral blood cell, monocytes, and neutrophils had 1.72-, 3.13-, and 2.73-fold increases after CB exposure for 90d, thymus and spleen displayed slightly morphologic changes. The early apoptotic thymocytes had a 2.36-fold increases after CB treatment compared with the control. CB induces the pulmonary toxicity, which includes the localized effect and the non-targeted adverse cardiovascular response of exposure to CB. Both directly and systemically, immune responses are interlinked, and thus toxicity can be considered as a combined effect of them.

**1214 Low Vitamin D Levels Are Associated with Increased Cardiovascular Responses following Diesel Exposures in Healthy Subjects**

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Cardiovascular disease accounts for over 17 million deaths per year. There is a large body of evidence suggesting that vitamin D deficiency is associated with cardiovascular disease after risk factors. Vitamin D deficiency might be associated with elevated cardiovascular responses in healthy people. Before, after, and 18 hrs following each exposure, blood samples were collected. Nine participants were males (69%), the average age of the participants was 27 years, and the majority of the participants were white (62%). The average vitamin D concentration before clean air exposures was 22.3 ng/mL, and the average vitamin D concentration before diesel exposures was 23.4 ng/mL (paired t-test; p = 0.44). Four participants were considered vitamin D deficient (vitamin D < 20 ng/mL), 7 participants had inadequate vitamin D levels (vitamin D between 21-29 ng/mL), and 2 were vitamin D sufficient (vitamin D > 30 ng/mL). Positive and significant associations were observed between baseline vitamin D concentrations and tPA (β = 6.93, 95% CI = -0.30, 13.57) and IL-4 (β = 0.01, 95% CI = 0.00, 0.01), while a negative and significant association was found with plasminogen (β = 2.84, 95% CI = 0.07, 0.61). At 0 hrs post- exposure, there were significant negative associations between baseline vitamin D concentrations and D-dimer (β = 0.02, 95% CI = -0.04, 0.00) and IL-8 (β = -0.03, 95% CI = -0.06, 0.00). Additionally, at 18 hrs post-exposure negative associations were found between baseline vitamin D concentrations and PAI-1 (β = -0.03, 95% CI = -0.07, 0.01) and TNF-α (β = -0.03, 95% CI = -0.06, 0.00). This suggests vitamin D deficiency might be associated with elevated cardiovascular responses in this cohort.

**1215 Effects of Asian Dust on Respiratory Symptoms in Children in Nagasaki and Fukuoka**

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Asian dust (AD) originates from inland China and is carried to Japan by the Westerlies, and anxiety over its effect on human health has been expressed. We evaluated the potential effects of AD on respiratory symptoms and allergy rates in children with and without bronchial asthma in Nagasaki and Fukuoka. In Nagasaki, 110 children with bronchial asthma under outpatient care at 4 hospitals were investigated annually in March-May from 2014 to 2016. In Fukuoka, 29 children with bronchial asthma receiving outpatient care at 6 hospitals and 137 children at 2 elementary schools were surveyed in April-June 2013. The peak expiratory flow (PEF) values measured daily in the morning and evening, information collected from diaries and medical interview forms were analyzed. The exposure to AD was quantified as the values indicated by the light detection and ranging system. In Nagasaki, no association was observed between AD and respiratory symptoms (including asthma attacks) in children with asthma. In Fukuoka, also, no decrease in PEF due to AD was noted. The results concerning the effects on the health of children with asthma were consistent between Nagasaki and Fukuoka. Many of the asthmatic children in this study used anti-asthmatic drugs over a long period, and so the symptoms may have been controlled adequately. Therefore, when the analysis was restricted to the asthmatic children not on long-term anti-asthmatic medication, a decrease (about 1%) in PEF associated with AD was observed. In children without asthma, also, a decrease in PEF (about 1%) coinciding with the arrival of AD was observed. The views expressed in this article are those of the authors and do not necessarily reflect those of the Ministry of the Environment, Japan.

**1216 Diesel Exhaust Particles Downregulate PI3K/Akt/mTOR Signaling and Mitochondrial Bioenergetics in a Novel Organotypic Model of the Airway Microenvironment**

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The pathophysiological importance of oxidative stress generation and induction of cytokines in diesel exhaust particle (DEP)-mediated inflammatory responses in fibroblasts, play a key role in maintaining tissue health and have the potential to play a central role in mediating bioenergetic and inflammatory endpoints following chemical exposure. Utilizing a novel 3D organotypic model of the human airway, we investigated whether DEP disrupts pulmonary and metabolic homeostasis in the airway microenvironment by impacting normal fibroblast functions through generation of oxidative stress and activation of the p38 MAPK pathway, a central mediator of cellular function. Confluent monolayers of bronchial epithelial HBEC grown on a collagen substrate in permeable Transwell inserts were exposed to DEP while healthy fibroblasts were present in the adjacent basolateral compartment. Here we show that an in vivo-like DEP exposure of airway epithelial cells strongly enhances phosphorylation of p38 as well as upstream MKK3/6 and downstream effectors MAPKAPK2, HSP27, and p35 in adjacent fibroblasts. Activation of the p38 pathway in fibroblasts was accompanied with the induction of the pro-inflammatory and oxidative stress genes interleukin-8 (IL-8) and heme oxygenase 1 (HMOX-1), respectively, which were attenuated by treatment with LY222820, an inhibitor of p38 kinase activity. We also examined the potential crossstalk between MAP kinases and the PI3K/Akt/mTOR pathway, known for regulating protein synthesis, bioenergetic, and tissue regeneration. DEP exposure decreased phosphorylation of PI3K, Akt, and mTOR in the fibroblasts while increasing phosphorylation of cell cycle regulator p27kip1 and Rb. Maximal mitochondrial respiratory rate was reduced in fibroblasts; however, these changes were not associated with alterations in gross morphology or cytotoxicity.
Silicosis is an inflammatory and fibrotic pulmonary disease caused by inhalation of silica particles. Lung macrophage plays a key role in the development and progression of inflammation and fibrosis process caused by silica particles. Bixin is a compound that is widely used as a natural food additive. And our previous studies have demonstrated that bixin could stimulate Nrf2 signaling pathway and alleviate inflammation in ventilation-induced acute lung injury as well as UV exposure-caused skin damage. To date, the mechanism of bixin in protecting silica-induced lung inflammation and fibrosis has not yet been studied. In this study, a murine silicosis model was established through silica intratracheal instillation. To elucidate the effects and mechanisms of bixin in silica-induced pulmonary inflammation and fibrosis, we treated the mice with bixin following silica instillation. Our results indicated that bixin treatment attenuated the accumulation of inflammatory cells, which significantly ameliorated pathological inflammation and fibrotic development in the lung. In addition, intraperitoneal (i.p.) injection of bixin in mice led to upregulation of Nrf2 response in the lung. The mechanism was further studied in the in vitro THP-1 cell model, where we showed that bixin activated Nrf2 signaling pathway via blocking KEAP1-mediated ubiquitination and degradation of Nrf2. Our work has brought insights into exploring anti-silicosis activities in daily consumption of natural products. In addition, our study also inspires the discovery of new beneficial effects of bixin and its application in the treatment of other inflammatory disease.

Air pollution has become a major factor concerning life expectancy and well-being. WHO estimated that 92% of the world population is affected by air pollutant concentrations that are above WHO air quality limits. An important factor in air pollution is particulate matter (PM), which has been focused heavily in many studies but often neglecting PM below 100 nm. We measured a non-road diesel engine emission effect simultaneously on A549 alveolar epithelial cell line in an air-liquid interface (ALI) exposure system and on a mouse inhalation exposure unit. Our ALI uses thermophoresis to deposit PM into cell. We used A549 that has been cultivated in inserts for 72 hours in 5% CO2 atmosphere and at 37°C. 24 h before exposure cells are allowed to form ALI Exposure was done in cells 1 h at 100% humidity with serum-free cell culture medium containing HEPES buffer. Instantaneously after exposure, medium was collected for cytotoxicity assays and cells were allowed to recover 24 h in fresh medium before further analysis. Mice were exposed in inhalation exposure chamber with 30% humidity. Control mice were at laminar airflow in an animal transfer station. Exposures for mice lasted for 4 h per day and were done for three consecutive days. Mice were euthanized and bronchoalveolar lavage fluid (BALF) were collected for analysis. A549 cells and mice BALF were analyzed with cell vitality using Dapi coloring, inflammatory response, and comet assay. Cell vitality staining showed no viability decrease in ALI samples. Whereas mice BALF cells showed significant decrease in vitality in exposed mice compared to unexposed. Inflammatory effect was at a significant level in A549 medium, but in BALF and mice serum the effect was mild. Comet assay showed that diesel emissions caused significant DNA fragmentation on A549 cells and in BALF when compared to controls. These results indicate that parallel ALI and animal inhalation exposure methods complement each other when toxicity of diesel engine emissions is assessed.

Exposure to particulate matter (PM), including mineral particles as well as organic particles/components may be linked to respiratory diseases, including asthma. This study assessed co-exposure effects of lipopolysaccharide (LPS) or Aspergillus fumigatus hyphae fragments (AFH) with mineral particle quartz (Min-U-Sil). The pro-inflammatory properties of these particles both individually and upon co-exposure were examined in human THP-1 monocytes (THP-1 Mo) and phorbol 12-myristate 13-acetate (PMA)-differentiated THP-1 (THP-1 Macrophages; THP-1 Ma). Combined effects of AFH and Min-U-Sil were further investigated in two primary macrophage models; human airway macrophages (AM)/sputum macrophages and peripheral human blood monocyte-derived macrophages (MDM), and in one human bronchial epithelial cell line (BEAS-2B). Exposures of either LPS and AFH alone increased the release of the pro-inflammatory cytokines interleukin-1 beta (IL-1β) and tumor necrosis factor alpha (TNF-α) in THP-1 Mo and THP-1 Ma at low doses, while Min-U-Sil induced IL-1β release only in THP-1 Ma. LPS or AFH co-exposed with Min-U-Sil showed both an enhanced/synergistic effect on IL-1β release from THP-1 Mo and THP-1 Ma. In contrast, the effect on TNF-α release was unchanged. There was no correspondingly increased mRNA expression of pro-IL-1β upon combined exposure. The AFH co-exposed with Min-U-Sil showed similar profound synergistic effects in AM, MDM, and BEAS-2B. The repression of mRNA expression of pro-inflammatory cytokines exposed to organic components, such as bacterial and fungal components, are more sensitive and give a stronger response to air pollution particles such as mineral particles when compared to unexposed cell. The synergistic response was caused by an enhanced release of IL-1β due to Min-U-Sil-induced activation of the inflammatory, and subsequent cleavage of LPS/AFH-induced pro-IL-1β to IL-1β.

Supporting a prominent functional role for fibroblasts in maintaining airway fibroblasts induces mitogenic crosstalk and overall downregulation of nutrient response, protein translation, and cell cycle progression, a pro-apoptotic ER stress response gene, were greater among donor with the I585I/V genotype (n=6) versus wild-type (I585I/I) donors with worse asthma symptom control. It was found that TRPA1 mRNA was expressed at higher levels among two donor samples of primary human lung epithelial cells with the I585V polymorphism of TRPV1 affected asthma symptom control. Specifically, the I585V variant was less responsive to the particle agonist coal fly ash, but heterozygosity for TRPV1 I585V was uniquely associated with worse asthma symptom control. It was found that TRPA1 mRNA was expressed at higher levels among two donor samples of primary human lung epithelial cells with the I585V/g genotype, which correlated with differences in IL-8 gene expression and secretion compared to wild-type expressing cells treated with coal fly ash (n=2). This study expanded upon these initial results and showed that the TRPA1 I585V/g genotype both increases the relative levels of TRPA1 expression as well as the relative sensitivity of primary human lung epithelial cells to diesel exhaust particles. Specifically, increases in the expression of mRNA for IL-8 and growth arrest and DNA damage-inducible transcript-3 (DDIT3), a pro-apoptotic ER stress response gene, were greater among donor cells with the I585V/g genotype (n=6) versus wild-type (I585I/I) donors (n=7). This work implies that the expression of TRPA1 in lung epithelial cells may contribute to the observed differences in asthma symptom control among subjects with the TRPA1 I585V/g genotype. Supported by ES017431 and GM121648.
Coal Fly Ash (CFA) is a byproduct of burning coal. It is found in building materials and is present in particulate air pollution. Exposure to CFA has adverse effects on human health. Transient receptor potential melastatin-8 (TRPM8) is activated by the chemical agonist menthol and also by CFA. CFA stimulates pro-inflammatory cytokine production by human primary bronchial epithelial cells via activation of TRPM8, but this finding was not replicated in mouse lungs. We constructed HEK-293 cells stably overexpressing human TRPM8, mouse TrpM8, and rat TrpM8. All three cell lines responded to the prototypical chemical agonist menthol. However, neither the mouse nor the rat TrpM8 overexpressing cells responded to CFA or a similar particle agonist, calcium oxide (CaO) nanoparticles. Reduced responses to CFA and CaO was also observed in cells stably overexpressing chicken and X. tropicalis TrpM8. Amino acid sequence alignment of human, mouse, rat, chicken, and X. tropicalis TrpM8 indicated several potentially important differences in the amino acid sequence of the extracellular pore-loop domain, which is important for TRPM8 tetramer formation and activation of human TRPV1 by CFA. We hypothesized that specific amino acids in the pore-helix region would regulate the species-specific activation of TRPM8 by CFA and related calcium-rich particles. Mutagenesis of amino acids of the human and mouse TRPM8 channels revealed that specific residues of the pore-loop domain did indeed determine species-specific activation. Mouse serine 927 and human threonine 932 were identified as important residues in the response of TRPM8 to CFA. Our results provide new insights on how particles activate human TRPM8 and provide clues for how insoluble particles may activate other TRP channels. This will aid in the development of better models for studying particle effects such as health risks associated with CFA or similar materials. Support: ES017431.

First responders and Lower Manhattan residents exposed to the World Trade Center dust (WTC dust) have been plagued by upper and lower respiratory ailments. Using anti-inflammatory drugs, this study explored the molecular pathways involved in these WTC dust-induced pulmonary ailments. WTC dust (63µg) suspended in saline was intranasally instilled in male C57BL/6 mice. Immediately post-exposure, mice were injected intraperitoneally with saline, dexamethasone (0.1mg/kg), or Drug-X (5mg/kg). Drug X is a quinolone antibiotic, which was used to treat urinary tract infections and chancroid. Drug X was found to be a highly selective IRAK1 kinase inhibitor with low cytotoxicity. New indications of Drug X have been identified for treating inflammation. Left lungs were lavaged 24 hr post-exposure for evaluation of inflammation (PMN number), and right lungs were used for RNA sequencing analysis. WTC dust-exposed mice had increased PMN infiltration (17.4±1.7%), and increased inflammatory IL1α, IL12, IL10, IL12, IL17A, and TNFα. Dexamethasone exposed mice had increased PMN infiltration (17.4±1.7%), and increased inflammatory IL1α, IL12, IL10, IL12, IL17A, and TNFα. Dexamethasone and Drug X decreased PMN in the lungs by 22 and 42%, respectively. Using a Poisson regression model, Drug X was 26% more effective in reducing WTC-induced lung inflammation (95% CI: 3.43%; p<0.001). Preliminary dynamic compound-target-pathway network analysis revealed that WTC lung inflammation most likely involved CDC-like kinase 1 (Ck1, involved in pre-mRNA processing), and the cell cycle-regulated proteins cyclin-dependent kinase 1 (Cdk1) and aurora kinase B (Aurkb). In addition, serine/threonine-protein kinase Chk1 (Chek1) and Mgc proto-oncogene protein (Myc) were also affected. Thus, WTC dust exposure may enhance cell proliferation and potentially cause lung cancer, and may help to explain the higher rate of adverse respiratory health effects being observed many years after the WTC dust exposure.
Large cities in the world are known to have many sources of air pollution and thus elevated pollutant levels. Although previous studies have looked at health effects of rapid changes in air pollution on residents, no studies have looked into the impact on the respiratory and cardiovascular systems when individuals travel abroad to cities with air pollution levels different from their home city. Travelers are exposed to varying levels of air pollution in a new environment within minutes to hours, which may lead to adverse health outcomes. This study’s goal was to examine the impact on the respiratory and cardiovascular systems due to a change in the environment when traveling to a city in another country, with a main focus on changing air pollution levels. The study recruited subjects traveling from NYC to cities abroad including London, Beijing, Shanghai, Prague, and Mexico City. Participants were pre-trained to measure lung function, blood pressure, and heart rate for 1 week before departure, during the stay, and after returning from the city abroad. Participants also carried an “Airbeam,” a portable, low-cost PM2.5 monitoring instrument, to measure personal exposure levels. The Airbeams were tested against a PDR DataRam 1500 monitor and the standard gravimetric filter method for comparison and calibration. Results from the pilot study have shown that overall, subjects who traveled to cities that were more polluted than NYC had a significant decrease in lung function as compared to their pre-travel values, whereas there was no significant difference in subjects who traveled to “clean” cities (total n=20). The maximum mean reduction in FEV1 and PEF for subjects traveling to “polluted” cities were 8% and 20%, respectively, when compared with NYC means. Systolic blood pressure levels were significantly increased in some subjects who traveled to cities whose PM levels were significantly higher than NYC. Levels in the travel abroad city were correlated with changes in lung function. The Airbeam low-cost sensors showed inter-instrument variability and required calibration of individual units. The results of this pilot study show that travel to cities with significantly higher pollution than one’s home city can have acute impacts on the cardiopulmonary system and support a concern for the potential for adverse health effects due to air pollution while traveling abroad.

Inhalation Exposure to Traffic-Generated Pollutants Results in Increased Plasma Angiotensin II and Angiotensin II Type 1 Receptor Expression in the Vasculature, Kidneys, and Adipocytes of C57Bl/6 Wildtype Mice

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Multiple studies in the literature have revealed a clear association between exposure to traffic-generated air pollution and exacerbation of cardiovascular disease (CVD). More recently, a few studies have shown that obesity exacerbates the association between chronic exposure to air pollution and prevalence of CVD. Additionally, exposure to traffic-generated pollution has been shown to induce or exacerbate obesity in both children and adults. To date, very limited information exists on the effects of inhaled pollutants on adipocytes/adipocyte signaling. The renin-angiotensin system (RAS), when dysregulated, is known to mediate pathogenesis in the renal and cardiovascular system, as well as in adipocytes, primarily through angiotensin (Ang) II signaling via the Ang II Type 1 (AT1) receptor. Thus, we hypothesize that MVE-exposure in adipocytes, primarily through angiotensin (Ang) II signaling via the AT1 receptor, results in altered RAS signaling and altered AT1 receptor expression. Ang II Type 1 (AT1) receptor. Thus, we hypothesize that MVE-exposure in adipocytes, primarily through angiotensin (Ang) II signaling via the AT1 receptor, results in altered RAS signaling and altered AT1 receptor expression. Therefore, we investigated the effects of inhaled ubiquitous air pollutants—wood smoke (WS) or mixed diesel and gasoline vehicle exhaust (MVE)—on alterations in inflammation, and on microbiota profiles in the intestine of atherosclerotic apolipoprotein E knockout (ApoE−/−) mice. Male 8-week-old ApoE−/− mice on a high-fat diet were exposed to either MVE (300 µg/m³ PM: 250 µg/m³ PM diesel engine + 50 µg/m³ PM gasoline engine emissions), WS (~450 µg/m³ PM), or filtered air (FA) for 6 hr/d, 7d/wk, for 50 d. RT-qPCR was used to quantitate expression of inflammatory factors tumor necrosis factor (TNF)-α and interleukin (IL)-1β in the intestines. Plasma cortisol levels were assessed via ELISA from ApoE−/− mice on a separate study that saw the same diet and MVE exposure conditions/durations, which was statistically increased in MVE-exposed mice, compared to FA controls. Microbial profiling of the intestine was done using Illumina 16S sequencing of V4 16S rRNA PCR amplicons. We observed a significant increase in TLR-4 mRNA in the intestines of MVE and WS-exposed (1.5-fold and 2.5 fold, respectively), and IL-1β mRNA expression (~1.4-fold induction for both exposures), while only WS exposure resulted in significantly increased TNF-α mRNA expression (~1.5-fold), compared to FA controls. Both WS and MVE-exposure resulted in decreased intestinal bacterial diversity, as well as alterations in microbiota profiles, including the Bacteroidites:Firmicutes ratio at the phyla level. Our findings show inhalation exposure to either MVE or WS have degrading effects on mouse mucosal integrity while altering microbiota profiles and diversity, which are associated with local and systemic inflammation. Supported by UNT RIG Grant GA9306 (AKL) for the analysis assays and NIH/NIEHS Grant ES014639 (MJC) for the mouse exposures/tissue.

Characterization of the Murine Alveolar Macrophage Response to In Vitro Ozone Exposure

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Exposure to ambient ozone is associated with numerous adverse health effects including airway inflammation, decreased respiratory function, and exacerbation of existing respiratory disease. Controlled exposure studies in humans have established that the response to ozone is characterized by large inter-individual and minimal intra-individual variation, suggestive of gene-environment interactions; however, the molecular basis of this variation is incompletely understood. Alveolar macrophages (AMs), a critical resident immune cell, are known to mediate aspects of ozone response. Though previous studies have extensively characterized the mouse strain-dependence of ozone response, little has been done to investigate how natural genetic variation may modify these responses through the modulation of AM function. Hence, we sought to develop an in vitro system to facilitate an analysis of the AM contribution to strain-specific ozone responses. More specifically, we developed a platform to perform an unbiased, genome-wide survey of the transcriptional responses induced by ozone in AMs. We obtained primary AMs from BALB/cJ mice, cultured them at air-liquid interface, and then exposed them to ozone (0.4 ppm x 4 h) or filtered air alone. Twenty-four hours after exposure, media and RNA were collected for cytokine measurements and whole-transcriptome RNA-seq, respectively. We detected increased MIP-1α at the protein level, and increased TNF expression at the mRNA level. Future studies will extend this approach to AMs from Collaborative Cross, a new mouse genetic reference population, in order to assess the contributions of AM to gene-environment interaction, and we will also compare the effect of in vitro vs. in vivo exposure.
Lipid Hydroperoxides as Mediators of Ozone-Induced Oxidative Changes in Human Airway Epithelial Cells

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Despite strict regulatory measures, millions of Americans are exposed to concentrations of tropospheric ozone (O3) that are associated with an increasingly broad range of health effects. The respiratory tract is the main target of ozone exposure, causing pulmonary function decrements and airway inflammation, which are thought to occur through oxidative mechanisms. While it is known that ozone oxidizes carbon-carbon double bonds in polyunsaturated fatty acids to produce lipid hydroperoxides, hydrogen peroxide (H2O2), and various aldehydes, the role of these intermediates in downstream responses is still not well understood. Previous studies have observed an increase in the glutathione redox potential (EGSH), a marker of intracellular oxidative state, in human airway epithelial cells (HAEC) exposed to ozone. In this study we investigated the role of lipid hydroperoxides as a key mediator in ozone-induced oxidative responses in HAEC. We hypothesized that lipid hydroperoxides contribute to increases in the glutathione redox potential (EGSH) during ozone exposure. We utilized live-cell confocal imaging and the fluorogenic sensor roGFP to monitor EGSH in BEAS-2B cells exposed to the lipid hydroperoxide HpODE in real time. Exposure to HpODE induced a rapid, dose-dependent increase in EGSH. Overexpression of catalase was effective in blunting EGSH changes induced by H2O2, but not those induced by HpODE. shRNA-mediated knock-down of glutathione peroxidase 4 (GPx4), the primary enzyme responsible for reduction of lipid hydroperoxides, suppressed the HpODE-induced EGSH changes in HAEC, indicating this enzyme is critical in reducing lipid hydroperoxides at the expense of reduced glutathione. Taken together, these results suggest that lipid hydroperoxides contribute to redox changes induced by ozone exposure of HAEC. This abstract of a proposed presentation does not necessarily represent US EPA policy.

Bittersweet: Real-Time, Dynamic Changes in Blood Glucose Levels during an Acute Ozone Exposure in Rats

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In humans and rats, acute exposures to ozone have been shown to activate the sympathetic-adrenal-medullary and hypothalamic-pituitary-adrenal axes to induce multi-organ metabolic alterations, including impaired glucose homeostasis. These findings have largely been gleaned from one-time snapshots of responses measured after exposure. The complexity of such responses, however, requires a multidimensional view of in-life, dynamic biomolecular changes across time for accurate elucidation of etiology. The purpose of this study was to use new radio-telemetry technology capable of providing real-time quantitative glucose and core body temperature data to assess the impacts of ozone exposure across time and exposure level. We hypothesized that ozone exposure will impair normal circadian glucose rhythms in a concentration-dependent manner. Male, 13-week-old Wistar-Kyoto rats (n=8), implanted with telemeters capable of monitoring glucose and body temperature in conscious and unrestrained rats, were exposed to 0.0, 0.2, 0.4, and 0.8 ppm ozone for 4h/day, 1 day/week for 4 consecutive weeks. A crossover design was used wherein all 8 implanted animals were exposed to each concentration with continuous monitoring of blood glucose levels, core body temperature, and locomotor activity prior to, during, and following ozone exposure for the entire 4-week period. Exposure to 0.8 ppm ozone caused a precipitous increase in blood glucose levels as core body temperature simultaneously decreased, approximately 1.5h after the beginning of exposure. Glucose tolerance testing performed immediately after exposure to filtered air and 0.8 ppm ozone further revealed ozone-induced glucose tolerance. These data for the first time demonstrate the real-time temporal dynamics of ozone-induced hyperglycemia and glucose intolerance, which can be more reliably used to establish biological plausibility for epidemiologic findings and develop more predictive computational models of the biological effects of environmental stressors. This abstract does not reflect US EPA policy.

Ultrafine PM and O3 Act Synergistically to Induce Airway Injury in Aged Rats with Cardiovascular Disease


Elderly individuals with cardiovascular disease (CVD) have increased risk for adverse events precipitated by air pollutant exposure. In order to model this effect, the present investigation examined the interaction of ultrafine particulate matter (UFPm) and ozone (O3) in aged normotensive and hypertensive rats. Normal (NW) and spontaneously hypertensive (SH) aged (10-14 mos) male Wistar-Kyoto rats (350-700 g) were used in this study. Four exposure regimens included (1) filtered air (FA); (2) 1.0 ppm O3; (3) 250-300 μg/m³ UFPm, and (4) 1.0 ppm O3 and 250-300 μg/m³ UFPm (UFPm+ O3). Flame-generated particles (mean aerodynamic diameter = 74,0 nm) utilized were described previously (Lee et al., J Appl Physiol [1985], 2010 Oct;109[4]:1115-24). The experimental protocol was (1) 60-minute control period, (2) 6-hour exposure period, and (3) 8-hour recovery period. Rats were then euthanized and lungs were formalin fixed by intratracheal instillation. Standard, paraffin-embedded, hematoxylin, and eosin histopathology sections were evaluated for large airway epithelial loss (EL), epithelial necrosis (EN), and exudate (Ex) in the terminal bronchioles, macrophage infiltration (MOI), edema (Ed), and neutrophils (N) in the alveolar ducts and intravascular fibrin-neutrophil complexes (IVFNc) in parenchymal vessels in a blind fashion by a board-certified pathologist using a semiquantitative grading scale (0-5). Data were analyzed using a Kruskal-Wallis test with Dunn-Bonferroni post-hoc comparisons. Within NW groups there were significant (p ≤ 0.001) effects of UFPm+ O3 compared to FA for all pathology endpoints except for IVFNc. Within SH groups there were significance effects of UFPm+ O3 compared to FA for all pathology endpoints (p ≤ 0.001) except for IVFNc. There were significant differences between NW and SH strains for EN in the O3 exposure group (p = 0.04) and IVFNc in the UFPm+ O3 exposure group (p = 0.006). There was a synergistic interaction between UFPm and O3 exposure in both strains. The SH group was uniquely susceptible to intravascular inflammation with UFPm+ O3. The increased presence of IVFNcs in the SH group is consistent with increased thrombus formation and increased cardiovascular risk in an aged population with preexisting CVD.
Air pollutant exposure has been associated with excess cardiac mortality in elderly individuals with cardiovascular disease (CVD). In order to model this effect, the present investigation examined the synergistic interactions of ultrafine particulate matter (UFPM) and ozone (O3) in aged normotensive and hypertensive rats. Normal (NW) and spontaneously hypertensive (SH) aged (10-14 mos) male Wistar-Kyoto rats (350-700 g) were used in this study. Four exposure regimens included: (1) filtered air (FA); (2) 1.0 ppm O3; (3) 250-300 μg/m³ UFPM, and (4) 1.0 ppm O3 and 250-300 μg/m³ UFPM (UFPM+O3). Flame-generated particles (mean aerodynamic diameter = 74.0 nm) utilized were described previously (Lee et al., J Appl Physiol. 2010;109(4):1115-24). At least two weeks prior to exposure, rats were surgically implanted with telemetry units. Experimental protocol: (1) 60-minute control period, (2) 6-hour exposure period, and (3) 8-hour recovery period. Rats were then euthanized and hearts formalin fixed. Standard, paraffin-embedded, hemoxylin and eosin histopathology sections were evaluated for organized necrosis (ON), acute cellular necrosis (ACN), and hypercontractility (HC) in a blind fashion by a board certified pathologist using a semiquantitative grading scale (0-5). Data were analyzed using a Kruskal-Wallis test with Dunn-Bonferroni post-hoc comparisons. Heart rate variability (HRV) was evaluated using time and frequency domain measures. Data were analyzed using mixed model three-way analysis of variance with repeated measures followed by Bonferroni post-hoc comparisons. UFPM+O3 induced significant ON, ACN and HC in SH rats compared to FA. Within the NW groups there were significant effects of UFPM+O3 compared to FA for HRV parameters. Within the SH groups there were significant effects of O2 and UFPM+O3 compared to FA and between the NW and SH strains with O3 for HRV parameters. UFPM+O3 and PM alone induced myocardial injury in rats with CVD. There was a synergistic interaction between UFPM and O3 exposure on HRV in the NW strain. The myocardial injury and HRV responses to oxidant stress in the SH rats indicates that CVD results in increased susceptibility to air pollutants.
1237 Long-Acting β2 Adrenergic Agonists Potentiate Ozone-Induced Lung Injury and Inflammation
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Ozone (O3), a ubiquitous air pollutant, disproportionally affects asthmatics. We have shown (1) that O3-induced lung injury and inflammation are associated with increased circulating epinephrine and corticosterone, and (2) that antagonists of β-adrenergic receptors (BAR) and glucocorticoid receptors (GR) attenuate many of the O3-induced effects. Agonists of both BAR and GR are used to reduce asthma-associated bronchoconstriction and inflammation, respectively. We hypothesized that treatment of healthy rats with long-acting β2 agonist (LABA; clenbuterol; CLEN; and GR agonist (dexamethasone; DEX)) would further increase O3-induced changes in lymphoid tissues, and exacerbate pulmonary vascular leakage and inflammation. Male 12-week-old Wistar-Kyoto rats were injected daily with saline, CLEN (0.004 [low dose] or 0.02 [high dose]) mg/kg, i.p., or DEX (0.02 [low dose] or 0.1 [high dose]) mg/kg, i.p.. Dual therapy was often prescribed in patients, a subset of rats received both drugs (CLEN+DEX; at 0.005 [low dose] or 0.02 [high dose]) mg/kg, i.p.). Drug treatment began one day prior to and each day before start of air or 0.8 ppm O3 exposure, 4hr/day x 2 days. O3, DEX, and CLEN+DEX, but not CLEN alone, reduced thymus and spleen weights and circulating lymphocyte numbers. However, drug treatmentalone had no significant pulmonary effects in air-exposed rats. High dose CLEN exacerbated O3-induced pulmonary protein leakage and neutrophil influx in bronchoalveolar lavage fluid (~4-fold), whereas DEX-alone did not modify O3-induced lung injury or inflammation. When both CLEN+DEX were administered, O3-induced injury and inflammation were increased only ~2-fold. In conclusion, our results show that systemic administration of DEX resulted in depletion of lymphoid tissues and circulating lymphocytes, not unlike that induced by O3 exposure, but DEX treatment did not modify O3-induced pulmonary protein leakage or neutrophil influx. However, LABA monotherapy potentiated O3-induced pulmonary protein leakage and inflammation in rats. When dual therapy was used, this potentiation was reduced to nearly half. By extension, it is possible that those individuals receiving LABA or a combination of LABA and steroids may be more sensitive to air pollution-induced pulmonary vascular leakage and inflammation. This abstract does not reflect US EPA policy.

1238 Scavenger Receptor B-I Mitigates Ozone-Induced Pulmonary Inflammation through Efferocytosis
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Ozone (O3) is a criteria air pollutant known to increase lung injury and exacerbate pulmonary diseases. After injury, the tissue resolution in the lung is driven in part by macrophage efferocytosis to restore homeostasis. Efferocytosis is the phagocytosis of apoptotic cells, which prevents secondary necrosis and upregulates anti-inflammatory cytokine production. Scavenger Receptor B-I (SR-BI) is a class B scavenger receptor that mediates macrophage (Mφ) efferocytosis through Src/PI3K/Rac1 activation in multiple models of cardiovascular disease. Currently, it is unknown if O3 exposure impairs efferocytosis and whether SR-BI is critical in mediating pulmonary efferocytosis. We hypothesize that O3 exposure induces efferocytosis and SR-BI attenuates the inflammatory effects of O3 on the lungs by mediating efferocytosis through the Src/PI3K/Rac1 pathway. To assess the pulmonary effects of SR-BI on O3 exposure, we exposed C57Bl/6J (WT) male mice and SR-BI-/- mice to filtered air (FA) or 1 ppm O3. Mice were necropsied 24 hrs post exposure, lungs were lavaged for bronchoalveolar fluid (BAL) to analyze protein, cytokines, and immune cell infiltration. To evaluate the effects of SR-BI on efferocytosis following O3 exposure, WT or SR-BI-/- mice were exposed to FA or 1 ppm O3. Apoptotic thymocytes were instilled oropharyngeally (o.p.) 24 hrs post exposure. The phagocyte index (PI) was calculated by counting the number of BAL Mφs that phagocytosed thymocytes compared to the number of Mφs without apoptotic cell uptake. O3 exposure in SR-BI-/- mice increased BAL GR+ cells, IL-6, G-CSF, MIP-2, KC, and neutrophil cell counts in the BAL compared to the WT mice 24 hrs post exposure. Exposure to O3 impaired efferocytosis by displaying a decreased PI compared to the FA control 24 hrs post exposure. SR-BI-/- mice displayed further impairment of efferocytosis when compared to WT controls. SR-BI protects against the pro-inflammatory effects of O3 exposure and mediates pulmonary Mφ efferocytosis of apoptotic cells. Data from this project will bridge the knowledge gap about the harmful effects of O3 inhalation and how SR-BI mitigates pulmonary inflammation. Future studies will define the molecular mechanisms by which SR-BI mediates efferocytosis.

1239 Alveolar Macrophages but Not Exudative Macrophages Are Increased following Acute Ozone Challenge
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Ozone (O3) alters the pulmonary immune system, which is important to subsequent lung injury development and immune responses. Pulmonary macrophages are essential to these processes in the lung as either alveolar, interstitial, or inflammatory monocyte-derived (also known as exudative) populations. Clear identification of pulmonary macrophages is, therefore, critical to understanding their unique functional roles. To perform this, we used a recently developed flow cytometry panel and characterized the macrophage cell populations following O3 exposure. We exposed 10-12-week-old male and female C57BL/6J mice to 2ppm O3 or filtered air (FA) for 3h. At 24, 48, and 72h following exposure, the mice underwent tissue harvest to obtain single-cell solutions. Cells were stained and flow cytometry was performed using a BD LSRII. The data were analyzed using Flojo software. Flow cytometry of lung tissue following O3 identified all the major immune cells in a single reaction. Macrophages were defined as alveolar (CD45+ Ly6G+ Ly6C+ CD44+ CD11b+ Siglec F-), interstitial (CD45+ Ly6G+ Ly6C+ CD44+ CD11b+ Siglec F+) or exudative (CD45+ Ly6G- Ly6C- CD11b+ Siglec F+). At 24h post-O3 exposure as a percentage of live CD45+, Ly6-G (non-PMNs) cells, there were increased alveolar and interstitial macrophages in male and female mice without evidence of exudative macrophages. At 48 and 72h following exposure, alveolar macrophages continued to be elevated in the male and female mice, though the differences between FA and O3 were greater in the female mice. Intersitial macrophages were non-statistically elevated at 48h and had returned to baseline by 72h post-exposure. No significant evidence of exudative macrophages presence was evident at either of these time points. Alveolar and interstitial macrophages increased following O3 exposure without significant contribution of inflammatory monoocyte-derived exudative macrophages. This supports focus on these specific macrophage populations as potential modulators of O3 responses. Furthermore, this highlights the potential of detailed flow cytometry characterization of the pulmonary immune cell compartment as a tool for evaluating toxicant responses.

1240 Suppression of Ozone-Induced Macrophage Activation and Oxidative Stress by Valproic Acid
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Ozone is a ubiquitous urban air pollutant known to damage the lung, causing alveolar epithelial barrier dysfunction, inflammation, and oxidative stress. This is associated with an accumulation of proinflammatory/cytotoxic and anti-inflammatory/wound repair macrophages in the lung, which have been implicated in the pathogenic response to ozone. Valproic acid (VPA) is a short-chain fatty acid with anti-inflammatory and antioxidant activity. In these studies we assessed the ability of VPA to mitigate ozone-induced oxidative stress and inflammatory macrophage activation. Mice were treated with air or ozone (0.8 ppm, 3 h) in whole-body exposure chambers, followed by VPA (300 mg/kg/day, i.p.) or PBS control beginning 30 min later. Bronchoalveolar lavage and lung tissue were collected 48 h later and analyzed for markers of macrophage activation and oxidative stress. Ozone exposure resulted in increased numbers of macrophages in the lung. Flow cytometric analysis revealed that these cells consisted of CD11b+Ly6G/F4/80+Ly6C+ pro-inflammatory and CD11b+Ly6G/F4/80+Ly6C+ anti-inflammatory macrophages. CD11b+Ly6G/F4/80+Ly6C+ monocytic myeloid-derived suppressor cells (MDSC) were also identified in the lung after ozone exposure. Treatment of mice with VPA resulted in decreased numbers of pro-inflammatory macrophages in the lung, with no change in anti-inflammatory macrophages or monocytic MDSC. VPA-mediated decreases in pro-inflammatory macrophage populations were associated with downregulation of ozone-induced expression of heme oxygenase-1, 1, a marker of oxidative stress. Ozone-induced increases in the generation of 4-hydroxynonenal, a lipid peroxidation end product, were also reduced by VPA. These data...
Dietary DHA Mitigates Ozone-Induced Pulmonary Inflammation and Reductions in Specialized Pro-Resolving Mediators

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Ozone (O₃), a criteria air pollutant, causes significant pulmonary morbidity and mortality annually. Obesity and metabolic disorders increase the susceptibility to the health effects of O₃. Currently, it is unknown why these populations are more susceptible. Our previous studies indicate O₃ exposure decreases pulmonary specialized pro-resolving mediators (SPMs), which promote resolution of immune responses. In obesity, the SPMs and, specifically, docosahexaenoic acid (DHA)-derived SPMs are decreased. Therefore, this could serve as a link between obesity and altered immune responses. Obesity is associated with consumption of a western diet (WD). We hypothesize that DHA supplementation increases pulmonary SPM levels, mitigating ozone-induced pulmonary inflammation. 5-week-old C57Bl/6J male mice were fed a normal diet (ND), a WD (45% fat) or a WD+DHA (43%+2% DHA). After 6 weeks, mice were exposed for 3 hr to filtered air (FA) or 1 ppm O₃. Pulmonary tissue lipid mediators were quantified using LC-MS/MS. In the ND group, O₃ exposure significantly increased BAL macrophages (Mₚ), pulmonary mRNA pro-inflammatory cytokine expression, and total BAL protein. This was associated with a decrease in pulmonary levels of SPMs 14(S)-hydroxy DHA (14-HDHA) and resolvins D and E (RvD, RvE) in the WD group. O₃ exposure increased BAL Mₚs, cytokine expression, and BAL protein. O₃ exposure did not change pulmonary 14-HDHA levels. In O₃-exposed mice, DHA significantly reduced pulmonary CCL2, CCL3, IL-6, and IL-1β expression. Compared to WD, DHA reduced BAL total protein and increased pulmonary 14-HDHA and resolvins DS levels, which are known to reduce pro-inflammatory cytokine expression. Unexpectedly, WD and ND have the same impact on O₃-induced inflammation. DHA increases pulmonary SPMs and SPM precursors, resulting in a decrease in pulmonary pro-inflammatory cytokine expression. These data support the novel idea that a DHA-supplemented diet may mitigate O₃-induced pulmonary responses by increasing pulmonary SPMs.

Suppression of Ozone-Induced Inflammation and Oxidative Stress by Ethyl Nitrite

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Ozone (O₃) is a pulmonary irritant known to cause lung injury, oxidative stress, and altered pulmonary mechanics. We previously showed that this is associated with an accumulation of pro-inflammatory macrophages in the lung, which contribute to ozone toxicity. Macrophage activation toward a pro-inflammatory phenotype is dependent on NF-κB. Nitric oxide is known to downregulate NF-κB, either through direct nitrosylation of the p65 subunit or indirectly through suppression of translocation. We hypothesized that administration of the NO+ donor ethyl nitrite (ENO) would reduce NF-κB signaling through increased nitrosylation and hence limit O₃-mediated inflammation via reduced pro-inflammatory macrophage activation. Female mice were exposed to 0.8 ppm of O₃ or air for 3 hr; this was followed 1 hr later by 0.0125% ENO (50 µL) intranasally. Mice were euthanized 48 hr later. Alveolar and tissue-associated macrophages were collected by bronchoalveolar lavage and lung digestion followed by magnetic sorting for F4/80+ cells, respectively. Left lung lobes were either inflation fixed for histology or snap frozen. Flow cytometric analysis demonstrated that administration of ENO reduced O₃-induced expression of the proinflammatory marker Ly6C in both alveolar and tissue-associated macrophages; O₃-induced upregulation of Ym1, a marker of anti-inflammatory macrophages, was also reduced. This was associated with reduced epithelial cell damage, lipid peroxidation, and expression of downstream inflammatory signals, including cytochrome b5, 4-hydroxynonenal, and I1L-1β. These data suggest that nitric oxide can play a protective role in the toxicity of O₃ by limiting inflammatory macrophage activation. Supported by NIH grants ES004738, ES005022, and HL086621.

Ozone Exposure Results in Altered Lung Microbiome Diversity and Growth Pattern

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The lung contains a significant microbiome within its lining fluid, which may be important in disease and potentially the response to inhaled toxicants. We analyzed the effects of inhaled ozone on the lung microbiome, exposing C57Bl6/J mice to air or 0.8 ppm ozone for 3 hr in whole-body chambers; bronchoalveolar lavage fluid (BAL) was collected 24, 48, and 72 hr later. Changes in the microbiome were assessed using an Oxford Nanopore (ONT) MinION system, which is accurate up to 97%. Bacterial rRNA operons were amplified using domain-specific forward primers in the 16S rRNA subunit, domain-specific reverse primers within the 23S rRNA subunit, and a High Fidelity Taq polymerase. This rRNA operon profiling approach yields an amplicon containing both ribosomal subunits for phylogenetic assignment (4,200 bp of sequence). Plus the ITS region (500+ bp) containing species/strain information. Replicate bacterial rRNA operon profiles were run. The rRNA operons were barcoded and sequenced in a 12-hr run on the MinION. We obtained 87,238 raw reads, and 11,788 were identified by MinKnow software. Sequences were analyzed using a simplified data analysis pipeline based on DMetaBlast against an NCBI 16S rRNA database via Genious software. Analysis using the Simpson diversity index showed a decrease in microbial diversity 24 hrs post-ozone, from 3.8 to 2.2, a time that we have demonstrated is associated with accumulation of pro-inflammatory macrophages. Subsequently, coordinate with the appearance of anti-inflammatory macrophages, microbial diversity rebounded to levels greater than control (5.6 vs 3.8); this was maintained for 72 hr. We speculated that the initial loss of diversity results from loss of normal microbes as a consequence of inflammation and oxidative stress; the later increases result from altered growth conditions within the lung lining fluid, and expansion of previously under-represented species. Thus, we assessed the active bacteria using a single isotope-labeling protocol. We found that only BAL from ozone-exposed animals is capable of incorporating 14C into the archael carrier band upon 16S rRNA amplification, consistent with our hypothesis that ozone alters the available carbon sources. We speculate that these alterations in the microbiome lead to increased susceptibility to disease. Supported by NIH grants HL 086621, ES005022 and ES004738.

Maternal Exposure to Polycyclic Aromatic Hydrocarbons in South Texas

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In this study, we characterized personal exposure to polycyclic aromatic hydrocarbons (PAHs) in pregnant women residing in Hidalgo County, where childhood asthma prevalence and hospitalization rates are among the highest in the state of Texas. Participants (n=17) in their third trimester of pregnancy were recruited from Rio Grande Regional Women’s Clinics located throughout Hidalgo County. Individuals provided three non-consecutive urine samples for biomarker analysis. We developed an isotope dilution liquid chromatography mass spectrometry method to quantify 1-hydroxypyrene (1-OHP), a common phenoil metabolite of pyrene that is often used as a biomarker to assess overall PAH exposure. In our study, 1-OHP was detected in 88% of the samples at a limit of detection (LOD) of 0.01 ng/mL urine. Individual concentration values were normalized by creatinine concentrations. Mean 1-OHP levels were 0.310 ± 0.395 (SD) with a median level of 0.213 ng/mg creat. Results illustrate that the measurement of the study population had a measurable exposure to PAHs. Values were in range of those in previously published studies for non-smoking individuals. Further analysis is ongoing to correlate urine biomarkers with additional measures of PAH and particulate matter level exposure collected from active sampling.
At present, no effective therapies exist to reduce the high mortality associated with dialysis-dependent acute kidney injury (AKI-D). Given the anuria associated with such patients, serum biomarkers may be useful in understanding the pathophysiological processes involved with AKI and assessing the severity of injury, and may point to novel therapeutic targets. In this study, Slow Off-rate Modified Aptamers (SOMA) can proteomic platform was used to profile 1,305 proteins in serum samples of patients enrolled in the Veterans Affairs-National Institutes of Health (VA/NH) Acute Renal Failure Trial Network (ATN) study. Study day-1 serum samples from 100 patients, and day-8 samples from 107 patients were analyzed by SOMAscan. From day-1 samples, 33 proteins were increased and 21 proteins were decreased when comparing samples of patients who died in the first 8 days versus patients who survived > 8 days. From day-8 samples, 76 proteins were increased and 78 proteins were decreased in patients who died between 8-28 days as compared to patients who survived >28 days. Higher serum levels of fibroblast growth factor 23 (FGF23), tissue plasminogen activator (tPA), neutrophil collagenase (MMP8), and soluble uricase plasminogen activated receptor (suPAR) on day 1, when stratified by tertiles, associated with higher mortality before and after adjusting for other potential risk factors including age, gender, cardiovascular SOFA score, congestive heart failure, and presence of diabetes. Upper tertile levels of FGF23, tPA and interleukin-6 (IL-6) on day 8 were associated with increased mortality. Ingenuity Pathway Analysis of the day-1 data revealed increased inflammation and increased coagulation in patients who died early (<8 days), and identified IL-6 as one of the top upstream regulators of inflammatory proteins. On day 8, mortality was associated with pathways of increased cell death and inflammation. The results indicate these serum proteins that may not only be important in the pathogenesis, but also helpful to discriminate AKI-D patients with high mortality.

Incidence of kidney failure is on the increase; unfortunately, traditional renal function markers are equivocal, especially at the early stage until end-stage renal disease, when a kidney transplant becomes inevitable. Hence, the need for early and more sensitive markers of renal damage indicating the presence of covert renal damage in occupational lead toxicity is imperative. This work is proposing diagnostic methods that could predict the development of chronic renal failure (CRF), especially in occupational lead-exposed subjects combining results of conventional and new biomarkers of kidney damage based on renal operating characteristics (ROC) area under the curve (AUC). Traditional renal function markers (plasma creatinine, urea, and uric acid) (TRF) were determined in one hundred each of lead-exposed subjects (LES) and non-exposed, non-nephrotic adults (control) along with sixty chronic renal failure patients (CRF) (all age-matched) using standard spectrophotometric methods. Blood lead level (Pb) was determined in one hundred each of lead-exposed subjects (LES) and control and non-exposed, non-nephrotic adults (control) along with sixty chronic renal failure patients (CRF) (all age-matched) using standard spectrophotometric methods. Blood lead level (Pb) was determined in one hundred each of lead-exposed subjects (LES) and non-exposed, non-nephrotic adults (control) along with sixty chronic renal failure patients (CRF) (all age-matched) using standard spectrophotometric methods. Blood lead level (Pb) was determined in one hundred each of lead-exposed subjects (LES) and non-exposed, non-nephrotic adults (control) along with sixty chronic renal failure patients (CRF) (all age-matched) using standard spectrophotometric methods. Blood lead level (Pb) was determined in one hundred each of lead-exposed subjects (LES) and non-exposed, non-nephrotic adults (control) along with sixty chronic renal failure patients (CRF) (all age-matched) using standard spectrophotometric methods. Blood lead level (Pb) was determined in one hundred each of lead-exposed subjects (LES) and non-exposed, non-nephrotic adults (control) along with sixty chronic renal failure patients (CRF) (all age-matched) using standard spectrophotometric methods. Blood lead level (Pb) was determined in one hundred each of lead-exposed subjects (LES) and non-exposed, non-nephrotic adults (control) along with sixty chronic renal failure patients (CRF) (all age-matched) using standard spectrophotometric methods. Blood lead level (Pb) was determined in one hundred each of lead-exposed subjects (LES) and non-exposed, non-nephrotic adults (control) along with sixty chronic renal failure patients (CRF) (all age-matched) using standard spectrophotometric methods. Blood lead level (Pb) was determined in one hundred each of lead-exposed subjects (LES) and non-exposed, non-nephrotic adults (control) along with sixty chronic renal failure patients (CRF) (all age-matched) using standard spectrophotometric methods. Blood lead level (Pb) was determined in one hundred each of lead-exposed subjects (LES) and non-exposed, non-nephrotic adults (control) along with sixty chronic renal failure patients (CRF) (all age-matched) using standard spectrophotometric methods.

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Diesel exhaust (DE) as the major source of vehicle-emitted particle matter in ambient air impairs lung function. The objectives were to assess the contribution of local (e.g., FeNO and serum CC16) and systemic (e.g., serum CRP and IL-6) inflammation to DE-induced lung function impairment using a unique cohort of diesel engine testers (DET, n=137) and non-DETs (n=127). Urinary metabolites, FeNO, serum markers, and spirometry were assessed. A 19% reduction in CC16 and a 94% increase in CRP were identified in DETs compared to non-DETs (all P<0.001), which were further corroborated by showing a dose-response relationship with internal dose for DE exposure (all Ps<0.04) and a time-course relationship with DE exposure history (all Ps<0.005). Mediation analysis showed that 43% of the difference in FEV1 between DETs and non-DETs can be explained by circulating CC16 and CRP (permuted P<0.001). An inverse dose-dependent relationship between FeNO and systemic markers does not support a single mechanism for DE-induced lung toxicity. A range of 95% lower bounds of benchmark dose (BMD) of 0.315 to 0.9676 μg/g creatinine for urinary pheranths was recommended for regulatory risk assessment. Local and systemic inflammation may be a key process that contributes to the subsequent development of obstructive lung disease in DE-exposed populations.

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Exposure to Pyrethroid and Organophosphate Insecticides among Adolescent Applicators and Non-Applicators

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Agricultural practices commonly include the application of several pesticides throughout a season, however, research seldom examines the impact of these multiple exposures. Previous studies have hypothesized a organophosphate (OP) mediated inhibition of the detoxification of pyrethroids (PYR) as a result of OPs inhibiting carboxylesterases responsible for hydrolyzing PYRs, resulting in PYR bioaccumulation. Exposures to PYR and OP insecticides were examined as part of a longitudinal study and metabolites (MTB) from three pesticides, λ-Cyhalothrin and α-Cypermethrin, class II PYRs, and chlorpyrifos (CPF), an OP, were examined before, during, and after the PYR application period. The goals of this study were to investigate if the urinary δ-glucuronide (D7 and D10) and trans-DCCA levels increased during the PYR application cycle and to examine the interaction between PYR and OP exposure on MTB levels. Urine samples from nine test sessions before, during and after the PYR application cycle were analyzed from adolescents employed (n=40) and not employed (n=58) by the Ministry of Agriculture (MOA). Negative ion chemical ionization gas chromatography mass spectrometry was used to analyze the samples for five MTBs: λ-Cyhalothrin (λ-Cyhal), MTB for λ-Cyhalothrin; cis-trans-3-(2,2-dichlorovinyl)-1,2-dimethylcyclopanecarboxylic acid (trans-DCCA), MTB for α-Cypermethrin; 3-phenoxypyridinol (3-PBO), a non-specific PYR MTB; and 3,6-trichloro-2-pyridinol (TCPy), MTB for CPF. Applicators had significantly higher MTB levels for all pesticides compared to non-applicants (p-values < 0.01). 3-PBO and trans-DCCA levels increased during the PYR application cycle and then continued to increase after spray ended. TCPy levels were significantly higher than the four PYR MTB (p-values < 0.0001) in both groups. TCPy and the four PYR MTB levels were examined to determine if there was inhibition in PYR biotransformation; however, higher TCPy levels were associated with higher PYR MTB levels, not demonstrating inhibition (p value < 0.001). PYR levels never returned to baseline in both groups which could be due to prior OP exposure slowing OP biotransformation and resulting elimination of PYR metabolites or to continued exposure. Further investigation into the interactive effects between PYRs and OPs are needed.

Evaluation of Prodromal and Reversibility Effects on Novel Biomarkers of Nephrotoxicity in Cynomolgus Monkeys Treated with Gentamicin

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Very few studies have been published on kidney safety biomarkers in Cynomolgus monkey. This study was conducted to further evaluate the ability of novel biomarkers of nephrotoxicity to monitor the onset, progression, recovery of drug-induced kidney lesions, and to explore potential prodromal effects in this species. Groups of 4 or 6 males were given intramuscular daily injections of gentamicin at 10 mg/kg/day for 3 or 10 days and were sacrificed on Day 4, 11 or 25. Blood samples and 16-hour urine samples were collected on Day −7, −3, 2, 4, 7, 9, 11, 18 and 25. In parallel to conventional parameters, the urinary kidney biomarkers N-acetyl-β-D-glucosaminidase (NAG), microalbumin, retinol-binding protein 4 (RBP4), cystatin C, α1-microglobulin, clusterin and osteopontin were evaluated. Kidney microscopic findings consisted of minimal to mild tubular cell alteration, hyaline droplet formation and/or tubular cell degeneration/necrosis in 4/6 monkeys treated for 10 days on Day 11. There were no kidney lesions on Day 4 or 25. No increases in serum creatinine, blood urea nitrogen, urinary total protein and microalbumin were observed, except in 1/6 treated animals with mild tubular cell degeneration/necrosis on Day 11. NAG, RBP4 and clusterin were increased at similar levels from individual monkeys (2- and 3-fold, respectively), and almost returned to baseline levels during the recovery phase. On Day 4, similar NAG and RBP4 fold-increases were noted in the absence of kidney microscopic changes. A trend increase in osteopontin was noted from Day 7 and was followed by a partial decrease after treatment cessation. In this study, no significant cystatin C and α1-microglobulin changes were observed. These results indicate that the urinary NAG and RBP4 are sensitive biomarkers of nephrotoxicity in monkeys with potential prodromal added value, and which could be used to monitor the reversibility of drug-induced kidney lesions.

Kidney Injury Protein Biomarker Quantification in Canine Urine by Novel Multiplex-Immunoprecipitation Mass Spectrometry Assay

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1Janssen Pharmaceutical Research and Development LLC, Spring House, PA; 2Janssen Pharmaceutical Research and Development LLC, San Diego, CA; 3Natural and Medical Sciences Institute at the University Tübingen, Reutlingen, Germany; and 4Signatope GmbH, Reutlingen, Germany.

In this 10-day (D) study, tobramycin nephrotoxicity was monitored in male beagle dogs where normal renal urinary tubular injury biomarkers were analyzed and compared with histopathology and classical parameters. For this, caninespecific sandwich immunoassays were used along with a novel multipleximmunoprecipitation mass spectrometry (IP-LC/MS) assay which can be used across species. Animals received tobramycin (60 mg/kg, n=6) or vehicle (n=3) for ten days. Urine/serum were collected at pre-dosing (D-4) and on D1, D3, D7, and D10. Plasma was collected at 0.25, 0.5, 1, 2, 4, 6 and 24h post the first last administrations. There were no deaths/cclinical observations. Tobramycin plasma exposures with respect to mean Cmax, mean AUC4-24 and mean AUC× more were higher when compared to D1. The Tmax ranged from 0.3 to 1 hours and 0.5 to 2 hours on D1 and D9, respectively. Tobramycin-induced kidney lesions were characterized by minimal to marked proximal cortical tubular necrosis and interstitial fibrosis; minimal to slight acute interstitial inflammation and hyaline casts; and minimal mononuclear cell infiltration. No significant increases in urea nitrogen and serum creatinine were detected. When normalized to urinary creatinine, increases in urinary GGT (D7 and D10) and N-acetylβ-D-glucosaminidase (D10) were detected in dogs. Dogs versus controls were used to manufacture the powders indicating that TTTCA is likely a product of glucosinate metabolism in cruciferous plants. Our data strongly suggest that TTTCA is not only an exposure biomarker of CS2 but is also a promising biomarker of cruciferous vegetable intake in clinical trials. Furthermore, consumption of cruciferous vegetables should be taken into consideration as a confounder in CS2 monitoring within high-risk populations. This work was supported by P50 CA097190 and R35 CA197222.
outperformed by selected novel urinary biomarkers, indicating that the innovative multiplex IPLC/MS assay is a valuable platform for simultaneous detection of urine-based proximal cortical tubule injury biomarker changes in dogs and potentially in other species.

1253 Sex Differences in Excretion Levels of Urinary Biomarkers of Nephrotoxicity in Rats


Urinary biomarkers have been used widely in preclinical toxicity studies to detect dysfunctions and injuries of the kidney caused by drugs under development. While they have been well-studied for evaluating nephrotoxicity, knowledge of sex differences in excretion levels of urinary biomarkers remains inadequate. We conducted experiments focused on effects of endogenous sex hormones on urinary biomarkers using intact and castrated male and female rats. Comparisons of the urinary biomarker excretion levels between intact male and female rats at 5, 7, and 9 weeks of age revealed higher excretion levels of leucine aminopeptidase (LAP), γ-glutamyl transpeptidase (GGT), total protein, liver-type fatty acid-binding protein (L-FABP), cystatin C (Cys-C) and β2-microglobulin (β2-MG), and lower excretion level of kidney injury molecule 1 (Kim-1), in male rats as compared to female rats. Orchidectomized male rats showed lower urinary excretion levels of alkaline phosphatase (ALP), GGT, N-acetyl-β-D-glucosaminidase, cystatin C, total protein, L-FABP, Cys-C, β2-MG and neutrophil gelatinase-associated lipocalin, and higher urinary excretion levels of clusterin and Kim-1 than sham-operated male rats. On the other hand, no significant differences in the urinary biomarker excretion levels excluding ALP were observed between ovariectomized and sham-operated female rats. In the present study, we demonstrated the existence of sex differences in excretion levels of urinary biomarkers that are universally used in preclinical toxicity studies, and also that these differences, especially in relation to the urinary excretions of ALP, LAP, GGT, total protein, L-FABP, Cys-C, and β2-MG, may closely relate to the endogenous testosterone.

1254 Evaluation of Urinary Renal Safety Biomarkers in Non-Human Primates with Six Model Kidney Toxicants


Certain classes of drugs (e.g., antibiotics like aminoglycosides or cephalosporins, chemotherapeutic agents, and antivirals) are known to cause drug-induced kidney injury (DIKI) in patient populations. Due to the close evolutionary relationship with humans, non-human primates (NHP) are an important nonclinical model for assessing drug-induced injuries. NHP models afford an opportunity not only to study the type of injury induced by such drugs but also to evaluate the performance of translational safety biomarkers that may be useful for monitoring similar injuries in humans. Novel urinary kidney safety biomarkers have been shown to be more sensitive than the conventional renal function biomarkers, blood urea nitrogen (BUN) and serum creatinine (Scr). To characterize the types of kidney injury caused by common drugs and to assess the relative performance of a growing list of novel kidney safety biomarkers, studies were run in NHP using six compounds that are classified as non-class A, antivirals, amino-naproxen, cyclosporine, naproxen and cyclosporine. A comprehensive evaluation of nine urinary biomarkers (albumin, creatinin, cystatin C, Kim-1, lipocalin-2 or NGAL, N-acetyl-β-D-glucosaminidase (NAG), osteopontin (OPN or SPP1), retinol binding protein 4 (RBP4) and total protein) was performed on urine collected from these studies. Treatment with each of the six compounds resulted in kidney proximal tubule injury of various severities, and the performance of the urinary biomarkers was determined relative to the microscopic histomorphologic changes observed. Among the kidney injury biomarkers analyzed, Kim-1, creatinin, and albumin showed the highest overall performance for detecting drug-induced renal tubular injury in the NHP, and the majority of the biomarkers were able to detect the injury earlier than BUN or Scr. This comprehensive evaluation of six common nephrotoxic drugs characterized the histopathological changes in the kidney and demonstrated the monitoring of such changes using novel safety biomarkers, and provided additional supporting evidence for translating these biomarkers for use in clinical settings to further ensure patient safety.

1255 Alterations in the Expression of Shelterin Complex Genes in Crystalline Silica Exposed Rat Lungs


Occupational exposure to silica can result in a variety of pulmonary fibrosis and lung carcinoma through several complex mechanisms. Therefore, it is imperative to identify the key biomarkers of silica-induced pulmonary toxicity for the intervention of lung pathologies. Telomerases (the nucleoprotein structures with repetitive (TTAGGG) sequences at the end of chromosomes) are a molecular "clock of life" and alterations are associated with several chronic diseases. Shelterin complex protection of telomerase1 (POT1), telomeric repeat binding factor1 (TRF1), telomeric repeat binding factor2 (TRF2), TRF1-interacting nuclear factor2 (Tin2), TRF2-interacting telomeric protein (Rap1), and POT1 and Tin2-organizing protein (TPP1) play an important role in maintaining telomere length and integrity and any alteration in telomeres activate DNA damage machinery resulting in telomere attrition. The goal of this study was to assess the effect of crystalline silica exposure on the regulation of shelterin complex genes in an animal model. Male Fisher 344 rats were exposed by inhalation to Min-U-Sil 5 silica for 3, 6, and 12 weeks at a concentration of 15 mg/m³ for 6 hours/day for 5 consecutive days/week. After the final day of exposure the right lung was homogenized, total RNA was isolated and reverse transcribed to obtain cDNA, and expression of shelterin complex genes was assessed. At all time points after exposure, mRNA expression of POT1, TRF1, TRF2, Tin2, Rap1, and TPP1 were significantly decreased (p<0.05) in the silica-exposed animals compared to air controls, and the decrease observed were exposure time dependent. POT1 and TPP1 which mediates telomerase-dependent telomere extension were significantly decreased in exposed animals. In conclusion, our results suggested that silica inhalation promoted shelterin complex instability. This study indicated that measurement of expressions of shelterin genes involved in telomere regulation may serve as a potential biomarker for silica-induced pathology including carcinogenesis. In addition, changes in shelterin complex could potentially promote telomere end-to-end fusions and cancer formation.

1256 Evaluation of Di-Docosahexaenoyl Phosphate (22:6 BMP) as a Biomarker of Drug-Induced Phospholipidosis in Rodents


Drug induced phospholipidosis (DIP), a lysosomal phospholipid storage disorder, has been known as a side effect of cationic amphiphilic drugs. Recently di-docosahexaenoyl (C22:6)-bis(monoacglycerol)phosphate (22:6 BMP) has been proposed as a noninvasive and specific biomarker to monitor DIP in animals and human. In this study, amiodarone (AMD), a well-known phospholipidosis inducer, was administrated to rats to evaluate onset, dose-response and reversibility of 22:6 BMP in serum and urine. We also evaluated the specificity of 22:6 BMP by the analysis of rats treated with tetracycline (TC), a negative compound for DIP. In the 1st study, AMD was orally administered to male Crl:CD(SD) rats at dose of 150 mg/kg/day for 3, 7 or 10 days. Additional animals were dosed 150 mg/kg/day of AMD for 10 days followed by 13 days recovery period. In the 2nd study, AMD and TC were orally administrated for 7 days at doses of 16, 50 and 150 mg/kg/day of AMD or 2000 mg/kg/day of TC to male rats. In both rat studies, urine was collected overnight via metabolic cages after last dosing or at the end of recovery period. Blood and tissues were collected at the necropsy, and the tissues were used for microscopic examinations. Serum and urine 22:6 BMP were measured by LC/MS/MS. DIP-related histopathological changes were observed after administration of 150 mg/kg/day of AMD for 3, 7 or 10 days, which caused the increase of 22:6 BMP in both serum and urine. AMD-induced histopathological changes and the increase of 22:6 BMP were returned to normal after the recovery period. There were no histopathological changes at doses of 16 and 50 mg/kg/day of AMD for 7 days, and serum and urine 22:6 BMP were not increased significantly. Regarding the TC dosing group, slight to moderate fatty changes of hepatocyte were observed, but serum and urine 22:6 BMP were not increased. Serum and urine 22:6 BMP are considered to be enough sensitive, reversible and specific markers for DIP. They only increase significantly when the histopathological changes related to DIP are observed.
Cardiovascular diseases are the number-one cause of death worldwide. Much is known about cardiovascular diseases risk factors, including hypertension, high cholesterol, obesity, diabetes, physical inactivity, tobacco exposure, and unhealthy diets. Additionally, epidemiological and clinical data in the last ten years positively related air pollution exposure with cardiovascular diseases. More specifically, chronic air pollution, mostly originated from diesel exhaust, has been found to increase cardiovascular disease mortality and have significant effect on atherosclerosis. So far, little is known about molecular mechanism and biomarkers of diesel exhaust particle (DEP) effects on atherosclerosis development. In this study we applied in silico approach to a gene expression profile of human macrovascular endothelial cells (HMEC) treated with DEP to predict candidate biomarkers of air pollution-induced cardiovascular effects. From the whole-genome analysis, 528 significantly dysregulated genes were selected for further analysis. For the prediction of hypotheses, our expert knowledge system utilizes a network-based approach that considers manually curated prior knowledge of genes, proteins, and chemicals and their associations with each other and to thousands of pathologies. This expert knowledge system reports that DEP dysregulated genes play a role in cardiovascular pathologies, including atherosclerosis, hypertension, cardiotoxicity, endothelial cell testing, and vascular smooth muscle toxicity. Network analysis of atherosclerosis revealed involvement of 34 genes from DEP gene expression data. Centrality analysis ranked 14 of these genes by the importance in the mechanism of atherosclerosis development. Among these 14 genes, further testing on annotated public data readily identified 6 known and potential biomarkers for atherosclerosis and cardiovascular diseases, the highest ranked being DUSP1, TNFAIP3, and MMP10. These tests support validation of a systems biology approach to discovery of biomarkers for DEP effects on the cardiovascular system.

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1258 Serum 14,15-DHET, a Metabolite of Soluble Epoxide Hydrolase, Is an Early Biomarker to Predict Doxorubicin (DOX)-Induced Cardiotoxicity in Rats


Over 2.8 million women in the US in 2015 were subjected to breast cancer chemotherapy, which increased 5-year survival rate. However, chemotherapy anthracyclines, e.g., doxorubicin (DOX), cause cardiotoxicity and heart failure (34% of the patients). Early diagnosis followed by medical intervention is critical to prevent cardotoxicity. The aim of this study is to find whether serum hypertension biomarkers, 14,15-DHET (metabolite of soluble epoxide hydrolase), 20-HETE (metabolite of cytochrome P450 4A4/4F) and 8-isoprostane (non-enzymatic oxidative stress product) are early biomarkers to predict DOX-induced cardiotoxicity. Female Sprague-Dawley rats (n=6 per group) were treated with and without DOX (3 mg/kg /week, i.v. for 2 weeks) (no recovery group) and with a 2-week recovery period (2 week recovery group). Decreased ANP and BNP mRNA levels in the myocardium is a marker of hypertrophy. Whereas no increases of heart ANP and BNP mRNA levels were detected by RT-PCR after 2 weeks of DOX treatment, mRNA levels increased ~3-fold and 2.5-fold, respectively, after 2 week recovery. A hypertrophy and vacuolization study of heart tissues of the rats revealed that no damage occurred after the 2-week recovery. The qualitative and mRNA analyses after DOX treatment suggested that the rats in the 2-week recovery group did not experience cardiotoxicity. Early increase of the BNP mRNA level without cell hypertrophy followed by decreased BNP mRNA levels after extended recovery period with cell damage were correlated with DOX-treated rats. The results demonstrated that the fatty acids were not affected after two weeks of DOX treatment (no recovery). However, after 2 week recovery, when cardiotoxicity was still not detected, levels of 14,15-DHET (p<0.05) and 8-isoprostane (p<0.05), but not 20-HETE levels, were increased compared to the control group. The results suggest that 14,15-DHET and 8-isoprostane are early biomarkers to predict DOX-induced cardiotoxicity.

1259 Air Pollution-Induced Cardiovascular Effects: Bioinformatics Approach to Biomarker Identification


The aim of these studies was to determine the utility of candidate biomarkers in the assessment of platelet activation and stress; two endpoints adding to the interpretation of preclinical toxicology studies. Platelet activation is usually measured in vitro, using whole blood, by flow cytometry. This requirement for special sampling and instrumentation limits the use of these assessments for in vivo studies. This study qualified the performance of assays for fibrinogen, d-dimer, p-selectin, and 14,15-DHET, biomarkers that reflect in vivo platelet activation and clot formation and are present in plasma, which is readily collected and analyzed. Samples taken from cynomolgus monkeys and human donors were incubated with activators ADP, TRAP-6, and thrombin, processed to platelet lysates and incubated with an immunoassay for fibrinogen, d-dimer, and 14,15-DHET. The results for both species reflect fibrinogen and d-dimer are potential biomarkers of clot formation and breakdown. A cynomolgus monkey cross-reactive p-selectin assay was not identified. For stress biomarkers, IL-6 and cortisol were examined in plasma. Salivary cortisol levels, which are potentially less stressful to collect, were also measured to determine if those levels accurately reflect plasma levels. The following stressors were evaluated: cynomolgus monkeys during acclimatization following shipment, and beagle dogs before and during facility transfer. The stressors failed to elicit an increase in IL-6 to above the lower limit of quantification for either species. For monkeys, there was no relationship between cortisol levels and acclimatization, nor between individual plasma and saliva cortisol values. This could be due to rapid acclimatization and the effects of social dominance. For dogs, both plasma and saliva cortisol levels increased roughly 2-fold on the day that they were moved, and then decreased back to baseline levels. The results for both species reflect veterinary observation. In summary, these data suggest that fibrinogen and d-dimer are potential biomarkers of clot formation and breakdown in vivo in cynomolgus monkeys, and that plasma and salivary cortisol are potential biomarkers of stress in beagle dogs, but not cynomolgus monkeys, using the given stressors. The qualification of the assays used in this study enables their use in toxicology studies to provide additional interpretation to other findings.
1261 Changes in Whole Blood Gene Expression Due to Exposure to Bromobenzene or Allyl Alcohol

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Exposure to toxic industrial chemicals resulting in organ injury is an occupational hazard for many workers, community members, and military personnel. Bromobenzene and allyl alcohol are two such chemicals which may cause injury to the liver and kidney. Peripheral blood is an accessible biofluid containing a wealth of information. Transcriptomic analysis of blood has shown that gene expression changes are reflective of physiological and pathological events in different tissues. Here, we examined gene expression in the whole blood of rats exposed to bromobenzene or allyl alcohol to elucidate the mechanisms of toxicity, as well as to identify potential biomarkers of steatohepatitis induced by bromobenzene or fibrosis induced by allyl alcohol. Rats (n=5) were treated with 78.5, 157, or 314 mg/kg of bromobenzene, 45 mg/kg of allyl alcohol, or vehicle control daily for up to 5 days. Blood samples were collected at days 2-5 after the final exposure. Whole blood transcriptomics were assessed by next-generation sequencing. Differential gene expression was determined using edger and enrichment analyses were performed. Exposure to the toxicants induced gene expression changes at each time point. Interestingly, more genes were changing in the early time points due to exposure to bromobenzene than after 5 days, while more genes were differentially expressed at the later time points due to exposure to allyl alcohol. Many of the same canonical pathways, such as actin cytoskeleton signaling and acute phase signaling, were enriched by both chemicals, however the chemicals differed in whether they activated or inhibited the pathway. The transcriptomic analysis also suggests that both chemicals inhibit PPARα/RXRα activation. In conclusion, we tested rats for steatohepatitis and fibrosis inducing chemical and measured gene expression changes in whole blood daily for four days. We identified modulated molecular pathways and functions common and unique to each chemical, providing insight into the molecular difference between steatohepatitis and fibrosis as well as identifying potential biomarkers specific to these different liver injuries. Research was conducted in compliance with the Animal Welfare Act, and all other federal requirements. The views expressed are those of the authors and do not constitute endorsement by the US Army.

1262 Expression and Release of Hepatic Biomarkers from Primary Human Hepatocyte Spheroids

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Drug-induced liver injury is a major obstacle to the development of new medicines, and is associated with significant patient risk. Detection of hepatic liabilities is complicated by species differences in drug metabolism as well as a lack of mechanistic biomarkers. In vitro systems which better mimic human liver physiology may serve to bridge this gap. In the current study we therefore sought to determine how emerging clinical biomarkers were regulated in a spheroid model. Spheroids generated from cryopreserved primary human hepatocytes were treated with acetaminophen (APAP) or staurosporine (STA), which induce cell death via predominantly necrotic or apoptotic mechanisms respectively. Media and cells were harvested at 1, 2, 4, 4h, 24h and 72h and a panel of hepatic biomarkers including cytoketeron 18 (both full length (CK18) and a caspase-cleaved fragment (ccCK18)) and mir-122 were measured and compared to levels of cellular ATP. In addition, the expression and localization of these biomarkers was monitored via immunohistochemistry. Both compounds induced a time and dose dependent reduction in cellular ATP. This was followed by elevations in mir-122 and CK18 which were both detected in cell culture media at later time points (24-72h), suggesting a leakage of cellular contents. APAP treatment resulted in minimal production of ccCK18, whereas it was increased up to 5-fold after treatment with STA, confirming that the spheroids can be used to distinguish between apoptosis and necrosis. Detection of ccCK18 was improved significantly when analyzing cellular lysates rather than culture medium, likely due to very low constitutive amounts of the fragment present in healthy cells. At the same time increased expression of active caspase 3 in STA-treated spheroids was observed via immunohistochemistry, allowing the identification of specific apoptotic cells. Wider analysis of clinically relevant miRNA candidates as well as transcriptomic profiling will be now be performed in order to identify toxicological signatures of different drug-induced pathologies. The addition of non-parenchymal cells such as Kupffer and stellate cells will also be investigated as a possible route to better mimic in vivo physiology and model adverse reactions where multiple cell types are involved.

1263 Quantitative, Accessible, and Translational Biomarkers of Gastrointestinal Toxicity


Drug-induced toxicities to the gastrointestinal tract (GIT) encompass diverse pathologies, reflecting the heterogeneity in physiological function in this system. Clinically, adverse drug reactions include: upper GIT erosions from non-steroidal anti-inflammatory drugs (NSAIDs) and dose-limiting lower GIT safety concerns such as diarrhea or colitis due to chemotherapeutics and immuno- oncology therapies. Qualification of quantitative and accessible GIT injury biomarkers with a mechanistic association to the injury and with translational relevance will enhance the ability to use preclinical toxicity studies to predict adverse clinical outcomes. Here we compare the performance of mechanistically diverse biomarkers of GI injury in multiple models for the first time with a two week mechanistic toxicity study in rats with the NSAID indomethacin (INDO) (up to 3 days), the chemotherapeutic methotrexate (MTX) (6 days) and the mucosal solvent dextran sulfate (DSS) (6 days) to respectively, evaluate the performance of plasma and fecal biomarkers of GIT injury due to: epithelial perforation, disruption of intestinal villus turnover and colitis. The biomarkers evaluated were plasma citrulline, intestinal fatty acid-binding protein, CD14 and lipopolysaccharide-binding protein and fecal lipocalin-2. These measurements were accompanied by terminal histopathology and genomic analysis and by standard clinical observations (COs), measurements of body weight (BW) and fecal hemoglobin (Hb). Standard measurements showed: significant BW loss and fecal Hb for INDO-treated animals by Day 3 and before Day 6. Watery feces, with slight BW loss, for MTX-treated animals (Day 6 on), accompanied by increased morbidity without recovery. No GIT-related COs or BW loss in the DSS-treated animals. However, a dose-dependent, mechanistically-coherent and organ-specific increase in histopathological findings and genomic expression was observed due to all treatments, demonstrating low sensitivity of standard in life measurements for specific GIT toxicities. In contrast, accessible biomarker responses matched the type, onset, severity, incidence and recovery of histopathology findings; consistent with the mechanistic link between biomarkers and injury. The biomarkers evaluated are clinically translatable and add sensitive, quantitative, mechanistic information to standard measurements of GIT injury.

1264 Mechanism of the Phenylhydrazine-Induced HbA1c Reduction in Rats

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Hemolytic anemia affects glycated hemoglobin (HbA1c) levels, which are widely used as an indicator of glucose control in patients with diabetes. However, there are limited experimental data examining the mechanism underlying HbA1c changes in hemolytic anemia. We examined HbA1c levels in three animal models of Sprague-Dawley rats (hemolysis model, blood loss model, and aplastic model) and compared old and young rat erythrocyte sensitivity to phenylhydrazine (hemolysis model, blood loss model, and aplastic model) and compared old and young rat erythrocyte sensitivity to phenylhydrazine in vitro. Based on the specific HbA1c decreases in the hemolytic model, the change in HbA1c was not affected by the change in the percentage of reticulocytes. Additionally, the in vitro study indicated that phenylhydrazine induced greater methemoglobin formation in old erythrocytes than that in young erythrocytes due to decreases in methemoglobin reductase activity. These findings indicate that the mechanism of decrease in HbA1c induced by phenylhydrazine is attributable to hemolysis, specifically occurring in old erythrocytes. These data suggest that HbA1c can be a sensitive biomarker for hemolysis, particularly hemolysis induced by oxidative stress. Importantly, these findings contribute to the understanding of the glycosylated hemoglobin process and the correct use of HbA1c as a screening tool for hemolysis and diabetes.
A new safety testing paradigm that relies on gene expression biomarker panels was developed to easily identify drug-induced tissue injuries prior to drug candidate selection. The approach has yielded positive impact on early compound de-risking, enabling drug advancement while reducing later probability of attrition. Further, defining doses associated with tissue injury has provided additional perspective to advancing dose and development of emerging mechanistic biology that may be impacted as a consequence of organ toxicity. Here we describe the development, qualification, and implementation of gene expression signatures that diagnose tissue degeneration/necrosis for use in early rat Safety Lead Optimization (SLO) tolerability studies. Microarray data were used to first identify approximately 400 genes that were discovered as being shared consistently across 4 prioritized tissues (liver, kidney, heart, skeletal muscle), following injuries induced by known toxicants and so were termed ‘universal’. Quantitative PCR on ~100 of those genes followed, and the approach was extended across 5 other tissues (pancreas, spleen, GI, bladder, and testes) where early toxicities are historically less common. The most consistent and robustly responding transcripts were selected, resulting in a final 12-gene set for each of 9 tissues, with some tissues supplemented with transcripts specific for a given tissue (e.g., Kim-1 for kidney). Mathematical algorithms were generated to convert each tissue’s gene expression fold changes for the 12 genes into a single metric, scaled between 0 and 1, and a positive threshold was set using a training set of approximately 60 compounds. Performance for all tissue signatures on an independent test set of approximately 30 rat studies yielded approximately 90% sensitivity and 100% specificity. Bundled together, we have incorporated these gene expression signature panels into a 5 day rat SLO derisking study, that provides rapid objective assessment of compound liabilities, benchmarks toxic doses, and guides selection of lead candidates to improve attrition without the necessity to perform time-consuming histopathology, which can be reserved for advancing more optimized compounds at later GLP development stages.

Impact of Stress on Histology, Hematology, Clinical Chemistry, and Biomarkers of Inflammation and Muscle-Injury in Rat


Stress may affect results and complicate data interpretation in safety assessment studies. Prednisone is a short-acting corticosteroid, which is known to mimic characteristics of a physiological stress response in animals. Studies with vehicle vs prednisone-treated rats were conducted to examine the impact of chemically-induced stress on the histology, hematology, clinical chemistry, and biomarkers of inflammation and muscle-injury. In the 1st study, rats received 2 or 4 doses of prednisone at 0.03 or 10 mg/kg. Blood was collected 24 hours after the final dose. No changes in the clinical chemistry or biomarker results for cardiac troponin I (cTnI), myosin light chain 3 (Myl3), monocyte chemotactic protein-1 (MCP1), thrombospondin-1 (TSP1), N-terminal pro cardiac troponin I (cTnI), myosin light chain 3 (Myl3), monocyte chemotactic protein-1 (MCP1), thrombospondin-1 (TSP1), N-terminal pro-cardiac troponin I (cTnI), myosin light chain 3 (Myl3), monocyte chemotactic protein-1 (MCP1), thrombospondin-1 (TSP1), N-terminal pro-cardiac troponin I (cTnI), myosin light chain 3 (Myl3), monocyte chemotactic protein-1 (MCP1), thrombospondin-1 (TSP1), N-terminal pro-cardiac troponin I (cTnI), myosin light chain 3 (Myl3), monocyte chemotactic protein-1 (MCP1), thrombospondin-1 (TSP1), N-terminal pro.
1269 Identification of Candidate Genes Associated with Breast Cancer Using Multiple High-Throughput Analysis

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Breast is one of the common cancer types prevalent in women worldwide. Molecular profiling of breast tumors may lead to the identification of gene sets or signatures that are associated with higher risk of metastasis and poor disease outcome for the patients. In the present study, we aim to analyse these gene signatures using high throughput put analysis. Breast cancer samples from the patients who haven’t sought chemotheraphy and underwent breast conservation surgery or total mastectomy were collected from Sri Venkateswara Medical College, Tirupati. Sub classification of the breast specimens were done by immunos- taining for estrogen receptor, progesterone receptor and human epi- dermal growth receptor. Gene expression analysis was performed using microarray analysis. Microarray profile for the patient samples were compared with the gene expression profile data available at the public domain databases Gene Expression Omnibus and Array express. Results from microarray analysis of the samples with a fold value of 0.6 showed that 4162 genes were up regulated and 9629 genes were down regu- lated. Among them, up regulated cancer pathway genes were 84 and down regulated cancer pathway genes were 140. Among the genes conferring high susceptibility to breast cancer, BRCA1 (fold value = 0.86), BRCA2 (fold value = 1.12), TP53 (fold value = 1.20), STK11 (fold value = 1.03), CDH1 (fold value = 9.09) were found to be up regulated whereas PTPN11 (fold value = 1.31) was found to be down regulated. In the case of moderate and low penetrance genes, ATM (fold value = 0.69), CHEK2 (fold value = 1.33), BRI1 (fold value = 2.28), TOX3 (fold value = 2.95), MAP3K1 (fold value = 0.68), FAM84B (fold value = 2.31), NEK10 (fold value = 0.83), CASP8 (fold value = 1.07), ES1 (fold value = 1.95) were found to be up regulated whereas PALB2 (fold value = -0.04), FGFR2 (fold value = 0.34), LSP1 (fold value = -1.08), COX11 (fold value = -0.93), TPNI1 (fold value = -0.20), NOTCH2 (fold value = -0.76), RAD51B (fold value = -0.14), FGR10 (fold value = -0.42) were found to be down regulated. Overall, results from the present study may provide fundamental aspects early prognosis and therapeutic strategies in breast cancer.

1270 Anti diabetic Activity of NR4A1 Ligands in C2C12 Muscle Cells

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Research in this laboratory has identified 1,1-bis (3'-indolyl)-1-(p-hydroxyphenyl) methane (DIM-C-pPhOH) as an NR4A1 ligand that acts as an antagonist and inhibits pro-oncogenic NR4A1-dependent, growth, survival, and migration of cancer cells and tumors. A series of butyrenes (3 or 3, 5-substituted) analogs of DIM-C-pPhOH have been prepared, and this second generation set of NR4A1 ligands typified by the 3-chloro- 5-methoxy analog (DIM-C-pPhOH-3-Cl-5-OCH3) is 4 to 10 times more potent than DIM-C-pPhOH. In this study we used C2C12 muscle cells to investigate the antidiabetic activity of NR4A1 ligands, which includes induction of GLUT4 and glucose uptake and induction of glycolytic gene expression. Treatment of C2C12 muscle cells with metformin or the NR4A1 ligand DIM-C-pPhOH induced NR4A1 and GLUT4 mRNA and protein expression. Similar results were observed with butyrenes (3 or 3, 5-substituted) analogs of DIM-C-pPhOH including DIM-C-pPhOH-3-Cl-5-OCH3, and the butyrenes analogs were more potent than metformin or DIM-C-pPhOH as inducers of NR4A1 and GLUT4 mRNA levels. Metformin and the bis-indole substituted analogs also induced expres- sion of several glucose synthesis genes and Rab4, which has previously been linked to enhancing cell membrane accumulation of GLUT4 and overall glucose uptake in C2C12 cells, and these responses were also observed after treatment with metformin and the NR4A1 ligands. The role of NR4A1 in mediating these responses was also confirmed by knockdown of NR4A1, which attenuated not only the bis-indole NR4A1 ligand but also the metformin-induced responses. Our results demon- strate for the first time that the bis-indole-derived NR4A1 ligands rep- resent a novel class of anti diabetic drugs that enhance glucose uptake in C2C12 muscle cells, and we also show that the antidiabetic effects of metformin in this cell line are NR4A1-dependent.

1271 Enzymatic Activities of PON1 as Predictors of Cardiovascular Disease: Which is the Ideal Biomarker?

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Paraoxonase 1 (PON1) has been extensively studied in the field of toxicology and more recently it has been well established as a cardioprotective agent in cardiovascular diseases (CVD). PON1 is a high density lipoprotein-associated enzyme that has antioxidant and anti-inflammatory activities towards organophosphate triesters, alylesters, lactones, among others. The aim of this study was to demonstrate which of the enzymatic activities of PON1 predict CVD the best. A case-control study was conducted in 356 volunteers from Institutes of Health in Nayarit, Mexico. The Case group was divided in two subgroups: cardiovascular risk factors group (CRF) and cardiovascular disease group (CVD). The study was approved by the Institutional Ethics Committee. Written informed consents were obtained from patients in compliance with Good Clinical Practice. PON1 activities were assessed in plasma samples using 4-chloromethylphenyl- nitrate (CMCPase), dihydroucamarin (LACase) and ethyl-paraoxon (PONase) as substrates. Our results showed that the LACase activity was 12 U/mL, 13 U/mL, and 9 U/mL in control, CRF and CVD groups, respectively. The CMCPase activity was 22.06 U/mL, 21.73 U/mL and 14.18 U/mL in control, CRF and CVD groups respectively. Comparing LACase and CMCPase activities, there were significant differences between control and CVD (p<0.0001), and between CRF and CVD (p<0.0001). No significant difference in PONase activity was found. Logistic anal- yses showed that the risk of having CVD decreases (RRR = 0.85, 95% CI 0.79-0.91) as the CMCPase activity increases. Also, the increase in LACase activity diminishes the risk of belonging to the CVD (RRR = 0.56, 95% CI 0.47-0.67). CMCPase or LACase activities had no influence on the risk of having CRF. Further, PONase activity had no influence on the risk of having CVD or CRF. These data together showed that LACase activity was the best predictor of CVD, followed by the CMCPase activity of PON1.

1272 DTMBA Metabolite as a New Biomarker of Exposure to Vinclozolin


Vinclozolin (V) is a fungicide with anti-androgenic properties whose metabolism is not fully understood, and data on urinary elimination of either V or its metabolites are limited. M3 metabolite has been used as biomarker of V exposure; nonetheless, it is a non-metabolite of V and would be generated during the processing of samples for analysis. Furthermore, there is no quantitative information about its generation from the metabolism of V by humans. Therefore, the kinetics of urinary elimination of V and its metabolites, after an oral dose (100 mg/kg) in adult male rats were investigated. V and its metabolites were extracted from urine, then enzymatically hydrolyzed using β-glucuronidase/sul- fate of H. pomatia, and analyzed by HPLC/DAD. Urinary pharmaco- kinetic parameters were calculated using the analute concentrations adjusted by creatinine levels. V and its metabolites DTMBA (formerly denoted as M5), M1, M2 were efficiently detected. The mean urine concentration of DTMBA was the best predictor of CVD, followed by the CMCPase activity of PON1.
Recently, study on circulating microRNAs (miRNAs) as potential biomarkers of drug-induced liver injury (DILI), has received increasing attention. It has been demonstrated that miR-122 and miR-192, which are liver enriched, could be potential biomarkers of DILI. However, these miRNAs cannot discern types of injuries. In this study, we identified plasma miRNAs that can distinguish different types of DILI, hepatic, biliary, or mixed cholestasis and steatosis, using deep sequencing. Male 6-week-old Sprague Dawley rats were treated with acetaminophen (APAP, 1500 mg/kg, p.o.) and thioacetamide (TAA, 100 mg/g, p.o.) for hepatic DILI models, o-naphthylisothiocyanate (ANIT, 150 mg/kg, p.o.) and 4,4'-methyleneedianiline (MDA, 250 mg/kg, p.o.) for cholestasis models, and carbon tetrachloride (CCL4, 300 mg/kg, p.o.) and dexamethasone (DEX, 15 mg/kg, p.o.) for steatosis models. Blood was collected at several time points, and miRNA was extracted from plasma for comprehensive expression analysis of small RNAs, in which more than 300 miRNAs were identified in each DILI model. The expression level was normalized with DESeq package of R software. To compare the expression profiles between different injury models, hierarchical clustering was performed, and we defined early, middle, and late stage of injury based on the cluster patterns. Through differential analysis, we characterized miRNAs that were specifically up- or down-regulated in each DILI model with traditional biomarkers, such as ALT and T-Bil, were observed in the late stage of injuries, whereas miRNA profiles were dramatically changed earlier than those traditional biomarkers. For example, in APAP-induced hepatocellular injury model, miR-let-7b-5p was up-regulated as early as 3 h after dosing, while significant change in ALT level was observed 24 h after. Thereafter, we focused on the DILI type-specific miRNAs which were up-regulated at early stage of injury, and performed RT-qPCR validation. As a result, miR-let-7b-5p for hepatic cellular injury models, miR-143-3p for cholestasis models, and miR-302-3p for steatosis models have been shown increase in the early stage of injuries. Our study suggests potential miRNA biomarker candidates which can detect early stage of DILI in type-specific manners.

Exosomes are nanovesicles present in many body fluids including urine. In the kidney they are secreted by different cell types and contain microRNAs (miRNAs) which change in number and profile depending on pathophysiological processes. They are therefore considered as promising urinary biomarkers for kidney injury. Yet a consensus on a standard method for exosome isolation and exosomal miRNA analysis is still lacking. Currently available isolation methods cause differences in miRNA yield and profile, and in microvesicle (MV) contaminations. Furthermore some require rather large urine volumes. We compared three exosomal miRNA isolation kits regarding their performance in small urine volumes from a rat disease model: (1) Size exclusion chromatography (SEC) columns (Izon) combined with the miRNeasy kit (Exiqon), (2) the precipitation-based mirCURRY Exosome and miRNA Isolation kit (Exiqon) and (3) the Urine exosome RNA isolation kit (Norgen Biotek). Single miRNAs were quantified using the mirCURRY LNA Universal miRNA PCR kit (Exiqon), and Mouse&Rat miRNome Panel I (Exiqon) was used for profiling. The amount of exosomes was quantified by western blotting (WB) for the exosomal proteins Alix and TSG101. Contamination with microvesicles (MV) was addressed with the ER marker protein calnexin, which is also present in MVs. Our results show that it was possible to isolate exosomal miRNA with all three methods: The miRNA yield was highest for the Norgen kit, followed by mirCURRY, but considerably lower for the SEC protocol. According to the WB results, the greatest amount of exosomes was obtained with the miCURRY kit, followed by SEC. MV contamination was present in both preparations to the same degree. WB evaluation of Norgen kit preparations was technically not possible, due to a component in this kit which prevented appropriate protein separation by gel electrophoresis. Based on the best performance in miRNA qPCR and WB, the miCURRY kit was used for miRNA profiling in 1 ml rat urine: Here, 195 out of 246 rat specific miRNAs present on the panel were detectable. In conclusion the miCURRY exosome isolation kit performed overall best in our comparison and was chosen for future assessment of exosomal miRNA profiles in kidney disease models.
MicroRNAs (miRNAs) are short non-coding RNA species that play a critical role in post-transcriptional regulation of gene expression. MiRNAs also serve as a promising source of early predictive biomarkers for different types of health outcomes, although there is limited information on target pathways linked to specific miRNAs and associated dose response metrics. In this case study, we measured liver miRNA alterations using next generation sequencing in male B6C3F1 mice that were exposed to a known liver tumorigen, di(2-ethylhexyl) phthalate (DEHP). For 7 and 28 days at doses of 0, 0.75K, 1.5K, 3K, or 6K ppm in feed. At highest dose tested, DEHP altered 61 miRNAs after 7 days and 171 miRNAs after 28 days of exposure, with 48 overlapping miRNAs. Analysis of these 48 common miRNAs indicated enrichment in pathways related to liver injury and cancer, most notably p53osioner-activated receptor alpha (PARRa) activation, which is the known tumorogenic mode-of-action for DEHP. Of the 48 persistent miRNAs, 10 exhibited a dose trend (two-tailed Jonckheere-Terpstra test p<0.05 for all). Four of these 10 miRNAs were linked to PARRa activation: mir-125-5p, -182-5p, -20a-5p, and -378a-3p. They were subsequently tested across a dose range for DEHP and two related (non-tumorogenic) phthalates, di-n-octyl phthalate (DNOP) and n-butyl benzyl phthalate (BBP), following 7-day exposures using digital drop PCR. CHESS-1 targets were based on transcriptional bench mark dose (BMD) analysis. The DEHP>BPP>DNOP and DEHP>DNOP>BPP for mir-182-5p and -378a-3p, respectively, correctly identifying DEHP as having the greatest potency of the test exposures. Point-of-departure (P0D) dose estimates for DEHP based on these miRNAs (average 163; range 126-202 mg/kg-day) were higher on average than previously calculated values for PARRa target genes. US male (AA) rats experienced a higher tumor incidence derived from the 2-year tumor data (hepatocellular carcinoma BMD = 71 mg/kg-day; hepatocellular adenoma + carcinoma = 35 mg/kg-day). These findings identify putative miRNA markers of PARRa activation. We suggest that early changes are related to type and early down norative chemicals based on transcriptional potency. However, a microRNA BMD estimates were less sensitive compared to most target genes and tumorogenic outcome. This abstract does not represent US EPA policy.

MicroRNAs as Biomarkers of Testicular Toxicity in the Rat


Testicular injury in non-clinical toxicity studies can adversely affect the male reproductive tract by targeting cell types of the testis. This injury can only be detected by measuring changes in testis weight or assessing testicular tissue microscopically. We investigated whether circulating miRNA (miR) levels could serve as biomarkers of testicular injury. miRs were measured in tissues of naive rats (n=4) to identify which miRs were enriched or specific to the testis, 1,3-dinitrobenzene (1,3-DNB; 0.6 mg/kg), carbendazim (CBZ; 400 mg/kg), ethylene glycol monomethyl ether (EGME; 200 mg/kg) or vehicle controls were administered orally to male Sprague-Dawley rats (n=3/group) for 2 or 6 consecutive days. Serum levels of candidate miRs and testosterone levels following toxicant administration were compared to serum miRs in controls. 1,3-DNB and CBZ induced testicular toxicity in all the rats, which was characterized microscopically. Serum testosterone levels were highly variable in 1,3-DNB, EGME and CBZ-treated rats and did not correlate with testicular toxicity. Using TaqMan array, miRs that were ≥ 2.0-fold change compared to vehicle control were identified. Sixty-seven miRs were differentially expressed in the 1,3-DNB Day 2-treated group, while 33 were altered relative to controls after 6 days. Four testicular specific/enriched miRs were detected in serum. For 1,3-DNB treatment, mir-743b was decreased by 10 fold (Day 2) and 4 fold (Day 6), and mir-202-3p was decreased by 2 fold but only at Day 6. Mir-34b was increased by 2 fold at Day 6 while mir-449a was increased by 2.8 fold at Day 2. EGME treatment showed only mir-449a was increased by 2 fold at Day 2 and 47 at Day 6. Mir-743b was increased by 4 fold (Day 2) and 5.6 fold (Day 6) with EGME treatment while mir-202-3p was increased by 2 fold at Day 2 only. Only mir-449a was decreased (12.7 fold) at Day 2 and unchanged at Day 6. In the CBZ-treatment group at Day 2, 39 miRs were differentially regulated after CBZ treatment. The testicular miR, mir-449a, was increased by 2 fold after 2 days of treatment. Mir-449a was decreased 29 fold at Day 2 with CBZ treatment. In conclusion, none of the testes specific/enriched miRs exhibited consistent patterns of change with the testicular toxicants used in this study. Work is ongoing to identify other non-testicular miRs as potential biomarkers of testicular toxicity.

MicroRNAs as Contributors to Prostate Cancer Disparities

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Prostate cancer (PCa) has been the most frequently diagnosed cancer and the second leading cause of cancer deaths among American men. African American (AA) men have higher incidence rates of PCa compared with Caucasian/European American (EA). In addition to socioeconomic and environmental factors, accumulating evidences suggested that biologic and genetic factors may account for part of the observed disparities and influence the PCa recurrence and aggressiveness in AA population. MicroRNAs (miRNAs) are a class of non-coding endogenous RNAs that have been identified to play a role in various types of cancers. In PCa, miRNA deregulation has been implicated in tumor initiation and progression through the regulation of the expression of target genes involved in multiple signaling pathways, including the ones contributing to tumor aggressiveness such as treatment resistance and metastasis development. Moreover, several studies revealed the observed disparities and influence the PCa recurrence and aggressiveness in AA population. In summary, our preliminary data suggest that miRNA-mRNA regulatory network may play a critical role in the PCa aggressiveness and drug resistance in AA patients, promoting the PCa disparities between AA and EA PCa.

Dose-Responsive Analysis of Early MicroRNA Alterations Linked to PPAR-Alpha Activation


EPA, Durham, NC; and

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Unique Set of Non-Coding RNAs Indicates Human and Animal Ovary Lifespan

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Ovary lifespan varies significantly across women due to genetic and environmental factors impacting fertility. Current screening tests for evaluating ovarian reserve are poorly sensitive and specific, with little predictive value. Herein, we report transcriptomic data that associate a cohort of non-coding RNA (ncRNA) markers into a signature of middle-aged ovaries. This unique set includes snorRNAs, miRNAs and one IncRNA. The power was apparent under conditions that affect ovary lifespan - including chronic low-dose exposure to ethylene thiourea. Expression analyses of this set confirmed that ovary lifespan varies across genetic backgrounds in mice and that response to environmental perturbation (diet) is dependent on genetic background. Finally, we report increased of miRNA143 and miRNA145 in follicular fluid of women with diminished ovarian reserve. Their levels inversely correlate with the hormonal profile and the number of the oocytes recruited upon hormonal stimulation, suggesting the assembled ncRNA signature will provide a useful diagnostic tool.

MicroRNAs Dysregulation during Inhibition of Hematopoietic Stem Cell Differentiation Induced by Benzene Metabolite Hydroquinone

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Analysis of the relationship of miRNAs and the inhibition of bone marrow cell proliferation and differentiation induced by benzene metabolites may help to clarify the mechanism of benzene poisoning. In this study, we screened the human hematopoietic progenitor cells (CD34+), isolated from human umbilical cord blood, with benzene metabolite hydroquinone (HQ) for different times and confirmed that HQ inhibits CD34+ proliferation and differentiation. We then screened differentially expressed miRNAs with microarray technology. As a result, we identified 58 differentially expressed miRNAs. Further determination of the miRNAs that
mediated benzene inhibition for mononuclear macrophages and erythrocyte cell differentiation by in vitro models, animal experiments, and benzene poisoning patient investigation. HQ inhibited the differentiation of HL-60 cells into mononuclear macrophages accompanied by an increase in miR-146a expression. We further demonstrated that miR-146 was involved in HQ inhibiting HL-60 cell differentiation into mononuclear macrophages through TRAF6-mediated NF-kB pathway. On the other hand, HQ inhibited the differentiation of K562 cells into erythrocytoid cells; the expression of miR-451a and miR-486 was downregulated. We also found that the decrease of erythrocyte count in peripheral blood was associated with miR-451a and miR-486 downregulation in bone marrow cells after C57BL/6 male mice exposure to benzene for 28 days. In addition, the expression of miR-451a and miR-486 in bone marrow cells was significantly downregulated in benzene poisoning patients with peripheral blood erythrocytes count decreasing. Taken together, our findings suggest that various miRNAs involved in benzene-inhibiting hematopoietic stem cell differentiation and the aberrant miRNAs regulations can potentially be used as biomarkers for benzene-induced leukemias.

### 1282 MiRNAs as Preclinical Predictive Biomarker for Characterization of Drug-Induced Cardiotoxicity


Cardiotoxicity in preclinical toxicology studies is one of the main reasons for drug candidate terminations. There is a need for more sensitive and predictive translational biomarkers. Here we report the findings from a five day dosing and one week recovery mechanistic toxicity study in Wistar Han rats. In this study, known myocardial toxicity causing drugs isoproterenol and doxorubicin were used to evaluate the performance of a panel of miRNAs alongside two internal compounds (BI 49668 and BI 689794) which were not pursued for development due to preclinical cardiotoxicity. Dosing concentrations and routes of administrations of reference and BI compounds were selected based on published literature and previous toxicological studies. To evaluate potential predictive biomarkers in plasma, a panel of miRNAs (using TaqMan qRT-PCR) was measured at various time points during dosing and during a recovery period. To enable additional comparisons of the performance of miRNA biomarkers, protein biomarkers and histopathological analysis, by diograms evaluations were also included in the study. Histopathological analysis at end of treatment showed that isoproterenol treatment induced the expected myocardial cell necrosis and inflammation. Interestingly, BI 689794 showed cardiac histopathological findings similar to that of compounds selected for screening and assessment in BI 689794 treated animals were shown to be dose dependent. Dose and time dependent changes in plasma for the soluble protein biomarkers and candidate miRNAs were also observed when compared with the histopathological findings. In the doxorubicin and BI 49668 treatment groups, both miRNA and protein biomarkers were increased in BI 689794 treated animals shown to be dose dependent. Dose and time dependent changes in plasma for the soluble protein biomarkers and candidate miRNAs were also observed when compared with the histopathological findings. In the doxorubicin and BI 49668 treatment groups, both miRNA and protein biomarkers were increased in BI 689794 treated animals shown to be dose dependent. Dose and time dependent changes in plasma for the soluble protein biomarkers and candidate miRNAs were also observed when compared with the histopathological findings. In the doxorubicin and BI 49668 treatment groups, both miRNA and protein biomarkers were increased in BI 689794 treated animals shown to be dose dependent.

### 1283 Regulation of Hepatic and Pulmonary Polycyclic Aromatic Hydrocarbons (PAHs) Metabolism through TRAF6-Dependent CYP1A1 Induction and the Associated Pulmonary Toxicity Elicited by Hyperoxia

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Polycyclic aromatic hydrocarbons (PAHs) are environmental pollutants with mutagenic and carcigenic properties. Among these PAHs, 3-methylcholanthrene (MC) is one of the most potent carcinogens. In this study, we tested the hypothesis that MC elicits persistent human CYP1A1 induction in vitro. We hypothesized that MC elicits persistent human CYP1A1 induction in vitro as well as lung tumorigenesis. Our laboratory has shown that cytochrome P450 1A1 (CYP1A1) plays a mechanistic role in the suppression of peripherally induced CYP1A1 and CYP1A2 and pulmonary mCYP1A1 was also observed in hGFP1A1/Cyp1a2-null mice groups compared to other mice. hGFPIA1/Cyp1a2-null mice also showed higher heart weight to body weight ratio than hGFPIA1 mice. These results suggested that human CYP1A1 has a major role in tumor induction and CYP1A2 plays a mechanistic role in the suppression of persistent induction of hCYP1A1 as well as lung tumorigenesis in humanized transgenic mice. These results are highly relevant to human lung carcinogenesis. Future studies will be undertaken to select CYP1A2 and candidate miRNAs used to develop novel drugs for the prevention and/or treatment of lung cancer in humans.

### 1284 AHRε and a Critical Aromatic Hydrocarbon Response Element in AhR-Dependent CYP1A1 Induction and the Associated Pulmonary Toxicity Elicited by Hyperoxia

C. Chu, and B. Moorthy. Baylor College of Medicine, Houston, TX.

Excess oxygen is routinely given to preterm newborns with pulmonary insufficiency, as well as to adults suffering from respiratory distress. However, hyperoxia causes lung damage, and this probably due to the formation of reactive oxygen species (ROS). Hyperoxia also induces cytochrome P450 enzyme, CYP1A1 in lungs, and exhibits a protective role against hyperoxia in experimental animals and in cell cultures. Hyperoxia induction of CYP1A1 involves activation of aryl hydrocarbon receptor (AhR), dimerization of AhR and AhR nuclear translocator (ARNT), and the binding of AhR to aromatic hydrocarbon response elements (AHREs) in the promoter/enhancer regions of CYP1A1. In this study, we tested the hypothesis that hyperoxia induces CYP1A1 in H358 pulmonary cells, and that specific AHREs on the CYP1A1 promoter contribute to CYP1A1 induction, in relation to cell toxicity. We observed that 95% oxygen significantly increased ROS (2.42-fold after 72 h incubation), caused apoptosis, and impaired MTT viability (by 32.1% after 48 h incubation) of H358 human pulmonary cells. However, in CYP1A1-H358, a cell line stably overexpressed CYP1A1, hyperoxia-induced cytotoxicity was greatly attenuated. The ROS increase was down to 3.1% at 72 h, the increase of the caspase 3/7 activities was significantly lower, and the decrease of MTT viability was lowered to 12.6%. Luciferase reporter assay showed that hyperoxia induced CYP1A1 promoter activity up to 135%. Induction of CYP1A1 mRNA and enzyme activity was observed in H358 cells, but the induction declined after 24 h. Mutation of AHRε474, but not other tested putative AHREs in the CYP1A1 promoter region, abolished most of the hyperoxia-induced CYP1A1 promoter activity. Electrophoretic mobility shift assay (EMSA) showed that mutation of AHRε474 significantly impaired the formation of complex between CYP1A1 promoter and AhR/ARNT elicited by MC. Chromatin immunoprecipitation in this study demonstrated the pivotal role of AHRε474 in both MC- and hyperoxia-induced CYP1A1 promoter/AhR/ARNT super complex. Our results supported the hypothesis that hyperoxia induced CYP1A1 through binding of AhR/ARNT, to a significant extent, with AHRε474 in the human CYP1A1 promoter/enhancer region. Further studies on the mechanisms of regulation of CYP1A1 by hyperoxia could lead to the development of novel drugs for the prevention and/or treatment of BPDS and ALL/ARDS in humans.
and ethanol increased serum triglyceride, which was also observed in WT mice but not in the CYP2A5 KO mice. The urine levels of cotinine, a nicotine metabolite, were lower in the CYP2A5 KO mice than in the WT mice. These results suggest that CYP2A5 contributes to the nicotine enhancing effects on alcoholic fatty liver through metabolizing nicotine. To further test whether CYP2A5-produced nicotine metabolites contribute to the nicotine enhancing effects on alcoholic fatty liver, cotinine was added to the ethanol liquid diet to replace nicotine. Like nicotine, cotinine enhanced alcoholic fatty liver, which was also observed in WT mice but not in the CYP2A5 KO mice. Nitrotyrosine and malondialdehyde (MDA), markers of oxidative/nitrosative stress, were increased by alcohol feeding and were further increased by nicotine and cotinine. These results suggest that nicotine enhances alcoholic fatty liver in a CYP2A5-dependent manner, which is relevant with oxidative stress resulted from CYP2A5-mediated metabolism of nicotine and cotinine.

1288 Chiral Polychlorinated Biphenyls (PCBs) Are Metabolized to Hydroxylated Metabolites by Human CYP2A6, CYP2B6, and CYP2E1

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Exposure to chiral polychlorinated biphenyls (PCBs) has been associated with neurodevelopmental disorders. Their hydroxylated metabolites (OH-PCBs) are also chiral and potentially toxic to the developing brain; however, the formation of OH-PCBs by human cytochrome P450 isoforms is poorly investigated. To address this knowledge gap, we tested the hypothesis that the biotransformation of 2,2',3,4,4'-pentachlorobiphenyl (PCB 91), 2,2',3,5,5'-pentachlorobiphenyl (PCB 95), 2,2',3,6,6'-hexachlorobiphenyl (PCB 123), and 2,2',4,4',5,5'-hexachlorobiphenyl (PCB 136) is mediated by different human cytochrome P450 isoforms. ADMET Predictor and MetaDrug software were initially used to predict cytochrome P450 isoforms involved in the metabolism of OH-PCBs in silico. These predictions were then validated using CYP1A2, CYP2A6, CYP2B6, CYP2E1 and CYP3A4 in the metabolism of chiral PCBs. Subsequent metabolism studies with recombinant human enzymes demonstrated that CYP2A6 and CYP2B6 oxidized PCB 91 and PCB 132 in meta position and that CYP2A6 oxidized PCB 95 and PCB 136, and formed meta hydroxylated metabolites. Traces of para hydroxylated PCB metabolites were detected in incubations with CYP2E1, whereas no hydroxylated metabolites were detected in incubations with CYP1A2 or CYP3A4. These findings suggest that CYP2A6 and CYP2B6 play an important role in the oxidation of neurotoxic, chiral PCB 95 and PCB 132. Further studies are needed to characterize the enantioselectivity of the oxidation of PCBs by both cytochrome P450 isoforms and assess the toxicity of the resulting OH-PCBs. Supported by ES058605, ES013661 and ES027169. Disclaimer: The findings and conclusions in this presentation have not been formally disseminated by the CDC/ATSDR, the Agency for Toxic Substances and Disease Registry and should not be construed to represent any agency determination or policy.

1289 Enantioselective Formation of Hydroxylated 2,2',3,3',4,4'-Hexachlorobiphenyl (PCB 132) Metabolites by Human Liver Microsomes

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Persistent organic pollutants such as polychlorinated biphenyls (PCBs) affect human health because of their presence in the human diet and air. PCB congeners with 3 or 4 chlorine substituents in ortho position have been associated with neurodevelopmental disorders. Many of these neurotoxic congeners are axially chiral and exist as two stable rotational isomers, called atropisomers, which are non-superimposable mirror images of each other. Hydroxylated metabolites (OH-PCBs) of these PCBs are also chiral and potentially toxic to the developing brain. We hypothesized that the oxidation of PCB 132 by human liver microsomes is enantioselective and varies from individual to individual. Racemic PCB 132 (50 μM) was incubated with pooled (pHLM) or individual human liver microsomes (IHM) for 10 or 30 min at 37 °C. In addition, racemic pHLM (5 μM) was incubated with pooled (pHLM) for 2 h at 37 °C. Levels of 2,2',3,3',4,4'-hexachlorobiphenyl 3'-ol (3'-140; 1:2 shift product), 2,2',3,3',4,4'-hexachlorobiphenyl 5'-ol (5'-132) and 2,2',3,3',4,4'-hexachlorobiphenyl 4'-ol (4'-132) as well as enantiomeric fractions (EF) of PCB 132 were measured using GC-MS. Only 3'-140 EFs were higher compared to 5'-132 EFs, suggesting that PCB 132 is preferentially metabolized to 3'-140 by human liver microsomes.
4'-132 was only a minor metabolite of PCB 132. The second eluting atropisomer of PCB 132 was slightly enriched in 2 h incubations of pHLMs with the low PCB 132 concentration (5 µM). The formation of the first eluting atropisomer of 3'-140 was nearly enantiopure (EF > 0.8). The second eluting atropisomer of 5'-132 was enriched in all microsomal preparations investigated. EF values differed between pHLM preparations. However, all EFs were > 0.84 to 0.96 for 5'-132 and > 0.12 to 0.84 for 3'-140. These findings suggest that there are inter-individual differences in the enantioselective biotransformation of PCB 132 to OH-PCBs in humans.

Studies of the atroposelectivity of toxicity of PCB 132 and its hydroxylated metabolites are needed to determine the role of atroposelective metabolism in PCB-mediated developmental neurotoxicity in at-risk populations.

1290 Male Cyp2b-Null Mice Are Susceptible to Diet-Induced Obesity

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CYP2B metabolizes pesticides, plasticizers, unsaturated fats and more than 25% of drugs available in the market. Recent studies using the cytochrome P450 oxidoreductase knockout mouse treated with unsaturated fatty acids shows activation of CAR and a profound increase of Cypb10 presumably to ameliorate hepatic lipid accumulation. Data from our laboratory using RNAi-mediated Cypb2-knockdown mouse indicated that the older knockout mice were heavier and contain greater serum lipid concentrations, especially males. To investigate the role played by CYP2B in lipid metabolism we have developed a CYPB triple knockout lacking Cypb9b, 10 and 13 using CRISPR/Cas9. We treated them with a 6% fat diet for 10 weeks. The levels of total serum cholesterol, insulin tolerance, and weight gain were examined. Male but not female Cypb2-null mice weigh approximately 15% more than WT counterparts fed a HFD, primarily due to 54% increase in white adipose tissue. Cypb2-null male mice fed a normal diet had higher fasting plasma glucose levels during weeks 5 and 8 compared to WT male mice, but we did not see significant differences between males fed a high fat diet. Cypb2-null females fed a high fat diet appeared to have higher fasting blood glucose levels during week 2 compared to WT, but by weeks 5 and 8 there was no significant differences between the females. Serum parameters indicate that obesity is due to increased hepatic triacylglycerol. CYPB-null HFD-fed mice show more ketoadiposis. In addition, leptin, adiponectin, and cholesterol were increased by 75%, 17% and 7.3% respectively in HFD-fed Cyp2b-null male mice compared to HFD-fed WT mice. Cholesterol, primarily due to increased HDL in both normal diet and HFD fed male and female mice. Liver triglycerides were significantly higher than their similarly treated WT counterparts (ND and HFD), indicating a role for Cyp2b10, 10,13 in fatty acid metabolism regardless of diet. This probably contributed to the lower levels of serum triglycerides in Cypb2-null mice. We are currently determining changes in lipid metabolism genes such as Cypb10, 10, 13 in Cypb2-null mice. Overall, our data indicates that the repression or chemical inhibition of CYP2B may exacerbate metabolic disorders and cause obesity.

1291 The Role of CYP2B in the Metabolism of Unsaturated Fatty Acids

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CYPB26 metabolizes xenobiotic and endobiotic compounds, including drugs, environmental toxicants, steroids, and polycysnatosated fatty acids (PUFAs). Studies indicate that the constitutive androstane receptor (CAR), a potent inducer of Cypb2 has antidiabetic effects, recognizes increased concentrations of PUFAs, and regulates lipid metabolism, potentially through induction of CYPs. Several CYPs hydrolyze or epoxidegenate PUFAs, which is cardioprotective, dilates blood vessels and alters inflammation and pain. We examined the metabolism of PUFAs by hCYP2B6 baculosomes exposed to 30 µM linoleic acid (LA) or 25 µM arachidonic acid (ARA). hCYP2B6 baculosomes increased conversion of the 2 species of LA (29%), PC 40:6-CH3/PE 42:6 of DHA (52%) and PC 38:4-CH3/PG 36:3 of ARA (75%) increased in Cypb9/10/13-null mice. This data indicates that induced or induced-metabolite role of CYP2B. Thus, inhibitors that inhibit CYP2B6 may exacerbate obesity while reducing inflammation. RNAseq was recently performed on the livers from the HFD-fed WT and Cypb29/10/13-null mice. Changes in gene expression, particularly for fatty acid metabolism, gluconeogenesis, lipogenesis, insulin signaling, and inflammation will be evaluated. In conclusion, the Cypb2 enzymes are involved in fatty acid metabolism and obesity; therefore chemical inhibition of these enzymes may exacerbate obesity.

1292 Inhibition of CYP2B6 Does Not Necessarily Alter Toxicity Except in the Case of Chemicals with Known Active Metabolites

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CYP2B6, a member of the cytochrome P450 family, metabolizes many xenobiotics, including clinical drugs, environmental toxicants, and endobiotics such as testosteron and fatty acids. We used the Vivid® CYP2B6 Life Technologies, Grand Island, NY USA) to determine potent inhibitors of CYPB6 because these compounds have the potential to cause adverse drug reactions and alter endobiotic metabolism. CYP2B6 inhibitors with EC50s significantly lower than 1 µM include endosulfan, parathion, atrazine, and nonylphenol. Those with EC50s near 1 µM include triclosan, arachidonic acid (DHA), and eicosapentaenoic acid (EPA). Several endobiotics have EC50s near 1 µM. Inhibition may perturb metabolism of other chemicals, but it does not necessarily indicate key metabolism leading to detoxification or activation. Therefore, we compared toxicity between HepG2 cells and CYPB6-transfected HepG2 cells exposed to nonylphenol, clopidogrel, atrazine, parathion, chlorpyrifos, and triclosan as measured in 96-well plates via MTS assays. We are currently treating cells with endobiotics such as polycysnatosated fatty acids. Somewhat surprisingly the presence of CYPB6 decreased the toxicity of parathion nearly 50% and chlorpyr- ifos 25% probably through increased production of 3,5-trichloro-2-pyr- idinol and p-nitrophenol, relative to chlorpyrifos-oxon and paraoxon, respectively. This is interesting because the primary adverse effects of these chemicals is mediated through the nervous system. In contrast, the presence of CYPB6 in HepG2 cells slightly increased the toxicity of atrazine (10-15%). The other chemicals were not affected by the presence of CYPB6. In conclusion, inhibition of CYPB6 can lead to changes in toxicity for select chemicals.

1293 Humanized, Transgenic Caenorhabditis elegans to Study CYP2B1-Induced Toxicity


Cytochrome P450 2E1 (CYP2B1) metabolizes many small hydrophobic pollutants, drugs, and other xenobiotic compounds. CYP2B1 metabolizes results in detoxification and improved elimination or, paradoxically, bioactivation to reactive metabolites. Although most CYP2B1 research has focused on the endoplasmic reticulum-localized (microsomal) form of the enzyme, eCYP2B1, CYP2B1 also localizes to mitochondria. The mitochondrial form, mtCYP2B1, has been significantly less studied, but it appears that there is significant interindividual variation in organellar targeting. We hypothesize that mtCYP2B1 can drive mitochondrial dysfunction by generating reactive metabolites within mitochondria that damage mitochondrial DNA and/or proteins. To test this hypothesis, novel C. elegans nematode models have been generated that express human wild-type CYP2B1, cCYP2B1, and mtCYP2B1. Purified subcellular fractions from transgenic animals displayed robust CYP2B1 activity in mitochondria and microsomes compared to wild-type N2 nematodes which do not have any detectable CYP2B1 activity. CYP2B1 expression alone causes changes to mitochondrial morphology, manifesting in more fragmentation and disruption of mitochondrial networks compared to age-matched wild-type controls (p<0.001). Furthermore, a 48-hour exposure of adult, CYP2B1-expressing nematodes to the classic CYP2B1-activated drug acetaminophen resulted in significantly more lethality (25% at 3mM, 50% at 5mM) compared to wild-type N2 nematodes, which did not show any lethality up to 25mM acetaminophen. By contrast, wild-type larval nematodes were sensitive to acetaminophen-induced growth delay, possibly due to disruption of developmental signaling, while CYP2B1-expressing nematodes were
3 can restore expression in CYP3A5*3/*3 genotypes. This is of particular interest in that a 6β-hydroxycorticoesterone metabolite can stimulate the MR, which in the kidney leads to sodium retention and excretion of potassium, a potential feedback regulation through a G-quadruplex structure.

1296 Zebrafish CYP3C Regulation by the Aryl Hydrocarbon and Estrogen Receptors

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Mammalian Cytochrome P450 (CYP), family 3 enzymes are highly expressed in detoxification organs and play a critical role in xenobiotic metabolism. In mammals, CYP3s are responsible for the metabolism of over 50% of pharmaceuticals. The zebrafish is a commonly used model organism in biomedical studies, including drug screening. Zebrafish metabolite enzymes vary from that of mammals. In mammals, the CYP3 family contains only 1 subfamily (CYP3A subfamily) while CYP3 genes in fish are diversified and include novel subfamilies (CYP3B, CYP3C, CYP3D). The regulation and functional roles of CYP3B, 3C and 3D proteins are not clear. This study utilizes the zebrafish, which has CYP3A6S and 4 CYP3C genes, to characterize the regulation of novel CYP3 subfamily genes. CYP3Cs are highly expressed in zebrafish liver and/or intestine from at least one gender, suggesting a role in xenobiotic metabolism. Response elements for the aryl hydrocarbon (AHR) and estrogen receptor (ER) are found upstream of CYP3C genes, suggesting a role for multiple nuclear receptors in fish CYP gene regulation. We have exposed zebrafish to 0.001 - 1 microMolar of beta-naphthoflavone (AHR agonist) or 0.001 - 10 microMolar of estradiol (ER agonist) for 96 hours and assessed gene expression in liver, intestine, and gonads using quantitative PCR. Beta-naphthoflavone induced upregulation of CYP1A (positive control) in intestines at 0.4 - 1 microMolar but not in the liver. A dose depended up-regulation of all CYP3Cs was seen in one or more adult female zebrafish organs exposed to beta-naphthoflavone, supporting a role for AHR in regulation of these genes. Exposure to estradiol also caused an upregulation of vitellogenin in liver of both male and female fish at 0.001 - 0.1 microMolar. CYP3C3 and CYP3C4 were induced in the testis; CYP3C1 and CYP3C4 were slightly induced in the ovary. In females, CYP3Cs were induced in the liver while in males CYP3Cs were induced in the intestine after estradiol exposure. At high concentrations, estradiol appears to have a repressive role in CYP3 gene regulation in male and female gonads and female intestine. Understanding how these genes are regulated is important for fully characterizing the zebrafish metabolic capacity and providing insight on the CYP3 family in fish.

1297 Nobel Mechanism of Rodenticide (Warfarin) Resistance of Wild Rats in Tokyo: Enhanced Pentose Phosphate Pathway Causes Rapid Metabolism of Warfarin

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Anti-blood coagulation rodenticides, such as warfarin, have been used around the world. They inhibit vitamin K epoxide reductase (VKOR), necessary for producing several blood clotting factors, which results in lethal hemorrhage. However, heavy usage of them has led to the appearance of rodenticide-resistant rats. To eliminate them, second-generation-rodenticide have been developed, but their high toxicity caused secondary damage to wild species, especially raptors. Thus, it’s necessary to reveal the mechanisms of resistance to establish an effective elimination strategy. There are two major mechanisms of the resistance: I) mutation of the target enzyme of warfarin, VKOR, and II) enhanced metabolism of warfarin. However, there have been few studies regarding hepatic metabolism of warfarin. This study aimed to reveal hepatic warfarin metabolism in resistant rats. To achieve this, we established two closed colonies of roof rats (Rattus rattus). One was warfarin-susceptible rats (S rats) and the other was resistant rats (R rats). Liver perfusion of warfarin was performed to examine hepatic metabolism. The results showed R rats had enhanced hepatic warfarin hydroxylation activity compared to S rats. Then, in vitro warfarin metabolism assay was performed to investigate kinetic parameters of cytochrome P450 (CYPs), which hydroxylate warfarin. We analyzed fractions of liver perfuse from their liver. One was a microsomes (Ms) fraction containing CYPs; the other was Ms fraction, including CYPs and natural NADPH secretion ability. NADPH was added to Ms, and NADP was added to Ms. The Vmax of Ms of R rats showed a modest difference; however, that of Ms of R rats was significantly higher than that of S rats. These results indicated the enhanced metabolism shouldn’t be due to CYPs but NADPH production.
ability. We measured hepatic NADPH production activity by pentose phosphate pathway, a major source of NADPH. R-rats showed enhanced NADPH production compared with S-rats. This is the first report that enhanced pentose phosphate pathway to produce NADPH results in the rodenticide resistance by enhanced metabolism.

**1298 Cytochrome P450 Genes in Viruses and the Enigmatic P450s in Giant Viruses**

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Cytochromes P450 (CYP; P450) genes encode heme thiolate enzymes that occur in the Archaea, Bacteria, and Eukarya. The 2009 report of a P450 in an insect virus, Mimivirus, raised important new questions about P450 biodiversity. The mimivirus established a new type of virus, the nucleocyttoplasmic large double-stranded DNA viruses, or NCLDV; members of the NCLDV are increasingly being isolated and classified in a new order, the Megavirales (the giant viruses). We now report that multiple and unique P450 genes, including one for an unusual fusion protein, are widespread in three families in the Megavirales, the Mimiviridae, the Pandoraviridae, and Mollivirae. The P450 genes in a given viral lineage are closely related, and their molecular phylogeny tends to reflect viral phylogeny. Most of the viruses were found in or carried by acanthamoeba. Data on human patients infected with acanthamoeba raise the possibility of a role of these viruses in human breast tissue, they are polymorphic, and thought to have a role in the pathogenesis of breast cancer.

**1299 Using Budding Yeast to Assess P450-Dependent Toxicity of Breast Cancer-Associated Chemicals**

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Cytochrome P450 (CYP450) phase I enzymes metabolize xenobiotics into an inactive form. However, these enzymes also have the potential to produce reactive, toxic, or carcinogenic compounds. In this study, we are assessing the effect of CYPs on the toxicity of a list of chemicals using recombinant Saccharomyces cerevisiae, or baker’s yeast, as a model. We are using yeast because it is eukaryotic, and many human genes implicated in diseases have yeast orthologs. Also, the three endogenous CYPs, CYP1, CYP5, and CYP61, are involved in nitrile metabolism and xenobiotics. Therefore, the CYP of interest can be expressed in yeast.

**1300 Silensomes as a New Tool to Determine Human CYP450 Contributions (In Vitro Fm) in Drug Metabolism to Predict the Potential Risk of Drug-Drug Interactions**


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The pharmacokinetic (PK) of a drug can be influenced by many extrinsic and intrinsic factors specifically drug-drug interactions (DDI), i.e. drug transporters and metabolic enzymes. Cytochrome P450s (CYP450s) are the major enzymes contributing to the metabolism of small molecules. In order to manage the impact of extrinsic factors on new drug candidates (NCEs) PK, it is crucial to identify and quantify the contribution of CYP450s to its metabolism (fm). FDA and EMA recommend evaluating the in vitro fm, a key parameter, used to predict the impact of a co-administered drug on the clearance of the NCE. In vitro tools used routinely are good for a semi quantitative evaluation of the role of each CYP in the clearance and metabolism of a drug. Moreover, no single assay can provide a robust and quantitative in vitro fm value for different CYPs, it is therefore crucial to have a robust and accurate in vitro model. To address all these issues we have developed a new in vitro CYP phenotyping model, called Silensomes®. Silensomes® are human pooled liver microsomes (HLMs) irreversibly inactivated for one specific CYP450 using mechanism-based inhibitors. The target CYP450s are extensively and selectivity inhibited by the selected MBIs, while non-target CYPs are inhibited by less than 20% of their homologous control activities. This work includes CYP-Silensomes® for CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2D6 and CYP3A4 enzymes. We first characterise each CYP-Silensomes® batch then test their reproducibility and selectivity in predicting the in vitro fm of 20 drugs with in vivo fm values previously published. We found a good correlation between the in vitro fm values evaluated in CYP-Silensomes® and in vivo fm values. In this poster we demonstrate that, through ensuring a long-lasting, complete and specific CYP450 inhibition, CYP-Silensomes® constitute a reliable, easy-and-ready-to-use model that allows the accurate prediction of the in vivo contribution of the major human CYP450 to the metabolism of a drug. These results support the use of CYP1A2-, CYP2A6-, CYP2B6-, CYP2C8-, CYP2C9-, CYP2D6 and CYP3A4-Silensomes® to accurately predict in vitro fm values for use in early and development phases of NCE.

**1301 Characterizing Cryopreserved Cell Lines as an Alternative Hepatic Model for In Vitro CYP450 Induction Assays**

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US Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD. Sponsor: A. Ruff

Cytochrome P450 (CYP450) enzymes are responsible for the majority of drug metabolism. Drugs taken concomitantly may lead to competition for CYP450 active sites; thus, the FDA recommends screening candidate drugs for both CYP450 inhibition and induction interactions. The USAMRICD Absorption, Distribution, Metabolism, Excretion, and Toxicity Center of Excellence (ADMET CoE) currently has an established in vitro assay to evaluate CYP450 inhibition of candidate drug compounds; however, the need to screen for CYP450 induction persists. To further expand the ADMET CoE profiling capabilities, a CYP450 induction assay is being developed using alternative cell lines to primary human hepatocytes to more closely match the metabolic profile of CYP1A2 and CYP3A4 isoenzymes. Drug induction occurs when a drug increases the rate of production of CYP enzymes. As a result, the rates of metabolism of subsequent drugs increase, posing a concern for dosing and safety. Primary human hepatocytes are the gold standard for evaluating drug metabolism. However, they have inherent limitations such as a fastidious nature, significant lot-to-lot variability, and high costs. In this study, induction assessments using cryopreserved hepatocytes and two different cell lines, HepaRG and HepatoCells, were conducted to determine a more robust, cost-effective alternative to hepatocytes. Both cell lines were determined to have a subset of 30 compounds that were highly predictive of the in vivo situation, such as a fastidious nature, significant lot-to-lot variability, and high costs. In this study, induction assessments using cryopreserved hepatocytes and two different cell lines, HepaRG and HepatoCells, were conducted to determine a more robust, cost-effective alternative to hepatocytes. Both cell lines were determined to have a subset of 30 compounds that were highly predictive of the in vivo situation.
real-time quantitative PCR (RT-qPCR). Hepatocytes displayed an average fold change of 118.5 for CYP3A4 and 50.1 for CYP1A2, whereas HepaRG displayed changes of 19.4 and 19.5, respectively. Average values for CYP3A4 and CYP1A2 for HepatoCells were 47.6 and 45.0. Based on the data, HepatoCells most closely mimicked cryopreserved hepatocytes in comparison to HepaRG cells. Further data on optimization of assay parameters will be presented.

**1302** Evaluation of BPAF and BPS Metabolism in Primary Human Hepatocytes (PHHs) and Tx21 Cell Lines

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Bisphenols (BPs) are a group of chemical compounds that have potential endocrine disrupting health effects. However, characterization of their clearance from humans has been limited. The internal exposure and toxicokinetics (e.g., concentrations, half-life) of chemicals like BPs significantly influence their toxicity. To aid in the development of toxicokinetic models, particularly those used for in vitro to in vivo extrapolation of toxic21 data, we evaluate the clearance of two bisphenol analogues in PHHs and three Tx21 cell lines (HepG2, HEH293 and MCF-7 cells). Human hepatic metabolic clearance rates of 7-hydroxycoumarin (7-HC), as a model compound, bisphenol S (BPS) and bisphenol AF (BPAF), a bisphenol A analogue, were determined using suspensions of primary human hepatocytes (PHHs). For this, a 50-donor pooled lot of cryopreserved PHHs was used with initial cell viability of 87.6%. (Cellometer, AOPI staining). Incubations were performed in 96-well plates at a cell density of ~50,000 cells/well in a cell culture incubator (37°C with orbital shaking (2 rpm)) for 0, 15, 30, 60, 120, and 180 minutes (triplicate). LC-MS/MS analysis of acetone extracts was performed to monitor the depletion of parent compounds at concentrations of 1 and 10 µM to derive apparent half-life (T½) and Clearance (Cl) values. There was rapid depletion of 7-HC, at 1 µM (T½=35m, Cl=122.7 ml/min/kg) and 10 µM (T½=47m, Cl=58.0 ml/min/kg). BPS was metabolized at 1 µM (T½=51m, Cl=84.2 ml/min/kg) with lower clearance rates at 10 µM (T½=139m, Cl=30.7 ml/min/kg). In contrast, BPAF was unchanged in these assays at either concentration with estimated half-life of 2,736 minutes and corresponding low Cl=1.56 ml/min/kg at 1 µM, and no depletion with 10 µM BPAF. These data are comparable with literature reports. BPS was appreciably metabolized at 1 µM (T 1/2=139m, Clint=30.7 ml/min-kg) with lower clearance rates at 10 µM (T 1/2=51m, Clint=84.2 ml/min-kg) with lower clearance rates at 10 µM (T 1/2=139m, Clint=30.7 ml/min-kg). In contrast, BPAF was unchanged in these assays at either concentration with estimated half-life of 2,736 minutes and corresponding low Cl=1.56 ml/min/kg at 1 µM, and no depletion with 10 µM BPAF. These data are comparable with literature reports - the main literature metabolite and 3 additional metabolites were observed. Results for carboxyl (4 literature metabolites reported, 8 metabolites detected, 1 metabolite overlap) and paracetamol (4 literature metabolites reported, 5 metabolites observed, 1 metabolite overlap) had some notable differences, with the primary discrepancy being a lack of detected known phase II conjugate metabolites. Possible reasons for this discrepancy include literature metabolites originating from in vivo studies, differences in in vitro incubation conditions, and limitations of the selected analytical system/methods. Future work in this area will focus on enhanced analytical detection capabilities and more efficient interpretation strategies via newly-developed computational and chemoinformatics tools.

**1303** Induction of Cytochrome P450 Enzymes with Benzo[a]pyrene (BaP) and Environmental Mixtures of Polycyclic Aromatic Hydrocarbons (PAHs)

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Many polycyclic aromatic hydrocarbons (PAHs) are procarcinogenic requiring bioactivation to form the ultimate toxicant. PAHs are known for their ability to induce cytochrome P450 enzymes; however, specific PAH doses or time required following exposure causing enzymatic induction are not well known. Induced metabolism caused by repeated exposure to PAHs may alter metabolic rates having consequences for PAH clearance, bioactivation, and detoxification. The goal of this project was to quantify dose and time relationships of PAH enzymatic induction using benzo[a]pyrene (BaP) and a mixture of the top 10 most abundant PAHs found in the Portland Harbor Superfund Site (Supermix 10). B6129SF1/J mice were dosed by oral gavage with daily doses of BaP or Supermix 10 at 5 different doses 2-180 µmol/kg. Mice were euthanized at 6 hr, 24 hr, and 3 days following the initial PAH dose. Levels of active P450 enzymes were measured in hepatic microsomes with activity-based protein profiling. Both BaP and Supermix 10 induced P450 enzymes as early as 24 hr, as observed on SDS-PAGE gels. BaP was a more potent inducer than Supermix 10, as 60 µmol/kg BaP caused significant induction at 24 hr, while higher doses of Supermix 10 (180 µmol/kg) were required. Mass spectrometry identified CYP 1A2 and CYP 2AS as being particularly responsive to PAH induction. Rates of BaP and dibenzo(def,ghi)chrysene (DBC) metabolism will be measured with selected induced and control microsomes to quantify implications of observed metabolic induction. Metabolic rates will be integrated into physiologically based pharmacokinetic (PBPK) models to be used as a tool to predict implications of repeated PAH exposures on internal dosimetry and human risk. Funded by NIEHS Grant No. P42 ES016465.

**1304** A Novel Approach to Rapidly Identify Chemical Metabolites by Combining In Silico Prediction, In Vitro Generation, and Non-Targeted Analytical Detection


Predicting exposure to biologically active entities (parent compounds and/or metabolite(s)) is critical for interpreting in vitro concentration-effect relationships in an in vivo health context. Current workflows for predicting and identifying potentially bioactive metabolites are generally quite laborious/time consuming, tailor-made for pharmaceutical compounds, and/or optimized for metabolites generated mainly from CYP P450 mediated metabolism. To overcome these limitations, we combined: 1) in silico prediction of metabolic structures; 2) in vitro generation of human metabolites using primary human hepatocytes (which are capable of both phase I and phase II metabolism); and 3) high-resolution mass spectrometry to identify metabolites of several benchmark compounds (i.e., paracetamol, carbyxl, and paracetamol). In silico metabolite prediction was first performed using MetaSense (ACD Labs, Toronto) and the OECD QSAR toolbox 4.0 (LMC, Bulgaria). In vitro cell-based incubations were then carried out with human cryopreserved hepatocytes. The incubation medium was analyzed using an Agilent QTOF high-resolution mass spectrometer in a non-targeted fashion. MetaSense was then used to align spectral data with predicted metabolites. Finally, predicted and identified metabolites were compared to listings of known human metabolites as previously reported in the literature. Empirical results for methylparaben showed reasonable agreement - the main literature metabolite and 3 additional metabolites were observed. Results for carboxyl (4 literature metabolites reported, 8 metabolites detected, 1 metabolite overlap) and paracetamol (4 literature metabolites reported, 5 metabolites observed, 1 metabolite overlap) had some notable differences, with the primary discrepancy being a lack of detected known phase II conjugate metabolites. Possible reasons for this discrepancy include literature metabolites originating from in vivo studies, differences in in vitro incubation conditions, and limitations of the selected analytical system/methods. Future work in this area will focus on enhanced analytical detection capabilities and more efficient interpretation strategies via newly-developed computational and chemoinformatics tools.

**1305** Impact of NQO1 on Aristolochic Acid I DNA Adduct Formation in Human Renal In Vitro Systems


Aristolochic acid I (AAI) is a major constituent of several Aristolochia species, native in the Balkan region and component of traditional herbal Chinese medicine. AAI is considered to be the cause of Balkan endemic or Aristolochic acid nephropathy (BenN/AAN), a unique type of progressive renal fibrosis, frequently diagnosed together with upper urethral cancer. It is currently hypothesized that following enzymatic activation (e.g. NQO1), AAI metabolites react with genomic DNA to form persistent DNA adducts with deoxyadenosine (dA) and deoxyguanosine (dG), generating a unique mutational spectrum. The aim of this project was to investigate the importance of NQO1 in the metabolic pathway of AAI leading to highly reactive metabolites, responsible for DNA adduct formation and the formation of the AAI-DNA adducts. A sensitive UPLC-MS/MS method to quantify da-AAI adducts in DNA samples was established. Synthesized 14N-labeled and unlabeled da-AAI adduct standards in Zn++-catalyzed biomimetic reactions were used to verify the analysis. The presence/absence of NQO1 in renal cells was tested by Western Blot analysis and activity assays, confirming the absence of NQO1 in HEK293 cells and NQO1 presence in an hTERT immortalized human renal proximal epithelial cell line with a primary cell like phenotype (RPTEC/TERT1). The absence of NQO1 in HEK293 cells was exploited to generate a stable NQO1-GFP overexpressing HEK293 cell line to determine the importance of this NQO1 in the metabolic pathways of AAI activation. NQO1-GFP overexpressing HEK293 cells had demonstrable in vitro NQO1 metabolic activity. Yet despite this, NQO1-GFP overexpressing HEK293 cells did not present with higher da-AAI-adduct levels than control HEK293 cells. Moreover, da-AAI-adduct levels in all HEK293 cells with/out NQO1 were significantly lower.
than those observed in RPTEC/TERT1 cells following exposure to AAI. Although the current findings suggest a limited importance of NOQ1 in the metabolism of AAI to the highly reactive N-hydroxyaristolactam, future studies targeting additional phase I enzymes, i.e. cytochrome P450, may lead to the discovery of further metabolites. Altogether, human RPTEC/TERT1 cells appeared a more appropriate renal cell model for investigating mechanism(s) underlying AAI-mediated DNA adduction in vitro compared to HEK293 cells.

1306 Genetic Polymorphisms in the Canine Glutathione S-Transferase P1 (GSTP1) Gene Promoter

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Genetic polymorphisms within the glutathione S-transferase P1 (GSTP1) gene affect the elimination of toxic xenobiotics by the GSTP1 enzyme. In dogs, exposure to environmental chemicals that may be GSTP1 substrates is associated with cancer. The objectives of this study were to investigate the genetic variability in the GSTP1 promoter in a diverse population of 278 purebred dogs, compare the incidence of any variants found between breeds, and predict their effects on gene expression. To provide information on ancestral alleles, a number of wolves, coyotes, and foxes were also sequenced. Fifteen single nucleotide polymorphisms (SNPs) and two microsatellites were discovered. Three of these loci were only polymorphic in dogs while three other SNPs were unique to wolves and coyotes. The major allele at c.-46 is T in dogs but is C in the wild canids. The c.-185 delT variant was unique to dogs. The microsatellite *16*C in greyhounds, *6*A in labrador retrievers, *9*A in golden retrievers, and *8*A in standard poodles, Beagles, and Siberian huskies exhibited minimal haplotypic diversity. Compared to the simple 16*1 allele, the compound 16*2 allele (found in 12% of dogs) was the mechanism for the multiple microsatellites observed. Twenty-eight haplotypes were constructed in the dog, and an additional 8 were observed in wolves and coyotes. While the most common haplotype across breeds was the wild-type *17*A, other prevalent haplotypes included *3*A (11%) in greyhounds, *6*A (16%) in labrador retrievers, *9*A (16%) in golden retrievers, and *8*A (19%) in standard poodles. Dogs and other canids exhibit extensive variation in GSTP1 transcript. Dogs and other canids exhibit extensive variation in the GSTP1 promoter. Genetic polymorphisms within distinct haplotypes prevalent in certain breeds can affect GSTP1 expression and carcinogenic detoxification, and thus may be useful as genetic markers for cancer in dogs. Reporter studies are currently underway to assess the effects of these variants on GSTP1 function.

1307 Enzyme- and Site-Specific Functional Characterization of Glutathione S-Transferases by Activity-Based Protein Profiling


Glutathione S-transferases are comprised of a highly diverse family of phase II drug metabolizing enzymes whose function is the catalysis of glutathione conjugation to a variety of endo- and xenobiotics, including reactive oxygen species generated by endogenous metabolism. Cytosolic GSTs are comprised of seven distinct classes of enzymes, all of which contain two active sites: the conserved, glutathione-binding "G" site, and the variable substrate-binding "H" site. To date, the contribution of GSTs to drug metabolism has largely been elucidated via measurement of expression (mRNA and protein data) and formation of glutathione-substrate conjugates (including commercially available GST substrates). These methods, while useful, cannot measure enzyme-specific activities. Therefore, we developed two activity-based probes that irreversibly bind GST active sites and report on their activity within tissue lysates from myriad organs. The probes successfully targeted active GSTs from all mammalian cytosolic GST classes. After validating their efficacy using a series of competitive GST inhibitors, we utilized these probes to detect enzyme-specific changes in active intestinal GSTs resulting from diet-induced obesity. We detected significant increases in active GSTM4, M1, and P1 enzymes in response to an obesogenic diet. Changes in the physiological state of an organism such as obesity, have been shown to result in concomitant changes in drug metabolism, making it difficult to determine appropriate dosages for often unrelated health problems. We assert that further research using these activity-based probes will advance our understanding of mammalian phase II drug metabolism. Funded by NIEHS Grant No. P42 ES016465.

1308 Genotoxicity and Expression of Stemness Genes after Benzo[a]pyrene Treatment of the Gastric Cancer SNU-1 Cell Line

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Gastric cancer (GC) is highly metastatic and tumorigenic, and is the third leading cause of global cancer-related death. Benzo[a]pyrene (BaP), an IARC Group I human carcinogen, is a ubiquitous environmental carcinogen. Exposure to BaP is epidemiologically associated with gastric cancer and induces gastric tumours in laboratory animals. BaP is known to be metabolically activated to DNA damaging derivatives by CYP1 family enzymes, however, the mechanisms whereby BaP induces gastric tumours remains unclear. Here we have used a human gastric cancer cell line, SNU-1 cells, to explore the mechanisms of BaP toxicity. BaP induced DNA damage in treated SNU-1 cells, as assessed by a micronucleus assay. The treatment induced a number of established BaP-AhR associated genes (CYPs) but also genes commonly associated with epithelial to mesenchymal transition and cell stemness (e.g. Oct3/4 and SNAIL-1). These observations implied that the SNU-1 cell line is phenotypically plastic and is capable of expressing side populations with stem cell properties. Using flow cytometry, we identified several side populations with a more stem-like phenotype, including populations with elevated CD44 and CD166. Upon culture, these enriched side-populations lost expression of their CD markers and stem-like phenotype and regressed towards the parental SNU-1 phenotype. We suggest the relative plasticity of gastric cancer cells is a contributing factor in BaP induced carcinogenicity.

1309 The Effect of Interleukins 8 and 10 on Carcinogen Activation and DNA Damage in Colorectal Cancer Cells

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Colorectal cancer (CRC) is a key cause of illness and death worldwide, accounting for more than 9% of all occurrence of cancer. Lifestyle factors such as diet, smoking and alcohol are known to contribute to the incidence of CRC and inflammation occurring in the colonic microenvironment supports development and progression of cancer. Exposure to dietary pro-carcinogens such as benzo[a]pyrene (BaP) and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) can lead to the formation of DNA adducts, causing DNA damage and mutations in epithelial cells, mediated through cytochrome P450 (CYP) enzymes. Additionally, the presence of inflammatory mediators in the microenvironment appears to promote tumorigenicity as cytokines and their receptors function as regulators of metastatic cancer. Amid these, expression of IL-8 and its receptor CXCR2 feature prominently in colon cancer. On the other hand, IL-10 an immunoregulatory cytokine appears to have dual function as pro-angiogenic and also as an immunosuppressive within the tumour microenvironment. To determine whether IL-8 and IL-10 can influence the activation and DNA damage potential of chemical carcinogens, we investigated their effects on BaP and PhIP in the Gastric Cancer SNU-1 Cell Line. We found that cytokine treatment of HCT116 significantly increased cell proliferation and promoted cell migration using a wound-closing assay. These treatments also induced CYP1A1, CYP1B1 and CYP2E1 expression, thereby potentially promoting the metabolic activation of pro-carcinogens such as BaP and PhIP. Whilst BaP and PhIP treatment of HCT116 cells caused pronounced dose-dependent genotoxicity, treatment with IL-8 or IL-10 individually did not lead to increased genotoxic damage, as assessed by a micronucleus assay. Surprisingly, we found that treatment of HCT116 cells with BaP and PhIP induced a constitutive expression of IL-8 and IL-10, promoting an inflammatory microenvironment. These data indicate that the ability of dietary carcinogens such as BaP or PhIP to damage DNA is also associated with an inflammatory response with induced cytokine mediator release from colonic epithelia cells and this encourages the development of meta-static phenotype within the microenvironment.
Lung cancer is the leading cause of cancer-related deaths in the United States. Polycyclic aromatic hydrocarbons (PAHs) are a group of chemicals that increase the risk of lung cancer in humans. PAHs induce phase I and II metabolism enzymes through aryl hydrocarbon receptor (AhR)-dependent and independent pathways. Cytochrome P450 (CYPs) enzymes play a key role in these phase I enzymes and promote detoxification of PAHs. However, the CYP1 family is also involved in the bioactivation of PAHs, leading to the formation of reactive intermediates that can form mutagenic DNA adducts. In this study, we hypothesized that CYP1 enzymes play a pro-carcinogenic role in 3-methylcholanthrene (MC)-mediated carcinogenesis and tumorigenesis. To test this hypothesis, C57BL/6J (WT) and CYP1A1/1A2/1B1-triple knockout (CYP1-3KO) mice were exposed to MC (100μM/kg) or corn oil (vehicle) via a single intraperitoneal injection. Liver and lung tissues were harvested at 1, 8, and 15 days post exposure. Protein levels were measured via western blotting; RNA levels were measured via RT-qPCR. Genomic MC-DNA adducts were analyzed by the nuclease P1-enhanced 32P-postlabeling. We found that MC caused significant induction of hepatic CYP1A1/1A2 gene expression in WT but not CYP1-3KO mice at each time point. WT and CYP1-3KO mice showed similar induction of hepatic NAD(P)H dehydrogenase quinone 1/2 (NQO1/2) transcription after MC treatment. Interestingly, CYP1-3KO mice showed prolonged hepatic AhRR RNA expression compared to wild-type controls throughout the 8-day time point (p=0.00012). MC treatment caused formation of DNA adducts in liver and lung for up to 15 days post MC, however, adducts were markedly diminished in the 3KO mice (~80%). The significant reduction in MC-DNA adducts in the liver and lungs of 3KO mice provide direct evidence of the mechanistic role of these enzymes in the formation of DNA adducts by MC. The persistent AhRR transcription in the 3KO mice suggest that the AhR is persistently activated, likely due to high levels of parent MC and/or the metabolites that metabolize MC (i.e., CYP1) are absent in these mice. These results are highly relevant to PAH-mediated carcinogenesis, as DNA adducts represent the initiation phase of carcinogenesis. Further studies to inhibit these enzymes could lead to the development of novel drugs for the prevention of lung cancer in humans by PAHs.
were classified for sensitivity towards ER stress which could be related to their background such as their disease status. Both up and down-regulated gene networks could be identified for each individual and their overlap. Furthermore, the variance among individuals in the degree of activation of one of the three UPR branches related to activation of apoptotic signaling could be identified. Moreover, distinction could be made between sets crucial in the initial phase and in the late phase of UPR activation. In conclusion, profiling of the inter-individual variance in chemical-induced UPR activation will aid in the improved understanding of the variance in susceptibility towards DILI among patients.

1314 Aryl Hydrocarbon Receptor (AhR) Mediated Short-Term Effects of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) on Bile Acid Homeostasis in Wild-Type and AhR-Null Mice
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The effects of the most potent aryl hydrocarbon receptor (AhR) agonist, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on bile acid (BA) homeostasis was examined in male and female wild-type and AhR-null mice shortly after 4-day exposure, rather than at a later time when secondary non-AhR dependent effects are more likely to occur. TCDD had similar effects on BA homeostasis in male and female mice. TCDD approximately halved the concentration of total- (Σ) BAs in liver (all major BA categories, with the exception of non-6,12-OH BAs), without decreasing the expression of the rate limiting BA synthetic enzyme (Cyp7a1) or altering the major BA regulatory pathways (FXR) in liver and intestine. Even though the Σ-BAs in liver were markedly decreased, the Σ-BAs excreted into bile were not altered. TCDD decreased the relative amount of 12-OH BAs (CA, TCA, TDCA, DCA) in bile and increased the biliary excretion of TCDD and its metabolites (TaMCA, TUDCA); this was likely due to the decreased Cyp8b1 (12α-hydroxylase) in liver. The concentration of Σ-BAs in serum was not altered by TCDD, indicating that serum BAs do not reflect BA status in liver. However, proportions of individual BAs in serum reflected the decreased expression of Cyp8b1. All of these TCDD-induced changes in BA homeostasis were absent in AhR-null mice. In summary, TCDD through the AhR decreases markedly BA concentration of Σ-BAs in serum reflected the decreased expression of Cyp8b1. All of these TCDD-induced changes in BA homeostasis were absent in AhR-null mice. Non-AhR dependent effects are more likely to occur. TCDD had similar effects on BA homeostasis in male and female mice. TCDD approximately halved the concentration of total- (Σ) BAs in liver (all major BA categories, with the exception of non-6,12-OH BAs), without decreasing the expression of the rate limiting BA synthetic enzyme (Cyp7a1) or altering the major BA regulatory pathways (FXR) in liver and intestine. Even though the Σ-BAs in liver were markedly decreased, the Σ-BAs excreted into bile were not altered. TCDD decreased the relative amount of 12-OH BAs (CA, TCA, TDCA, DCA) in bile and increased the biliary excretion of TCDD and its metabolites (TaMCA, TUDCA); this was likely due to the decreased Cyp8b1 (12α-hydroxylase) in liver. The concentration of Σ-BAs in serum was not altered by TCDD, indicating that serum BAs do not reflect BA status in liver. However, proportions of individual BAs in serum reflected the decreased expression of Cyp8b1. All of these TCDD-induced changes in BA homeostasis were absent in AhR-null mice. In summary, TCDD through the AhR decreases markedly BA concentration of Σ-BAs in serum reflected the decreased expression of Cyp8b1. All of these TCDD-induced changes in BA homeostasis were absent in AhR-null mice.

1315 Mitofusin-2 Deacetylation by Sirtuin-1 Mitigates Ischemia/Reperfusion Injury in Aged Livers

Hepatocellular depletion of sirtuin 1 (SIRT1) is a causative factor for cell death by prolonged ischemia. Here we investigated potential alterations in SIRT1 and its mitochondrial interactor and deacetylation target mitofusin-2 (MFN2) in aged livers, and its impact on ischemia/reperfusion (I/R) sensitivity and impaired reparative mechanisms. Young (3 month) and old (23-36 month) C57BL/6 mice were subjected to hepatic ischemia/reperfusion in vivo. Primary hepatocytes from each age group were also exposed to simulated in vitro I/R. Biochemical, genetic and imaging analysis were performed to assess cell death, autophagy flux, and mitochondrial function. Compared to young mice, old mice showed increased mortality and greater hepatocyte cell death following mild I/R injury. Biochemical analysis indicated a near-complete I/R-dependent loss of both SIRT1 and MFN2, which did not occur in young cells. Adenoviral overexpression of either SIRT1 or MFN2 alone in old hepatocytes failed to mitigate I/R injury, while co-overexpression of MFN2 and SIRT1 was significantly protective, by enhancing autophagic flux and preventing mitochondrial permeability transition (MPT) onset during reperfusion. Mutagenesis of putative SIRT1 target lysine residues on MFN2 implicated C-terminal lysines (K655 and K662) for mediating SIRT1-dependent autophagy activation. The SIRT1-MFN2 axis is pivotal during I/R recovery and may be novel therapeutic targets to reduce I/R injury in aged livers.

1316 Use of a Population-Guided Approach to Identify Novel Genetic Modulators of TCDD-Induced Liver Toxicity
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2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is a persistent and pervasive environmental toxicant that has been linked to wide array of disease states including cancer, immunosuppression, and metabolic syndrome. TCDD-induced toxicity is known to be primarily mediated through the aryl hydrocarbon receptor (AhR), a ligand-activated transcription factor. Though our understanding of AhR-regulated signaling has expanded, much remains unknown about how downstream gene dysregulation leads to TCDD-induced diseases. Previous studies have indicated that individuals respond differently to TCDD. Here, a genetically diverse mouse panel of 14 strains were dosed with 0.1, 1, or 100 ng/kg of TCDD for 10 consecutive days. At the end of dosing, liver samples were collected and the expression levels of 9 AhR-target genes for each strain and dose was measured. Notably, there were definite patterns of strain-specific expression that did not always correspond to the AhR allele found in the specific mouse line. The gene expression data were utilized to identify quantitative trait loci (QTL) in the mouse genome associated with the differing AhR-mediated responses. Statistically significant hits (p<0.05) were found on chromosome 2, 9, and X with overlaps across multiple AhR-target genes. While these overlapping significant regions may indicate variants associated with the inter-strain differences, they may also indicate novel genetic modulators of AhR-mediated gene transcription in the liver. As such, further analysis of the genes located within these QTLs may aid in identifying which sub-populations of humans with altered TCDD sensitivity and suggest possible mechanisms that explain these variant sensitivities.

1317 Direct-Acting Antivirals for Chronic Hepatitis C Treatment: Can “Rule-of-Two” Model Predict Potential for Liver Injury?
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Chronic hepatitis C is a major public health burden both in the United States and also globally. Direct acting antivirals (DAAs) provide treatment options with proven efficacy for chronic hepatitis C virus (HCV) infection and majority of patients can be successfully cured with these therapies. Postmarketing experience has shown that certain DAAs containing regimens are associated with significant hepatotoxicity in patients with advanced liver disease. This further indicates the need for additional tools to predict or identify signals associated with drug-induced liver injury during clinical drug development. Here, we retrospectively investigated whether the previously reported “rule-of-two” model, namely oral medications given high dose (i.e. ≥ 100 mg/day) and of high lipophilicity (i.e. logP ≥ 3), could predict the risk of hepatotoxicity associated with the use of DAAs. The “rule-of-two” model successfully identified three DAA regimens (i.e. asunaprevir, simeprevir and Viekira Pak) associated with hepatotoxicity, and the model’s analysis of DAAs is generally in agreement with the hepatotoxic risk observed in the post-marketing surveillance. Our study suggested the “rule-of-two” model has the potential as an additional tool in the comprehensive assessment of clinical trial data to detect signals associated with drug-induced liver injury during clinical drug development. Here, we retrospectively investigated whether the previously reported “rule-of-two” model, namely oral medications given high dose (i.e. ≥ 100 mg/day) and of high lipophilicity (i.e. logP ≥ 3), could predict the risk of hepatotoxicity associated with the use of DAAs. The “rule-of-two” model successfully identified three DAA regimens (i.e. asunaprevir, simeprevir and Viekira Pak) associated with hepatotoxicity, and the model’s analysis of DAAs is generally in agreement with the hepatotoxic risk observed in the post-marketing surveillance. Our study suggested the “rule-of-two” model has the potential as an additional tool in the comprehensive assessment of clinical trial data to detect signals associated with drug-induced liver injury during clinical drug development.

1318 Integrated Transcriptomics and Metabolomics Analysis of Human Hepatoma HepaRG Cells Exposed to PCB 126

The development of toxicant-associated fatty liver disease is complex and cannot be reduced to a unique chemical-receptor interaction. A better knowledge of liver response to toxic compounds at the molecular level is needed in order to establish adverse outcome pathways. We have determined transcriptome and metabolome signatures of the effects of a 10-day exposure to polychlorinated biphenyl 126 (PCB 126, at 100 µM, 10 nM and 1 µM) in differentiated HepaRG cells. The metabolome platform detected 802 metabolites. An OPLS-DA model (R2X = 0.177, R2Y = 0.769, and Q2 = 0.58) properly classified all samples.
A total of 30 metabolites had their levels disturbed by the PCB 126 treatment (p < 0.05, one-way ANOVA test adjusted for multiple comparison with Fisher’s least significant difference). There was a dose-dependent response to PCB 126 exposure, with changes in cell wall constituents (ceramides and sphingomyelins), energy production (glycolysis and carbohydrate pathways), tryptophan and glutathione metabolism, and free fatty acid levels. The most significant effect was a decrease in free long chain fatty acids (LCFAs) and polysaturated fatty acids (PUFAs) levels. These changes are consistent with a general liver toxicity phenotype expected from PCB 126 exposure. Transcriptome profiles were then analysed using illumina-based RNA sequencing. The data was processed with the new Tukey protocol (Hilut2, Stringtie, Ballgown). A standard linear model-based comparison of transcript abundance revealed that a total of 2, 17 and 219 transcripts had their levels altered at the concentrations of 100 pM, 10 nM and 1 uM, respectively (q<0.05, fold changes +/- 1.5). Gene functions altered were representative of an activation of xenobiotic metabolism by dioxin-like compounds. In conclusion, our data confirms that HepaRG cells are a sensitive model to assess hepatotoxicity. The most sensitive biomarker reflecting the effects of PCB 126 in our study was the disturbance of long-chain polysaturated fatty acids status.

**1319 Using Points of Departure from Partial Concentration-Response Relationships for Cytotoxic TNS-Drug Interaction to Classify Drugs According to Their Potential to Cause Idiosyncratic, Drug-Induced Liver Injury**

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Idiosyncratic, drug-induced liver injury (IDILI) typically affects a small fraction of patients when it occurs, although the effects can be serious and include death. Recent results suggest that tumor necrosis factor-alpha (TNF) interacts with drugs that cause IDILI to kill hepatocytes. Using a set of 24 drugs, we demonstrated previously that cytokotixity in the presence of TNF is used to classify drugs into voltage dependent regions. To confirm the sensitivity of the approach in preclinical evaluation of drug candidates, we hypothesized that successful classification could be accomplished using only data from the low end of the CRRs. We tested this hypothesis using the same data set as previously modeled; however, concentration-response data were censored above the EC50 to conduct CRR modeling using only the low-dose region. Multiple curve-fitting models were compared for their ability to fit the low-dose region for each CRR. Akaike Information Criteria (AIC) were used to select an optimal fitting model for each drug to be used to estimate a point of departure (POD) for the CRR. PODs were estimated for each drug in the absence and presence of TNF coexposure (covariates 1 and 2). These PODs, the difference between PODs (covariate 3), and the ratio of PODs for vehicle and TNF-treated cells (covariate 4) were used to create statistical classification models. Performance of the models was evaluated using receiver operating characteristic (ROC) analysis. Individually, covariates 1 through 4 yielded areas under the ROC curves (AUCs) of 0.72, 0.79, 0.83, and 0.83, respectively. The classification model incorporating both PODs (i.e., covariates 1 and 2) classified drugs with high selectivity and specificity and an AUC of 0.96. These results suggest that models employing covariates comprising PODs estimated from incomplete CRRs can accurately classify drugs according to their IDILI potential. Supported by NIH grant R01 DK112695.

**1321 Methionine Alleviates Cholesterol-Induced Increases in Inflammatory Markers in Sprague-Dawley Rats**

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Cardiovascular disease (CVD) is the leading cause of death worldwide. Western diets are mainly composed of meat and meat-based products which are rich in both cholesterol (Cho) and methionine (Met). Homocysteine (Hcy) is an intermediate in the metabolism of Met and consumption of high levels of Met has been shown to increase circulating levels of Hcy. Both Cho and Hcy are documented risk factors for CVD/atherosclerosis, an inflammation-based disease. The goal of the present studies was to test whether the combination of Met+Cho exacerbated the inflammatory effects of Met or Cho individually. After 35 days of ad libitum consumption of diets high in either Met or Cho, or a combination of both, body weight, energy intake and serum leptin were significantly lowered in the rats fed Met or a combination of Met+Cho. In the liver, genes for pro-inflammatory cytokines (IL-1, TNF, Mip-1) and Klf-2 were upregulated in rats fed the Cho diet compared to controls and the other dietary groups. In contrast, Met alone or Met+Cho had no detectable effect on expression of inflammatory markers in the liver. Moreover, maximum hepatic accumulation of triglycerides was seen in rats fed Cho diet which was reduced in rats fed either Met or Met+Cho. Thus, based on hepatic accumulation and bile control of hepatic inflammatory markers, our findings make a strong case that Cho alone represents a more significant risk factor than high Met. Further, high dietary Met ameliorates the adverse effects produced by Cho alone.

**1322 Chronic Polychlorinated Biphenyl (PCB) Exposure Disrupts Hepatic, Xenobiotic, and Intermediary Metabolism and Increases Hepatic Inflammation/Injury**

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Polychlorinated biphenyls (PCBs) are endocrine and metabolism disrupting chemicals. Epidemiological studies have shown that PCB exposure is associated with steatohepatitis and diabetes. The aim of this study was to investigate whether chronic PCB exposure could disrupt hepatic metabolism and cause liver injury in a two-hit mouse model. Eight week old male C57BL/6 mice were fed a high fat diet (42% fat) and treated with either Aroclor 1260 (20 mg/kg p.o), a NDL PCB mixture, or PCB126 (20 mL/kg p.o), a single DL PCB congener, or vehicle control for 12 weeks. The mixture and doses used were based on human bio-accumulation patterns which show high levels of heavily chlorinated NDL PCBs and relatively low levels of DL PCBs. After euthanasia, tissues were obtained for analyses. As anticipated, AhR target gene, Cyp1a2 was induced by PCB126 exposure, while the constitutive androstane receptor (CAR) target gene, Cyp2b10 was induced in mice treated with Aroclor 1260. Neither body weight nor liver weight was changed with PCB treatment, but the epididymal fat weight was increased in the Aroclor 1260 group. Histological data showed that the extent of hepatic steatosis, triglycerides and lipid levels were similar in all groups. ALT was increased by Aroclor 1260 exposure, while AST was decreased by cholestatic liver injury. In this model, impaired bile flow elicits hepatocyte injury and inflammation, leading to myofibroblast activation and collagen deposition in the periportal and portal regions of the liver. The goal of this project was to determine how AhR activation by TCDD impacts the progression of liver fibrosis in BDL mice. We hypothesized that TCDD treatment would increase inflammation and myofibroblast activation in BDL mice and exacerbate the development of fibrosis. To test this, male C57BL/6 mice were treated with TCDD (20 μg/kg) or peanut oil (vehicle) one day prior to BDL or sham surgery. Mice were euthanized 3, 7, or 14 days post-surgery. Results indicate that TCDD treatment exacerbated liver injury and inflammation in BDL mice, based on elevated collagen content levels of alanine aminotransferase and increased inflammatory cell infiltration to the liver. TCDD was found to prolong and increase liver damage and inflammation 14 days after BDL surgery. Furthermore, exposure to TCDD increased mRNA levels of myofibroblast activation markers, aSMA, Col1α1, and Colα1. Despite these increases, TCDD collagen content levels of liver fibrosis was similar in vehicle- and TCDD-treated BDL mice. These results support the notion that TCDD increases myofibroblast activation in vivo, yet whether this occurs through a direct or indirect mechanism remains unclear. In addition, the finding that TCDD increased collagen transcript levels but not hepatic collagen content raises the possibility that TCDD enhances extracellular matrix turnover, which would presumably limit collagen deposition despite increased liver injury and inflammation.
PCB126 exposure. Ar126 treated animals had increased lobular inflammation and expression of pro-inflammatory cytokines (Tnfa and Cxcl8). Hepatic genes related to fatty acid synthesis (Srebp1, Fasn, and Scd1) and lipoxigenase (Lipox) expression were increased in Ar126 treated animals. Glucose and insulin tolerance were not affected by PCB exposure. However, mice treated with PCB126 had lower fasting blood glucose associated with decreased expression of the glucose regulatory gene, Gck1. Chronic PCB exposure disrupts hepatic xenobiotic and intermediary metabolism, to influence hepatic inflammation and injury. These data suggest that DI and NDLC PCB exhibited slightly different effects which could be due to, in part, to differential receptor activation.

1323 TCD2 Alternates Polysaturated Fatty Acid Metabolism and Eicosanoid Biosynthesis in Female Sprague Dawley Rats

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2,3,7,8-tetrachlorodibenzop-dioxin (TCD2) is a potent aryl hydrocarbon receptor (AhR) agonist that elicits a broad spectrum of dose-dependent effects in the liver, including hepatic lipid accumulation coupled with inflammation at higher doses. We hypothesized that changes in polysaturated fatty acid (PUFA) metabolism would be coupled with dysregulation of inflammatory lipid mediators and play an integral role in TCD2-mediated hepatotoxicity in female Sprague-Dawley (SD) rats. Female SD rats were gavaged with sesame oil vehicle or TCD2 (0.01-10 µg/kg) every 4 days for 28 days. The integration of hepatic RNA-Seq data with untargeted metabolomics identified dose-dependent changes in linoleic acid (LA) and arachidonic acid (AA) metabolism. AA and LA are metabolized via cyclooxygenase, lipoxigenase, and cytochrome P450 epoxidiase/hydroxylation pathways. TCD2 elicited dose-dependent hepatic gene expression changes in all eicosanoid biosynthetic pathways with corresponding changes in PUFA and their eicosanoid metabolites. Overall, there was a dose-dependent increase in total ω-6 PUFA’s, while total ω-3 PUFAs decreased resulting in a dose-dependent increase in the hepatic ω-6:ω-3 PUFA ratio, as reported in human NAFLD. More specifically, TCD2 increased pro-inflammatory eicosanoids, including leukotriene (LT) B4 (3-fold), LTb (5-fold), and LTC4 (1.3-fold) and decreased cysteinyl LTs (CysLT, 13-fold) and LTb (2-fold) and decreased AA utilization by lipoxygenases. Accordingly, phospholipase A2 (P2a) was induced 6-fold consistent with increased AA biosynthesis, while AA utilization by lipoxygenases Alox5 (2-fold), Alox12 (2-fold), and Alox15 (10-fold) for the synthesis of LTs also increased. Hypothesized that TCD2 activates AhR, which mediates the toxic effects of environmental pollutants such as dioxins. AhR has also important endogenous functions, notably in vascular remodeling and THPS toxicity but not THP toxicity was significantly increased in the absence of amino acids in the medium. However, when methanal was added to the medium with amino acids, the toxicity of both THPS and THP was reduced. The hypothesis that conjugation of THPS with amino acids occurs only in the presence of methanal and protects against toxicity, possibly by decreasing the free THPS concentration. Morphological examination revealed the presence of microvillus in the cell cytoplasm without any obvious zone-specificity. The metabolism of glutathione peroxidase and superoxide dismutase 1 and 2 was not altered, indicating that other mechanisms than oxidative stress lead to liver toxicity and morphological changes observed. In conclusion, our results indicate that THPS toxicity might be due to THP’s and their acid conjugation mediated by methanal protects against this toxicity.

1324 Flutamide Interaction with Bile Acid and Tumor Necrosis Factor-Alpha in Human Hepatocytes: Implication for the Pathogenesis of Cholestatic Drug-Induced Liver Injury

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Drug induced liver injury (DILI) is a major cause of acute liver failure in the United States and a primary reason for regulatory actions, such as drug recall or withdrawal. In most cases of DILI, patients are idiopathic (IDILI), and many appear to involve an immune response. Several IDILI-associated drugs interacted synergistically with tumor necrosis factor-alpha (TNF-α) to cause cytokite toxicity in HepG2 cells (Maiuri et al., JPET 362: 459, 2017), but the response appeared more robust with drugs that cause hepatic rather than systemic toxicity. For example, flutamide (FLUT) causes cholestatic IDILI and showed no synergy with TNF-α in HepG2 cells. FLUT can inhibit transport of bile acids (BAs). It has been suggested that drug-induced cholestatic injury involves inhibition of BA transport, and it seemed possible that the lack of responsive changes in FA transport was due to the absence of transport of BAs. Accordingly, we hypothesized that intrahepatic accumulation of bile acids caused by FLUT initiates cell stress that interacts with TNF-α, resulting in cytokitotoxicity. To test this hypothesis, FLUT cytokitotoxicity was evaluated in human hepatocytes in sandwich culture in the presence and absence of TNF-α and/or the BA, glycochenodeoxycholic acid (GCDA). Cytokitotoxicity was measured as lactate dehydrogenase (LDH) release and decreased intracellular ATP. Hepatocytes were exposed for 24 hr to various concentrations of FLUT (0-100 µM) and to vehicles or TNF-α (15ng/mL) and/or GCDA (300 µM). These concentrations of TNF-α and GCDA were nontoxic by themselves. FLUT alone caused no cytokitotoxicity; however, modest but statistically significant cytokitotoxicity occurred from FLUT in the presence of the TNF/GCDA combination, and the effects of the cytokine and the BA appeared to be additive. These data support the hypothesis that drug-induced accumulation of BAs in hepatocytes causes cell stress that adds to TNF-α-induced activation of stress pathways resulting in liver injury. Supported by a grant from IONTOX and by NIH grant R01 DK12665.

1325 Mechanism of Liver Toxicity of the Biocide Tetrakis(Hydroxymethyl)Phosphonium Sulphate

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The biocide tetrakis(hydroxymethyl)phosphonium sulphate (THPS) is associated with hepatotoxicity according to several animal studies, but the mechanisms behind are still unknown. In water, THPS exists in a reversible equilibrium with tris(hydroxymethyl)phosphine (THP). Also, THPS is known to degrade rapidly and spontaneously in biological material. The biocide tetrakis(hydroxymethyl)phosphonium sulphate (THPS) and other downstream metabolites, and can also form conjugates with amino acids. The goal of this study was to identify the ultimate culprit of THPS-induced liver toxicity. Therefore we tested the toxicity of THPS, THPO and THP on rat perfusion-liver slices (PCLS). The slice viability was determined by means of Alamar Blue and LDH leakage. The morphological examination was performed on paraffin sections stained with hematoxylin and eosin. The expression of genes known to play a role in oxidative stress was studied. THPS showed hepatotoxicity with a TC50 between 70µM and 100µM. THSP showed only moderate toxicity (TC50 200 µM) as did THPO and was not toxic up to 124µM. It was hypothesized that THPS actively binds to plasma proteins and amino acids with methanal acting as a linker in this reaction. Accordingly, THPS toxicity but not THP toxicity was significantly increased in the absence of amino acids in the medium. However, when methanal was added to the medium with amino acids, the toxicity of both THPS and THP was reduced. The hypothesis that conjugation of THPS with amino acids occurs only in the presence of methanal and protects against toxicity, possibly by decreasing the free THPS concentration. Morphological examination revealed the presence of microvillus in the cell cytoplasm without any obvious zone-specificity. The metabolism of glutathione peroxidase and superoxide dismutase 1 and 2 was not altered, indicating that other mechanisms than oxidative stress lead to liver toxicity and morphological changes observed. In conclusion, our results indicate that THPS toxicity might be due to THPS and/or THP and that amino acid conjugation mediated by methanal protects against this toxicity.

1326 Gender-Specific Role of the Aryl Hydrocarbon Receptor in Cellular Senescence during Liver Aging

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Cellular senescence is a fundamental cellular stress response limiting the proliferation of damaged cells but also driving inflammation with potential deleterious consequences in age-related liver diseases. The Aryl Hydrocarbon Receptor (AhR) is a ligand-activated transcription factor that mediates the toxic effects of environmental pollutants such as dioxins. AhR has also important endogenous functions, notably in vasculature and liver development, as it can influence cell biological endpoints such as growth or differentiation. The goal of the present study was to gain new insights into the potential role of AhR in liver aging and cellular senescence through the characterisation of AhRKO and WT mice of both gender at different ages (26, 39 and 52 weeks). Contrary to WT mice, AhRKO mice did not gain weight with aging. AhRKO mice display smaller liver weight and lower alanine amino transferase serum levels than WT littermates, as previously reported, regardless of the age. Liver histology confirmed the presence of a mild periportal fibrosis in AhRKO mice. Accordingly, hepatic mRNA levels of pro-fibrotic markers (Col1α1, Act2) were higher in AhRKO than in WT mice, though they decreased with aging. Strikingly, plasma profiles of pro-inflammatory cytokines were exacerbated in AhRKO females compared to WT littermates or males, and proned to age-dependent modulation. Also, liver inflammation was more severe in aged-AhRKO females than in males. Remarkably, hepatic gene expression of oxidative stress markers (Nox2,
1327 Sitagliptin Exacerbates Expression of Inflammatory Markers in Sprague Dawley Rats Fed a High-Cholesterol Diet

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Sitagliptin, a dipeptidyl peptidase IV inhibitor, is an anti-diabetic drug. Independent of its hypoglycemic effects, sitagliptin is reported to reduce inflammation in addition to other health benefits. However, recent studies have demonstrated a risk of pancreatitis, hepatic injury and cardiac fibrosis associated with sitagliptin. Since hypercholesterolemia is responsible for the development and progression of atherosclerosis (an inflammation-based disease), studies were conducted in adult male Sprague Dawley rats to investigate whether sitagliptin exacerbates the effects of hypercholesterolemia. Using rats fed either a control or high cholesterol (Cho) diet, and gavaged daily with vehicle or sitagliptin, we found that high Cho increased hepatic expression of pro-inflammatory cytokines (Il1β, Tnf α, Mcp-1), and surprisingly, sitagliptin further increased the mRNA expression of each of these genes. High levels of Il1β were also seen in the serum of rats fed Cho and given sitagliptin. Analysis of serum metabolites revealed changes in purine metabolism. Reduced levels of uric acid (urate) and guanosine in sitagliptin-fed rats fed Cho is further suggestive of inflammation. Moreover, the high Cho produced a significant increase in serum Cho which was unaffected by sitagliptin. Serum triglycerides were reduced approximately 50% by sitagliptin in rats on Con diet but the effect was lost in rats on high Cho diet. Thus, sitagliptin increased expression of inflammatory markers in rats fed high Cho, in a manner that was independent of food intake or body weight. The diet x sitagliptin interaction with respect to inflammatory markers and lipid levels provides several interesting avenues for future studies.

1328 Bile Salt Supplementation for Detecting Drug-Induced, Cholestatic Liver Injury in Rats

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Drug-induced liver injury (DILI) is a problem that complicates clinical pharmacotherapy and drug development. Many clinical drugs are associated with inhibition of bile acid export pump (BSEP), which induces hepatic bile acid retention and leads to liver injury. In drug development process, conventional experimental animals hardly detect cholestatic liver injury of marketed drugs, probably due to species differences in bile acid compositions and susceptibility to cholestatic drugs between humans and rodents. In this study, we aimed to establish an animal model to detect liver injury of BSEP-inhibiting drugs. Five-week-old female Sprague-Dawley rats were orally treated with chenodeoxycholic acid (CDCA) sodium salt at a nontoxic dose every 24 hours together with typical cholestatic drugs, ketoconazole (KTZ) for 4 days or itronavir (RTV) for 5 days. The plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were measured as biomarkers. Liver samples were collected on day 5 for KTZ group and day 6 for RTV group. Then hematoxylin-eosin staining was conducted. We confirmed that CDCA alone treatment did not result in biomarker increases and histological changes. In KTZ and CDCA-co-treated group, ALT levels were elevated ranging from 200 to 400 U/L while hepatic inflammations, necrosis, and lipid droplet were observed. The ALT levels were increased in the liver of 52 weeks-old AhRKO females. Our study provides new evidence that AhR could protect from cellular senescence associated with age-related liver diseases in a gender-specific manner. Established a rat model of liver injury that is susceptible to cholestatic drugs with the oral bile acid supplementation. This model would be useful in nonclinical safety studies to examine cholestatic property of drugs which alone do not cause liver injury in experimental animals in vivo.

1329 Alcohol Metabolism in Non-Alcoholic Steatohepatitis

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Alcohol metabolism is a well-characterized biological process that is dominated by the alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) families. Nonalcoholic steatohepatitis (NASH) is the stage of nonalcoholic fatty liver disease (NAFLD), and is known to alter the metabolism and disposition of numerous drugs. Hepatic ADH and ALDH play critical roles in alcohol metabolism and other cellular metabolism processes, as well as the metabolism of clinical drugs. Therefore, the purpose of this study was to investigate the alterations of alcohol metabolism in a mouse model of NASH with focus to human NASH pathology. Expression and function of individual ADH and ALDH enzymes were examined in normal, steatosis, NASH (fatty) and NASH (not fatty) human liver samples. ALDH4A1 mRNA was significantly decreased in both NASH groups, while no significant changes were observed in the mRNA levels of other ADH and ALDH genes. The protein levels of ADH1A, ADH1B and ADH4 were each decreased in the NASH groups, which was consistent with a decreased overall ADH activity as measured by the oxidation of ethanol. In NASH, protein levels of ALDH2 and ALDH4A1 were significantly increased, but unchanged for ALDH1A1 and ALDH2B1. Interestingly, ALDH activity measured by multiple substrates was variable. There was no significant change in ALDH-mediated acetaldehyde oxidation, but ALDH-mediated hexanal oxidation was decreased in both NASH groups and octanal oxidation was decreased in the NASH (fatty) group. Furthermore, the significant accumulation of 4-hydroxynonenal (4-HNE) protein adduct was observed in NASH, indicating significant oxidative stress and a potential reduction in ALDH activity. Collectively, the alterations in ALDH expression and function indicate distinct regulatory mechanisms in NASH. ADH and ALDH expression and function are profoundly altered in the progression of NASH, which may have notable impact on the ADH and ALDH associated cellular metabolism processes and lead to significant alterations in drug metabolism mediated by these enzymes.

1330 A Novel Fibroblast Growth Factor 1 Variant Reverses Non-Alcoholic Fatty Liver Diseases in Type 2 Diabetes


Nonalcoholic fatty liver disease (NAFLD) is the most common chronic liver disorder and is strongly associated with type 2 diabetes (T2D). Our recently engineered FGF1 partial agonist, carrying triple mutations (FGF1ΔHBS) exhibits greatly reduced proliferative potential, while preserving the full metabolic activity of wild-type FGF1 (FGF1 WT) (Cell Rep. 2017; 20:1717-28). This study tests the therapeutic effects of FGF1ΔHBS on NAFLD in T2D and explores potential mechanisms. The results showed that pharmacological administration of FGF1ΔHBS on 9-month-old male db/db mice for 3 months almost completely normalized the blood glucose levels, insulin sensitivity, liver weight, hepatic lipid deposition and inflammation and liver function. Mechanistically, FGF1ΔHBS treatment prevented diabetes-induced hepatic oxidative stress by promoting nuclear translocation of the antioxidant transcription factor Nrf2 (nuclear factor erythroid 2-related factor 2) and elevating the expression of multiple enzymes. Nrf2 knockdown inhibited the expressions of hepatic hexokinase genes including FXS, SREBP-1, SCD-1, and reversed the suppressed activity of hepatic fatty acid oxidation pathways, including elevated expressions of PPARα and PGC-1α, and enhanced phosphorylation of AMPK and ACC. Hep2 cells treated with palmitate (Pal) mimicked the diabetic phenotype of hepatic oxidative damage and disordered lipid metabolism seen in db/db mice, all of which could be reversed by supplementing with FGF1ΔHBS. Knockdown of Nrf2 by siRNA completely abolished the anti-oxidative capacity of FGF1ΔHBS, but did not affect the beneficial effects of FGF1ΔHBS on Pal-induced lipid metabolic disorder. Furthermore, inhibition of AMPK by the inhibitor Compound C completely abolished FGF1ΔHBS ability to prevent Pal-induced hepatic lipotoxicity and lipid metabolic disorder, along with the inhibition of Nrf2-mediated anti-oxidative signaling pathway. Our findings demonstrate that, in addition to its ability to restore glucose-lowering and insulin-sensitizing effects, FGF1ΔHBS can reverse NAFLD in T2D, by its ability to up-regulate AMPK-mediated lipid metabolism and Nrf2 anti-oxidative pathway.
Mechanisms of Polyhexamethylene-Biguanide-Induced Mouse Liver Hemangiosarcomas
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Chronic exposure of mice to polyhexamethylene biguanide (PHMB) resulted in an increased incidence of liver hemangiosarcomas. The underlying mechanism for these PHMB tumors appears to be through a nongenotoxic mechanism. The present study examined several key pathways for hemangiosarcoma formation in PHMB-treated mice including hypoxia, macrophage activation, increased angiogenic growth factors, epigenetic modifiers and preclinical animal species. For example, Prescott and Wright1, demonstrated that PHMB activate Ppar-γ, induce hypoxia/oxidative stress which in turn activate Kupffer cells, producing increased vascular growth factors, which stimulate endothelial cell progenitor cells differentiation, and promote endothelial cell proliferation. Male C57BL6 mice were treated to PHMB via diet at doses of 0 (control), 400, 1200 or 4000 ppm for 7, 14, and 28 days. Real-time PCR gene expression analysis revealed that PHMB at the high dose induced Hif1α after 14 days and Hmgb1, a known marker of hypoxia, after 14 and 28 days. Kupffer cells (macrophages) activation was indicated by increased expression of Tnfα and Il6 at the high dose group. Though not all statistically significant, vascular growth factors Vegf, Angpt (angiopoietin) and Tgf-β, as well as Vegf receptors 1 & 2 were consistently increased following PHMB exposure. Similarly, eNOS, an angiogenesis inducer was also increased following treatment with PHMB. The endothelial differentiation /progenitor marker Cd34 and VE-cadherin were also significantly induced by PHMB at the medium and high dose levels after 14 and 28 days. In addition, Ppar-γ receptor and the Ppar-γ regulated gene, Cd36 was induced by PHMB in a dose-responsive manner. Tumor proliferation rate was determined by gene expression, and BrdU staining (ongoing). PHMB administration induced a dose-related increase of cMYC expression. What’s more, gene expression data were suggestive that oxidative stress (increased Nrf2, Sod1, and Hmox1) and nuclear receptor CAR (elevated Cyp2b10) might also be involved. PHMB also failed to induce hemolysis as determined by red blood cell numbers and hemoglobin (Hb) levels after treatment for up to 28 days. Together, the present study along with literature information suggested that PHMB activate Ppar-γ, induce hypoxia/oxidative stress which in turn activate Kupffer cells, producing increased vascular growth factors, which stimulate endothelial cell progenitor cells differentiation, and promote endothelial cell proliferation and finally development of hemangiosarcomas.

Assessment of Acetaminophen Glutathione Depletion under In Vivo-Like Pharmacokinetic Concentration Profiles Enabled by a Microfluidic Addition and Removal Device
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The pharmacokinetic (PK) profile is a determining factor in both the safety and efficacy of a drug or therapeutic regimen. PK profiles can vary significantly between patients and between humans and preclinical animal species. For example, Prescott and Wright1, demonstrated patients who suffer liver damage after acetaminophen (APAP) overdose exhibited an increase in both APAP plasma concentration and half-life. The plasma half-life for those with and without liver damage were 7.2 ± 0.7 hr and 2.9 ± 0.3 hr respectively. To model this effect using standard in vitro tests in which drug is added as a bolus is challenging as i) the concentration profile of the drug poorly mimics in vivo PK ii) the concentration of drug in the well may change in ways which are difficult to control due to cellular metabolism, binding to plasticware or degradation in solution. To provide a realistic in vitro model of APAP overdose profiles we developed a microfluidic device, known as the Microformulator (Viibre, Vanderbilt University), capable of precision addition and removal of medium from the wells, was used to create PK like concentration profiles in the wells of a microtiter plate. PK like concentration profiles were created by sequential addition and removal, where the duration of each step is set equal to the half-life. The accuracy of the profiles was verified using fluorescent probes. APAP was dosed onto primary human hepatocytes seeded in 96 well plates, either as a bolus or with PK like concentration profiles with a rise time of 4 hr and a decay half-life of either 7.2 or 2.9 hr. The maximal concentration reached was varied between 2 and 50mM. After 48 hr the cells were lysed and glutathione (GSH) levels measured. Bolus additions always resulted in greater GSH depletion than PK like profiles when comparing the maximal concentration in the PK profile to the concentration added as a bolus. For example at 20mM the GSH depletion was bolus 100%, PK 7.2 hr 94%, PK 2.9 hr 64%. Plotting the data against AUC, unified the database, indicating GSH depletion by APAP is AUC driven. This study demonstrates, the utility of a new tool for the generation of PK like concentration profiles in microtiter plates, and models clinical APAP overdose profiles in vitro. 1 Prescott LF, Wright N. The effects of hepatic and renal damage on paracetamol metabolism and excretion following overdosage. A pharmacokinetic study. Br. J. Pharmac (1973), 49, 602-613

HDAC3 Inhibition Prevents Liver Damage and Increases FGF21 Synthesis and Secretion to Aortic Protection in Diabetic Animals
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Vascular complications are common pathways associated with type 1 diabetes. Drugs are effectively lowering the incidence of microvascular complications, they are less effective in the treatment of macrovascular diseases, including atherosclerosis. In recent years, histone deacetylase enzyme (HDAC) inhibitors have been found to be successful in preventing atherosclerosis. To investigate the role of HDAC3 inhibition in preventing diabetic aortic pathologies, male OVE26 type 1 diabetic mice and age-matched wild-type mice were given the HDAC3 specific inhibitor RGFP966 or vehicle for 3 months. At this time, mice were sacrificed or maintained for additional 3 months without RGFP966 treatment. Levels of aortic inflammation and fibrosis, plasma and hepatic FGF21 levels were then determined. Additionally, hepatic miR-200a and Keap1 expression, Nrf2 nuclear translocation and antioxidant gene expression were measured. We found that HDAC3 inhibition significantly reduced aortic fibrosis and inflammation in OVE26 mice at both 3 and 6 months. Plasma FGF21 levels were significantly higher in RGF966 treated OVE26 mice compared to vehicle-treated OVE26 mice at both time-points. Because the liver is the major organ for FGF21 synthesis and secretion in diabetic animals, the effects of HDAC3 inhibition on hepatic FGF21 synthesis was examined. RGFP966 treatment significantly reduced hepatic pathologies associated with diabetes and increased FGF21 mRNA and protein expression. Furthermore, our data found that HDAC3 inhibition reduced miR-200a expression that was associated with a reduction of Keap1 protein levels. This resulted in an increase of Nrf2 nuclear translocation and an upregulation of antioxidant genes and FGF21 transcription. Our results indicate that HDAC3 inhibition promotes Nrf2 function to prevent diabetes-induced liver injury preserving hepatic FGF21 synthesis. The preservation of hepatic FGF21 synthesis and secretion ultimately leads to a reduction in diabetes-induced aorta pathologies.

Late Protective Effect of Netrin-1 in the Murine Acetaminophen Hepatotoxicity Model
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Acetaminophen (APAP) hepatotoxicity is the leading cause of acute liver failure in the United States; the current treatment option for APAP overdose is administration of N-acetylcysteine (NAC), which facilitates hepatic and renal damage on paracetamol metabolism and excretion following overdose. A pharmacokinetic study. Br. J. Pharmac (1973), 49, 602-613

The pharmacokinetic (PK) profile of a drug is a determining factor in both the safety and efficacy of a drug or therapeutic regimen. PK profiles can vary significantly between patients and between humans and preclinical animal species. However, NAC has a short early therapeutic window and novel strategies are needed for patients who typically present late to the hospital. This is relevant, since the recovery and regeneration capacity of the liver, which typically occurs late after injury, plays a critical role in patient survival after an APAP overdose. Netrin-1 is a laminin-related protein initially identified as a chemotactic agent for axon guidance, and recent work has demonstrated that Netrin-1 also promotes hepatic repair and regeneration during liver ischemia/reperfusion injury. The role of Netrin-1 in acetaminophen hepatotoxicity is unknown and this study evaluated this in vivo in a mouse model of APAP overdose. Male C57BL/6J mice were co-treated with exogenous Netrin-1 vehicle control, along with 300mg/kg APAP and euthanized at 6h and 24h. Significant elevations in ALT indicative of liver injury were seen in control mice at 6h, however, this injury was not significantly affected by Netrin-1 administration. Also, Netrin-1 treatment did not affect mitochondrial translocation of phospho-JNK, though translocation of Bax to the mitochondria and release of Smac and cytochrome c were blunted at 6h. Interestingly, however, Netrin-1 administration attenuated liver injury as seen by ALT levels and histology at 24h, at which time significant elevations in mRNA for the adenosine A2B receptor, one of the Netrin-1 receptors, was also seen. Thus, our data indicate a previously unrecognized role for Netrin-1 in attenuation of APAP hepatotoxicity, which could be mediated by prevention of mitochondrial dysfunction through the adenosine A2B receptor.
Diethylene glycol (DEG) is an industrial solvent, generally found in brake fluid and cooling fuel, that has been implicated in mass poisonings worldwide. The predominant metabolites of DEG are 2-hydroxythoxyacetic acid (2-HEAA) and diethylene glycol (DEG). Although proximal tubular necrosis is characteristic of DEG poisoning, hepatotoxicity has also been observed. DEG has been implicated as the metabolite responsible for the renal and hepatic toxicity seen during these poisonings due to its accumulation in these target organs. DEG has also been shown to increase intracellular levels of reactive oxygen species (ROS). We hypothesize that treatment with DGA would impact the ratio of reduced and oxidized glutathione (GSH/GSSG) in the target organs, particularly in the liver. GSH is an important antioxidant that helps to protect against damage caused by ROS, and changes in the ratio of GSH/GSSG can be indicative of oxidative stress. To test this hypothesis, an in vivo dosing study was conducted. Rats were divided into control, low dose (100 mg/kg of DGA by oral gavage), and high dose (300 mg/kg) groups. The liver of each animal was collected by flash freezing in liquid nitrogen, homogenized in acid-containing solutions, and analyzed via HPLC. There were no significant differences seen in the amount of GSH, GSSG, or in the GSH/GSSG ratio among the three dose groups, even though there was marked toxicity observed in the high dose animals. The results suggest that oxidative stress is not a mechanism by which DGA causes toxicity to the liver in vivo.

**1338 Perfluoronanoic Acid (PFNA) Activates Nrf2 by Suppressing Autophagy in Mouse Liver and Human Hepatoma Cells**

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Perfluorinated compounds (PFCs) are persistent environmental pollutants, which are commonly detected in human serum. Epidemiologic studies showed positive associations between higher serum levels of PFCs and greater risk of liver diseases, endocrine disorders, and immunotoxicity. Nuclear factor erythroid 2-related factor (Nrf2), which plays important anti-oxidative roles, can be activated by oxidative stress and/or autophagy repression to protect liver cells. Our preliminary data showed that perfluoronanoic acid (PFNA) activated Nrf2 in mouse liver and cultured human hepatoma cells. However, the underlying mechanism is unknown. The present study was designed to investigate whether PFNA activates Nrf2 signaling via oxidative stress and/or autophagy suppression. Our results showed that PFNA increased mRNA expression of Nrf2-targeting genes, such as glutamate-cysteine ligase catalytic subunit (Gclc), NAD(P)H:quinone dehydrogenase 1 (Nqo1) and heme oxygenase (Ho-1), and the nuclear level of Nrf2 protein in mouse liver as well as in cultured human Hep3B hepatoma cells. Seashore and flow cytometry analysis demonstrated that in cultured human Hep3B hepatoma cells, PFNA (10μM) did not induce apparent mitochondrial stress or oxidative stress. Therefore, PFNA-induced Nrf2 activation is not due to oxidative stress. We next determined the impact of PFNA on autophagy progression. PFNA time-dependently decreased the conversion of microtubule-associated protein 1 light chain 3 beta (LC3B-I) to LC3B-II in mouse liver, indicating that autophagy was repressed by PFNA exposure. Furthermore, in mouse liver and cultured human Hep3B hepatoma cells, PFNA largely increased p62 protein level in the cytosol. In summary, this study demonstrated that PFNA activates Nrf2, maybe mediated by autophagy suppression and subsequent cytosolic accumulation of p62 protein.

**1339 Absence of Mrp4 Impairs Lipid Homeostasis following Two-Thirds Partial Hepatectomy**

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The regenerating capacity of the liver is critical for tissue recovery and survival following chemical-induced injury. The Multidrug-resistance associated protein 4 (Mrp4), an ATP-binding cassette (ABC) efflux transporter, plays an important role in regulating both hepatotoxicity and adaptive changes during liver injury in mice. Hepatic Mrp4 expression is low under basal conditions, with remarkable induction reported during acetaminophen (APAP)-induced hepatotoxicity and cholestasis liver injury. Furthermore, previous work from our laboratory has shown that Mrp4 up-regulation is localized to proliferating hepatocytes following APAP overdose. However, the role of Mrp4 in liver regeneration has not been studied recently, with emphasis placed on lipid accumulations transient regeneration-associated steatosis (TRAS) in models that underwent two-thirds partial hepatectomy (PH), a model for liver regeneration. The present study evaluated the gene expression profile of several cell cycle markers and lipid homeostasis genes in both WT and Mrp4 KO mice at 24, 48 and 72 hr after PH. Hepatocyte proliferation was evaluated by measuring the gene expression of cell proliferation markers such as Ki67, cyclin D1 and D2. Induction of Ki67 mRNA was observed in both genotypes after surgery with no significant differences between

**1337 Mechanisms of Protein Adduct Release during Acetaminophen Hepatotoxicity: Role of Exosomes**

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Acetaminophen (APAP) hepatotoxicity is the leading cause of acute liver failure in the United States, and formation of adducts between the reactive APAP metabolite N-acetyl-p-benzoquinone imine (NAPQI) and cellular proteins are a critical feature of pathophysiologic. In addition, circulating protein adducts are biomarkers of APAP hepatotoxicity and have recently been the focus of a rapid bioassay for identification of an APAP overdose in humans. In spite of their clinical significance however, mechanisms of release of APAP-protein adducts from hepatocytes into the circulation are not well understood. Studies in hepatocytes in vitro show significant release of APAP-protein adducts into the medium prior to elevations in ALT, suggesting that adduct release occurs before extensive hepatocyte necrosis. Exosomes are discrete membrane bound vesicles, which package cellular cargo and function in extracellular transport. Clarification of their role in transport of APAP adducts is relevant since adduct packaging within these membrane bound vesicles could shield them from detection by anti-body based methods, resulting in under-estimation of circulating adduct levels. Hence, this study evaluated exosome release after APAP overdose in vivo in mice, in primary mouse hepatocytes and human HepaRG cells in culture from APAP overdose patients and examined their role in transport of APAP-protein adducts. Exosomes were isolated by ultra-centrifugation and characterized by size and protein composition, following which level of APAP-protein adducts were measured by HPLC. Significant elevations in circulating exosome numbers were observed 6 hours after APAP overdose in vivo and by 4 hours in primary mouse hepatocytes in culture. Circulating exosomes were also elevated in media from HepaRG cells by 24 hours after APAP exposure, an effect recapitulated in APAP overdose patients, where exosome numbers were elevated compared to healthy controls. Though APAP-protein adducts were elevated in circulation and media parallel to the increased exosome release, no detectable adducts were observed within exosomes. This suggests that though APAP overdose enhances exosome release from hepatocytes in mice and humans, it is not a significant mechanism of release of APAP-protein adducts into circulation.

**1336 Loss of Liver-Specific and Sexually Dimorphic Gene Expression by Aryl Hydrocarbon Receptor Activation in C57BL/6 Mice**


The aryl hydrocarbon receptor (AhR) is a highly conserved transcription factor that mediates a broad spectrum of species-, strain-, sex-, age-, tissue-, and cell-specific responses elicited by structurally diverse ligands including 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Dose-dependent effects on liver-specific and sexually dimorphic gene expression were examined in male and female mice gavaged with TCDD every 4 days for 28 or 92 days. RNA-seq data revealed the coordinated repression of 181 genes predominately expressed in the liver including albumin (Alb; 3.7-fold), methane dioxygenases (Kat la; 4.5-fold) α-fibrinogen (Fga; 14.5-fold), and β-fibrinogen (Fgb; 17.4-fold) in males with corresponding AhR enrichment at 2 hr. Liver-specific genes exhibiting sexually dimorphic expression also demonstrated diminished divergence between sexes. For example, male-biased Gsp1 was repressed 3.0-fold in males and induced 4.5-fold in females, which were confirmed at the protein level. Disrupted regulation was consistent with impaired GHR-JAK2-TAT5 signaling and inhibition of female specific CUX2-mediated transcription, as well as the repression of other key transcriptional regulators including Ghr, Stat5b, Bcl6, Hmna4, Hnf6, Foxa1/2/3, and Zhh2. Attenuated liver-specific and sexually dimorphic gene expression was concurrent with the induction of fetal genes such as alpha-fetoprotein (Afp; 15.0-fold). The results suggest AhR activation causes the loss of liver-specific and sexually dimorphic gene expression producing a functionally “de-differentiated” hepatic phenotype.
3340 Regulation of Long Non-Coding RNA Dino-Mediated P53 Activation by an Orphan Nuclear Receptor Nr2e3 in Acetaminophen-Induced Hepatotoxicity

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A damaged induced non-coding gene (DINO) is a unique long non-coding RNA that interacts with, and stabilizes p53 protein for proper activation of p53 signaling pathways. Here, we have newly revealed that by an orphan nuclear receptor NR2E3 play a role in maintaining active epigenetic status of DINO gene promoter by forming protein complex with Sp1 transcription factor. On the contrary, NR2E3 loss induced repressive DINO epigenetic status by recruiting histone demethylase LSD1 that turn off active histone mark H3K4me2, subsequent led to impaired DINO expression and p53 signaling pathway activation. In acetaminophen-induced animal liver injury model, Nr2e3 knockout mice recently generated in our laboratory exhibited more severe liver damage than wild type mice accompanied by increased necrosis with less apoptosis mainly due to defective DINO-p53 signals activation. Also, the liver damage in Nr2e3 knockout mice was not successfully reversed by antioxidant N-acetylcysteine treatment, comparing to an efficient reversal in wild type mice, indicating that DINO-p53 signaling axis is critical for the antioxidant-mediated recovery from acetaminophen-induced hepatotoxicity. Collectively, our new findings demonstrated that NR2E3 is an essential player for maintaining proper activation of p53 signaling pathways by sustaining active DINO epigenetic status and expression. The findings will provide fundamental scientific information that can be translationally applied for the improvement of prognosis and therapeutic strategies in various p53-related human diseases.

3342 Chronic TCDD Treatment Elevates Liver Fibrosis in Hepatocyte-Specific Ahr Knockout Mice

S. Rayavara Veerabhadraiah. Boise State University, Boise, ID. Sponsor: K. Mitchell

Accumulating evidence implicates a role for the aryl hydrocarbon receptor (AhR) in the development of liver fibrosis, which is a pathological wound healing response that is characterized by excessive synthesis of extracellular matrix molecules. During liver fibrosis, chronic injury and inflammation drive the activation of myofibroblasts, which produce collagen type I. We have previously reported that exposure to the exogenous AhR ligand 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) increased HSC activation in vitro and in mice treated with carbon tetrachloride. Furthermore, recent literature reports demonstrate that chronic TCDD administration alone elicits liver fibrosis. It is possible that AhR activation in HSCs alone can drive the activation of myofibroblasts and fibrogenesis. Therefore, we hypothesize that AhR activation by the exogenous agonist 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) increased HSC activation in vitro and in mice treated with carbon tetrachlore. Hence, exacerbated liver fibrosis.

3343 Exogenous AhR Activation Increases Myofibroblast Fibrogenic Activity in Mice with a Hepatocyte-Specific Ahr Deletion

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The aryl hydrocarbon receptor (AhR) is a soluble, ligand-activated transcription factor that has been implicated in fibrosis, which is a pathological wound healing response that is characterized by excessive synthesis of extracellular matrix molecules. During liver fibrosis, chronic injury and inflammation drive the activation of myofibroblasts, which produce collagen type I. We have previously reported that exposure to the exogenous AhR ligand 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) increases HSC activation in vitro and in the liver of mice chronically treated with carbon tetrachloride (CCL4). The goal of this project was to distinguish between the direct effects of TCDD on HSC activation and indirect effects that could occur through TCDD-induced hepatocyte damage. Cre-LoxP recombination was used to generate hepatocyte-specific AhR knockout mice (AhR∆hep). To elicit liver injury and fibrosis, these mice or control mice (AhRfl/fl) were treated with 1.0 ml/kg CCl4 every four days for 5 weeks. TCDD (100 μg/kg) or peanut oil vehicle was administered during the fifth week. Based on serum alanine aminotransferase levels, TCDD increased liver damage in CCl4-treated AhRfl/fl mice but had no effect in AhR∆hep mice. In addition, TCDD treatment increased hepatic inflammation in CCl4-treated mice on both backgrounds, but the number of inflammatory foci was twice as high in AhRfl/fl mice than in AhR∆hep mice. TCDD also increased markers of HSC activation in CCl4-treated mice, based on increased expression of α-smooth muscle actin and Colla1, but expression levels were approximately 3-fold lower in AhR∆hep mice compared to AhRfl/fl mice. Nevertheless, despite discrepancies in liver damage, inflammation and HSC activation, collagen content was remarkably similar in CCl4/DDC-treated AhRfl/fl and AhR∆hep mice. Collectively, these results indicate that hepatocyte damage augments - but is not absolutely required for - the increased myofibroblast activation observed in TCDD-treated mice. Data support the notion that TCDD either directly activates HSCs or else exacerbates inflammation, which subsequently leads to HSC activation.
Potential Cross-Talk between FXR and FGF19 Signaling Pathways in the Human Hepatic Stellate Cell Line LX-2

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Fibroblast growth factor 15 (Fgf15; FGF19 - human ortholog) is an endocrine FGF produced in the ileum in response to the activation of the nuclear receptor farnesoid X receptor (FXR) by its endogenous ligands, bile acids (BAs). BAs also activate other receptors and pathways including TGR5. Fgf15 acts as a crucial feedback factor on BA production by suppressing the hepatic expression of genes encoding BA synthesizing enzymes, Cyp7a1 and Cyp8b1. Our lab has previously shown that Fgf15 deficiency was protective against liver fibrosis in a high fat diet (HFD)-induced non-alcoholic steatohepatitis (NASH) mouse model. Despite the observed decrease in fibrosis in Fgf15 deficient mice with NASH, there was severe steatosis and inflammation indicating that Fgf15/19 may directly activate hepatic stellate cells (HSCs). FXR activation in HSCs has been shown to reduce the expression of collagen thus inhibiting fibrosis. Therefore, Fgf15 deficiency may protect against the development of hepatic fibrosis by increasing the total BA pool size and subsequently increasing FXR activation in HSCs. To differentiate the effects of Fgf15 signaling from FXR signaling, we treated the human hepatic stellate cell line, LX-2, with a BA that is a FXR agonist, chenodeoxycholic acid (CDCA), and/or recombinant FGF19. Effects on gene expression at mRNA and protein levels for HSC activation and proliferation were determined. We found that FGF19 induced the phosphorylation of hyperplipidemic messenger of fibroblast growth factor receptor (FGFR), STAT3, indicating that FGF19 could directly activate FGFRs in LX-2 cells. However, FGF19 treatment did not alter the gene expression of the HSC phenotypic marker genes, including COL1A1, aSMA, or PPARγ. As expected, CDCA up-regulated the expression of the chenodeoxycholic acid receptor, FXR, and its response genes, CYP3A7, ABHD5, and ATGL. Treatment with FGF19 led to decreased expression of the BA receptors, FXR and TGR5, while CDCA treatment increased the expression of the FGF19 co-receptor FGFR1 and β-KLOTHO. These findings indicate potential cross-regulation and feedback between these pathways in HSCs. As Fgf15 decreases the total BA pool size and leads to down-regulation of FXR in HSCs, we postulate that the partial protective benefit offered by FGF15 deficiency on the development of hepatic fibrosis may be due to increased FXR activity in HSCs.

Hepatotoxic Effects of Gugulipid® 2.5% following In Vivo Exposure in Sprague Dawley Rats and In Vitro Exposure in Human Liver Microsomes


Gum guggul extract (GGE), derived from the resin of Commiphora mukul, is marketed in the United States as a dietary supplement purported to treat hyperlipidemia, arthritis, obesity, and acne. The active constituents of GGE, the sterioisomers E- and Z-guggulsterone, are known to alter the activity of cytochrome P450 (CYP) enzymes, suggesting concern for herb-drug interactions following GGE exposure. GGE is also not recommended for use in individuals with liver disorders. Due to concerns for liver toxicity and inhibition of CYP activity following exposure to GGE, the National Toxicology Program (NTP) selected a dietary supplement currently on the market, Gugulipid® 2.5% granules, for toxicity evaluations. The subchronic effects of Gugulipid® 2.5% exposure were determined by targeted gene expression array in Sprague Dawley rats and the effects of Gugulipid® 2.5% or E- and Z-guggulsterones on enzyme and transporter activity were determined in vitro in human liver microsomes (HLM). Male and female SD rats were administered Gugulipid® 2.5% by oral gavage at 0, 62.5, 125, 250, 500, and 1000 mg/kg for 13 weeks. At study termination, rats were evaluated for general toxicity including liver weights, CYP activity, and clinical pathology. Exposure to 500 or 1000 mg/kg Gugulipid® 2.5% resulted in increased liver weights, dose-dependent increases in CYP3A4 and CYP2B1 activity, and decreased serum alkaline phosphatase (ALP) levels in both male and female rats. Serum bile salts were also decreased in female rats. Incubation of HLM with Gugulipid® 2.5% (normalized to 10 μM guggulsterones) caused marked inhibition of multiple CYP activities. E- or Z-guggulsterone alone inhibited CYP activity as well, albeit not to the same extent. When evaluated for effects on transporter activity, Gugulipid® 2.5% inhibited sodium/bile acid co-transporter Na+taurocholate (NTCP) activity and increased P-glycoprotein (Pgp) activity, suggesting Pgp as a key pump for xenobiotic compounds. Overall, in vivo and in vitro exposure to Gugulipid® 2.5% modulated hepatic enzymatic activity in both SD rats and HLM.

Perfluorooctanesulfonic Acid (PFOS) Worsens Hepatic Steatosis and Induces Fatty Acid Uptake in Mice Fed a High-Fat Diet and High-Fat to Low-Fat Diet Switch


Perfluorooctanesulfonic acid (PFOS) is part of a class of bio-accumulative pollutants, called perfluoroalkyl substances (PFAS). In mice, PFOS administration has been shown to increase liver weight and induce hepatic lipid accumulation. The aim of the study was to evaluate potential mechanisms of PFOS induced liver lipid accumulation. Mice were fed a standard chow low fat diet (LFD) or 60% Kcal high fat diet (HFD) for 12 weeks to establish significant body weight and food intake tolerance. Mice were then divided into two main groups; diet alone or diet containing 0.0003% PFOS. Furthermore, each group was subdivided into 3 groups: mice fed LFD, mice fed HFD, or mice fed HFD that were switched to a LFD to mimic the therapeutic treatment. The end study design resulted in a total of 6 treatment groups: (i) LFD, (ii) HFD-LFD, (iii) HFD, (iv) LFD + PFOS, (v) HFD-LFD+PFOS, and (vi) HFD+PFOS. For 10 weeks, mice were fed the new diets, and body weights were monitored. Serum lipids levels were measured in fasting blood. Liver lipid were isolated via chloroform: methanol extraction, and mRNA expression was determined by targeted bead array. HFD feeding for 14 weeks increased body weight, liver weight, white adipose tissue weight, serum lipids and liver lipids. HFD-LFD mice lost body weight and had similar tissue weights, serum lipids, and liver lipids as LFD mice at the end of the study. PFOS exposure in LFD mice reduced body weight and overall weight gain, decreased white adipose tissue weights, and increased liver triglycerides. PFOS exposure in the HFD-LFD group increased liver weight, decreased serum cholesterol, and increased free fatty acids. PFOS exposure in the HFD caused less overall weight gain, increased liver weight, decreased serum cholesterol, and increased liver lipids compared to HFD controls. Liver mRNA expression data shows that PFOS exposure modulates several genes: liver fatty acid translocase (Cd36 or Fat), cell death activator CIDE-A (Cidea), fatty acid binding protein 4 (Fabp4), glutathione S-transferase M3 (Gstm3), and 3-hydroxy-3-methylglutaryl-CoA synthase 1 (Hmgcs1). Western blot data shows PFOS exposure increased Cd36 and Fabp4 expression. The data suggests that PFOS exposure acts on liver lipid uptake, and exacerbates hepatic steatosis in mice induced by high fat diet feeding.

Low-Dose Perfluorononanoic Acid (PFNA) Alters Hepatic Gene Expression in a Mouse Model of Diet-Induced Obesity and Hepatic Steatosis


Perfluoronoctonanoic Acid (PFNA) is classified as a synthetic perfluorinated carboxylic acid with a nine-carbon backbone, that has been shown to cause adverse liver and insulin-signaling effects in rodents, and little is known about its effects on the human liver. PFNA is a member of the perfluoroalkyl substances family, which is a group of man-made compounds that are highly stable and of significant toxicological concern. The aim of this study was to evaluate whether PFNA exposure in combination with a moderately high-fat diet caused serum lipid changes and increased biomarkers of hepatic steatosis. For this study, 5-week-old male mice were fed a 10% Kcal (LFD) or 45% Kcal high fat diet (HFD) alone or in combination with PFNA (0.0003% in chow; LFD-PFNA and HFD-PFNA, respectively) for 12 weeks. The HFD increased body weight by 30%, white adipose tissue (WAT) by 100% and liver weight by 30%. The administration of PFNA slightly decreased body weight in the HFD mice by 85% and in the HFD mice by 82%. PFNA significantly decreased WAT tissue mass in both the LFD and HFD mice. HFD feeding increased hepatic total lipids and triglycerides, however no change was observed in cholesterol or NHEA. Hepatic total lipid content, NEFA, and cholesterol content were similar between controls and both PFNA treated groups. PFNA did not significantly decrease triglyceride content in the HFD fed mice. PFNA significantly increased liver weight and interacted with the HFD. A targeted gene expression array revealed that PFNA altered the expression of genes involved in hepatic lipid deposition. The fatty acid uptake genes Cd36, Lpl, Slc27a1 and Sreb1 were all significantly induced by PFNA administration in both HFD and LFD mice. This data suggests that an environmentally relevant dose of PFNA (0.0003%) may impact hepatic lipid accumulation. 

1344 Potential Cross-Talk between FXR and FGF19 Signaling Pathways in the Human Hepatic Stellate Cell Line LX-2

1345 Hepatotoxic Effects of Gugulipid® 2.5% following In Vivo Exposure in Sprague Dawley Rats and In Vitro Exposure in Human Liver Microsomes

1346 Perfluorooctanesulfonic Acid (PFOS) Worsens Hepatic Steatosis and Induces Fatty Acid Uptake in Mice Fed a High-Fat Diet and High-Fat to Low-Fat Diet Switch

1347 Low-Dose Perfluorononanoic Acid (PFNA) Alters Hepatic Gene Expression in a Mouse Model of Diet-Induced Obesity and Hepatic Steatosis
1348 The Role of Hepatocyte-Specific Farnesoid X Receptor (FXR) and Lipocalin 2 (Lcn2) in the Acute Phase Response

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Farnesoid X receptor (FXR) has emerged as a key nuclear receptor in regulating bile acid homeostasis, liver lipid metabolism, and most notably, liver inflammation. Specifically, FXR has been shown to be a key regulator of members of the lipocalin family of proteins. Lipocalins (Lcn) act as acute phase proteins (APPs) that are expressed and secreted by hepatocytes in response to systemic or local innate inflammatory response, collectively known as the hepatic acute phase response (APR). This study aims to define the role of hepatic FXR and Lcn in the APR using a mouse model with acute bacterial infection. Wild-type (C57BL/6), hepatocyte-specific FXR KO (FXR hep-/-), Lcn2 KO (Lcn2 hep-/-), and double knockout (DKO) mice were intraperitoneally injected with an acute dose of Escherichia coli (E. coli) or sterile phosphate buffered saline (PBS). In vivo bacterial counts in whole blood, liver and spleen were determined 24 hrs post-infection. Serum levels of liver injury markers were determined, and liver and spleen were collected for tissue-specific gene expression. It is hypothesized that hepatic FXR and Lcn protect against liver inflammation; therefore, deficiency of these genes will result in altered APR defined by either worsened bacterial infection and/or increased liver inflammation. All genotypes had significant increase in bacterial load with E. coli compared to their PBS controls. Liver injury was only noted in infected FXR hep-/- mice with significantly increased ALT (87.9 ± 35.1 U/L) and AST (70.4 ± 17.4 U/L) compared to Lcn2 hep-/- (109 ± 8 mg) and DKO (116 ± 20 mg) mice. Most significantly, DKO mice had lower ALT (13.7 ± 10.2 U/L) and AST (57.4 ± 17.4 U/L) compared to FXR hep-/- mice, suggesting Lcn2 may contribute to worsened liver injury in FXR hep-/- mice. These findings suggest a protective role of FXR in regulating the hepatic APR and/or APPs, overall supporting a larger role of FXR in the spectrum of disease known as nonalcoholic fatty liver disease (NAFLD). Chronic activation of the APR and dysregulation of APPs are factors of liver inflammation associated with the disease pathology of nonalcoholic steatohepatitis (NASH), in which FXR activation could protect against.

1349 Activation of Kupffer Cells and Bone Marrow-Derived Macrophages by the Fibrinolytic Enzyme, Plasmin

M. Rodriguez, K. Roth, and B. Copple, Michigan State University, East Lansing, MI. Sponsor: C. Rockwell

Studies have shown that inhibition of the fibrinolytic enzyme, plasmin, prevents macrophage activation after toxin-induced liver injury in mice. This decreases cytokine production and macrophage-mediated phagocytosis resulting in impaired liver regeneration. The mechanism by which plasmin regulates the activity of macrophages in the liver remains unknown. In the present studies, the hypothesis was tested that plasmin directly activates macrophages. To test this hypothesis, macrophages were isolated from mice and treated with plasmin. Treatment of bone marrow-derived macrophages and Kupffer cells with plasmin increased expression of tumor necrosis factor-a, Cxcl1 and Cxcl2. Upregulation of cytokines was inhibited by the plasmin inhibitor, tranexamic acid. Plasmin stimulated formation of extensive pseudopodia, and activated Erk1/2, Jnk, NF-kB and p38, while inhibiting activation of Akt. Previous studies have indicated an important role for the damage-associated molecular pattern molecule, high-mobility group B1 protein (HMGB1), in activation of macrophages after liver injury. Interestingly, in the present studies, HMGB1 alone did not directly activate macrophages, but it did synergistically enhance activation of macrophages by plasmin. Further, necrotic hepatocytes from wild-type mice enhanced activation of macrophages by plasmin whereas necrotic hepatocytes from hepatocyte-specific HMGB1 knockout mice had no effect on activation of macrophages by plasmin. Collectively, these studies indicate that plasmin is a key activator of macrophages in the liver after injury. Supported by NIH grant DK073566.

1350 A Novel In Vitro Liver Cell Culture Flow System Allowing Long-Term Metabolism and Hepatotoxicity Studies

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Hepatotoxicity is a concern when developing new small molecule pharmaceutical compounds. One of the major reasons for the withdrawal of a drug from the market is hepatotoxicity that typically does not appear immediately but after patients have had prolonged and repeated exposure to a drug. Animal models can be suitable for the detection of hepatotoxicity including long-term and repeat dosing studies to replicate this long-term phenomenon. However, animal models often do not accurately predict toxicity in humans due to species differences, e.g. differences in compound metabolism. In vitro models based on primary human hepatocytes (PHH) may be suitable to predict acute toxicity in humans. However, in vitro PHH undergo a rapid de-differentiation over the course of the first 7 days in culture that limits the ability to develop long-term repeat dosing models in vitro. Here we present an alternative in vitro model that is based on the culture of PHH in the Quasi Vivo Q900 flow system. The Q900 flow system consists of 6 chambers that can be connected in flexible configurations. A peristaltic pump generates a cell culture medium flow that mimics blood flow and ensures a steady supply of nutrients, growth factors and oxygen as well as removal of waste products and continuous dosing with test compounds. When culturing PHH in the Q900 flow system, we observed good cell viability and sustained albumin production for up to 14 days in culture. Healthy hepatocyte morphology was well maintained during the analysis period as assessed by phase contrast microscopy. In comparison to non-perfused cultures, the basal activity of the drug metabolism enzymes Cyp3A4, Cyp1A2 and Cyp2B6 was increased, e.g. by up to 10-fold for CYP1A2, as measured by mass spectroscopic analysis of the conversion of phenacetin to acetaminophen. After 72 h of rifampicin treatment, induced CYP3A4 activity was comparable between perfused and non-perfused cultures (assessed by luciferase assay and analysis of hydroxymidazolam formation). Furthermore, this system enabled fewer media changes as typically required in static cultures to enable build-up of potential toxic metabolites that would not be detected when media is refreshed daily. In summary, we present an in vitro liver cell culture flow system that improves longer-term stability of drug metabolism enzymes in PHH for a more human relevant in vitro system for repeat dosing of hepatotoxicants.

1351 Human Liver and Kidney Slices Maintained for Multiple Dosing and Recovery from Injury

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The extended viability of multi-cellular 3-dimensional human liver and kidney slices for up to 7 days expands the use of this in vitro model for studying pathways of injury and repair following multiple dosing. Human tissue was obtained from procurement agencies, according to accepted medical and ethical standards, as outlined by the Uniform Anatomical Gift Act. In culture, the organ slices are supported on a tita- nium mesh roller-insert, and the medium is replaced daily to mimic multiple dosing. The slices remain differentiated, are metabolically active, synthesize ATP and glutathione, while organelle integrity is maintained. By 72 hr of culture the organ slices begin to exhibit pathways of repair. Global gene expression profiling using U133A Affymetrix gene chip arrays revealed similarities in human liver and kidney slices, including the upregulation of extracellular matrix constituents, the proteolytic cascade IGFBP-5, and an up-regulation of growth factors. The up-regulation of several collagens, led to an increased deposition of collagen IV protein in both tissues. Differences in kidney slices included an up-regulation of the collagen catabolism matrix metalloproteinases. In this study, there was an up-regulation of genes encoding EGF, FGF-regulin and TGF-β1 in the human liver slices, and TGF-β1 in the human kidney slices. Activation of stellate cells in the liver was evident by the up-regulation of early (HSP47 and ß-crystallin) and late markers (αSMA and procollagen 1a1) of stellate cells. Kidney slices exhibited an up-regulation of markers of DNA synthesis and cell cycle progression, such as MCM, minichromosome maintenance, and the microtubule associated kinesins, as well as an up-regulation of cytokine IL-24 which is linked with wound healing, cell survival and proliferation. Morphological changes in liver slices included evidence of regeneration by day 3, anisokaryosis and scattered multinucleate hepatocytes that continued through day 6, with no evidence of progressive necrosis or fibrosis. Additionally,
bile production was evident in the liver slices. The human kidney slices exhibited progressive necrosis in spite of gene changes indicative of repair. These 3-dimensional multi-cellular organ slices maintain tissue architecture and function to predict pathways of injury and repair for several days and following multiple dosing.

### 1352 Non-Coding RNAs Function as Regulators in Acetaminophen-Induced Liver Injury: An Integrative Approach

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Acetaminophen (APAP) overdose is the leading cause of acute liver failure. MicroRNAs (miRNAs) and long non-coding RNAs (lncRNAs) are families of non-coding RNAs (ncRNAs) that control gene regulatory mechanisms epigenetically. To elucidate the roles of ncRNAs in APAP-induced pediatric liver injury, we systematically analyzed the gene and ncRNA expression profiles in APAP-treated HepaRG cells, and selected candidate ncRNAs that target the key drug metabolizing enzymes, transporters (DMETs) and DME regulators—nuclear receptors, and then investigated the underlying mechanisms by a series of biochemical or biological assays. We identified 2,757 genes, 47 miRNAs and 1,340 IncRNAs differentially expressed in APAP-treated HepaRG cells. The hsa-miR-224-5p, hsa-miR-320a, hsa-miR-449a, and hsa-miR-877-5p were predicted to target the CYP3A4, CYP3A4, and NR1I2 and the endogenous expression of these genes were suppressed by the transfection of corresponding miRNAs. RNA electrophoresis mobility shift assays and miRNA pull-down assays displayed direct interactions between miRNAs and their cognate mRNA transcripts. Importantly, experimental transfection of these miRNAs or antisense oligonucleotides (ASOs) for cognate miRNAs exhibited progressive necrosis in spite of gene changes indicative of repair. These 3-dimensional multi-cellular organ slices maintain tissue architecture and function to predict pathways of injury and repair for several days and following multiple dosing.

### 1353 Novel Liver Spheroids for High-Throughput Drug-Drug Interaction Screen by Next-Generation Sequencing

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For ADME, early prediction of Drug-drug interaction (DDI) risk prediction is needed, but available datasets don’t reach FDA approval. However, no accurate high throughput screen (HTS) assay is available for induction of Cytochrome P450 (CYP), the main family of drug-metabolizing enzymes and key players in DDI. Primary Human Hepatocytes (PHH) in 2D usually used in DDI screens feature loss of tissue-specific architecture, mechanical and biochemical cues, cell-to-cell and cell-to-matrix interactions and cell-metabolizing enzyme activities. This affects their metabolic functions and assays leading to relatively poor DDI risk reliability. We have developed “HepatoPearl”, a PHH 3D model produced in micrometric alginate capsules. Their architecture and function to predict pathways of injury and repair for several days and following multiple dosing.

### 1354 Diversity of Primary Hepatocytes Models Allow for Choosing the Best Tools for Hepatotoxicity Assessments

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Hepatotoxicity is a major issue for pharmaceutical companies due to it being a frequent cause of attrition of drug candidates. One of the major reasons for failures is the lack of accurate and predictive models of hepatoxicity in the early stages of drug development. There is therefore a need for in vitro hepatotoxicity screening models that are valid, robust and amenable to high throughput. An array of specific and representative read-outs, using high content imaging and flow cytometry, were established for analyzing compounds’ effects. Cytolysis was followed kinetically as a phenotypic readout of hepatotoxicity, while mitochondrial membrane potential, GSH depletion, or lipid contents measured by flow cytometry, help decipher hepatotoxicity mechanisms with excellent sensitivity. Hepatocyte primary cultures from three different species were used for different ADME studies. For example, this approach revealed that the human hepatocytes from rats is highly amenable to large screening campaigns. Primary human hepatocytes are also available in high numbers from deceased patients and constitute an excellent model for testing hepatotoxicity, with a higher cost. We have compared and validated selected hepatocytes against the three most reactive APAP hepatocytes in our array of specific readouts with the same compounds. We exemplify how the use of fresh rat hepatocyte based assays, in combination with the single cell level of analysis, represents a quality predictive model with a high level of sensitivity and specificity. However, primary human hepatocytes are only used for different ADME studies and they are highly relevant for modeling APAP-induced hepatotoxicity as mechanisms are similar in human and mice. Mice primary hepatocytes were used for screening potential protective compounds against APAP by using the GSH content readout and comparing with the effect of N-Acetylcysteine. We have therefore developed fully validated primary hepatocyte models from different species, combined with specific readouts, which allow for choosing the best model for a particular question.

### 1355 Utilization of Human-Focused 3D Liver Models in Conjunction with Multi-Parametric Cell Health Approaches for the Prediction of Drug-Induced Liver Injury

C. Dilworth. Cytorex, Alderley Park, United Kingdom. Sponsor: C. Strock

Recent pharmaceutical strategies to address drug induced liver injury (DILI) have focused primarily on multi-parametric approaches utilising conventional 2D cell culture methods with primary human hepatocytes (PHH) or HepG2 or HepaRG cells. A recent study has been published using a strategy for the prediction of human DILI utilising PHH 3D liver microspheres. Their approach compared responses in 2D versus 3D models to reference DILI compounds utilising a single biochemical endpoint, cellular ATP. We have further developed this DILI approach and developed a comprehensive panel of PHH and HepaRG 3D liver microspheres (MTs) utilising, in addition to ATP, a multiparametric confocal high content screen measuring a range of endpoints such as mitochondrial membrane potential, reactive oxygen species formation and GSH content. CYP450 characterisation microspheres showed significantly higher activities in HepaRG MTs compared to PHH MTs (CYP: 2.10 pmol/min/MT HepaRG, 80 pmol/mn/MT PHH). Following characterisation 40 DILI reference compounds (30 positive, 10 negative) were screened through each model and confocal high content was used in addition to ATP activity to measure cellular function. Prediction of human DILI was improved in the HepaRG MT when compared to the overall response in the human hepatocyte MTs. In addition prediction of DILI was increased further when a multiparametric approach was taken when compared to measurement of ATP. Compounds such as diclofenac and bosentan were not flagged as DILI positive (within 5-fold of Cmax) when ATP was used as the predictive marker, however using a multi-parametric approach these
were shown to be DILI positive. In addition a DILI positive response for fialuridine was only observed in the HepaRG model. The data presented here builds upon the current strategies to address human DILI. We have demonstrated that 3D MTs consisting of HepaRG cells gives an increased prediction of DILI. In addition the prediction of DILI is also significantly improved by the utilisation of multi-parametric approaches when compared to measuring ATP alone. These in vitro models are amendable to high throughput screening, are cost effective and as such could play an important role in mitigating DILI risk early in drug discovery. 1 Chem. Res. Toxicol. 2012 2067-82 2 Toxicol. In vitro 2016 33,63-70 3 Toxicol. In vitro 2015 30, 429-437 4 Arch. Toxicol. 2017 91, 2849-2863

1356 Assessment of the Gene Expression Signatures of Hepatotoxic and Non-Hepatotoxic Endothelial Receptor Antagonists in Primary Human Hepatocytes
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Impairment in the hepatobiliary elimination of bile acids (via inhibition of bile salt export pump (BSEP)) has emerged as a leading mechanism for clinical drug induced liver injury (DILI) associated with the structurally diverse endothelial receptor antagonists (ERAs) bosentan and sitaxsentan. In addition to BSEP inhibition, sitaxsentan is also known to undergo oxidative bioactivation by cytochrome P450 (CYP) 3A4 to yield protein reactive metabolites, which may represent an additional causative factor of clinical DILI. In contrast, evidence to date suggest little risk of DILI with the marketed ERAs ambrisentan and macitentan, which are used to treat pulmonary arterial hypertension. Incidentally, ambrisentan does not inhibit BSEP but macitentan inhibits the transporter with potency comparable to that of bosentan and sitaxsentan, and yet is not hepatotoxic. While the propensity for direct inhibition of hepatobiliary transporters by ERAs has been described using human hepatocytes, corresponding examination of the up- and/or down-regulation of major human drug metabolising enzyme (CYPs, UGTs, drug transporters (OATP, NTCP, MRP, BCRP, MDR)) and oxidative stress response ( NRF2 pathway) genes remains to be addressed. We recently reported the use of a custom TaqMan Gene Expression Array Card to differentiate nuclear receptor- mediated transcriptional regulation of drug metabolizing enzymes and transporters. In the present work, we have extended this methodology towards the comparison of the gene signature profile for the hepatotoxic and non-hepatotoxic ERAs in human hepatocytes. Varying concentrations of the test compounds were incubated with sandwich-cultured cryopreserved human hepatocytes (n=3 donors) for 48 h. Full dose-response curves were generated to enable differentiation by rank-ordered maximal effect (Emax) and/or half maximal concentration (EC50) for target genes. The results obtained from this analysis demonstrate unique gene signatures for structurally diverse ERAs with a trend towards differentiation of hepatotoxic and non-hepatotoxic molecules. This framework, in addition to the suite of existing in vitro trend towards differentiation of hepatotoxic and non-hepatotoxic molecules. This framework, in addition to the suite of existing in vitro tests. The liver is one of the first and most exposed organ to chemical damage, and thus can serve as a marker of potential systemic or target-specific toxicity elicited from repeated chemical exposure. This study provides an update on our understanding on mechanisms of toxicity elicited by selected commonly used chemical classes. It is focused on the construction of a hepatotoxicity decision tree based on more than 1300 different chemicals, their possible MOAs (including receptor binding activities), and their core chemical structural alerts (including reactive metabolites). Based on these principles, we have created a decision tree where we have grouped these chemicals into 30 different categories and more than 120 subcategories. This decision tree includes: 4 chemical categories associated with 9 different receptors, 10 chemical categories associated with reactive metabolites (RMs), as well as 14 chemical categories associated with similar structural features/MOA. We propose that this decision tree can be used to screen and identify potential liver toxicants based on their core structural alerts/features, and/or their reactive metabolites (RMs). The steps in the decision tree are very transparent, and could be used as a component of weight of evidence decisions based on SAR by identifying high quality suitable analogs, and thus toxicity data usable in read-across evaluations. This information can also be used to integrate chemical structure and MOA information within chemical classes, with the mechanistic explanations where possible, which could be used to improve the accuracy and consistency of SAR read across methods.

1357 Identifying Chemicals Based on Mode-of-Action/Metabolism/Structural Features Associated with Potential to Elicit Hepatotoxicity

A better understanding of mode of action (MOA) of chemicals of interest is important in the design/support of non-animal alternative methods, for example in supporting relevance of analogs used in Structure Activity Relationship (SAR)-based assessments, or for choosing appropriate in vitro tests. The liver is one of the first and most exposed organ to chemical damage, and thus can serve as a marker of potential systemic or target-specific toxicity resulting from repeated chemical exposure. This study refines an update on our understanding on mechanisms of toxicity elicited by selected commonly used chemical classes. It is focused on the construction of a hepatotoxicity decision tree based on more than 1300 different chemicals, their possible MOAs (including receptor binding activities), and their core chemical structural alerts (including reactive metabolites). Based on these principles, we have created a decision tree where we have grouped these chemicals into 30 different categories and more than 120 subcategories. This decision tree includes: 4 chemical categories associated with 9 different receptors, 10 chemical categories associated with reactive metabolites (RMs), as well as 14 chemical categories associated with similar structural features/MOA. We propose that this decision tree can be used to screen and identify potential liver toxicants based on their core structural alerts/features, and/or their reactive metabolites (RMs). The steps in the decision tree are very transparent, and could be used as a component of weight of evidence decisions based on SAR by identifying high quality suitable analogs, and thus toxicity data usable in read-across evaluations. This information can also be used to integrate chemical structure and MOA information within chemical classes, with the mechanistic explanations where possible, which could be used to improve the accuracy and consistency of SAR read across methods.

1358 Primary Human Hepatocyte 3D Spheroid Model for Predicting Drug-Induced Liver Injury

Primary human hepatocytes (PHHs) are considered the "gold standard" for liver biology and also the main target of drug induced liver injury (DILI). Drug development failures and post-launch withdrawals highlight the need for more physiologically relevant in vitro human models to improve liver toxicity prediction accuracy. Emerging 3D cell culture technologies can potentially fill the gap between preclinical tests and clinical observations by better mimicking the in vivo scenarios. In comparison to other 3D cell culture technologies, hepatoid culture is cost-effective and more compatible with high throughput toxicity screening in the early stages of drug discovery. More importantly, 3D spheroid culture has been shown to prolong the viability and functionality of PHHs up to several weeks and therefore allows for in vitro chronic hepatotoxicity tests with repeated dosing. In this presentation, we have tested more than 30 lots of cryopreserved PHH for 3D hepatotoxicity using Corning® Ultra-Low Attachment spheroid plates (96-well or 384-well format). About 50% of the PHH lots tested produced spheroids that can be used for 3D spheroid based assays. Three lots of 3D spheroid qualified PHH were used for both 2D (24 hour single dose) and 3D hepatotoxicity assays (three repeated dosing over 2 weeks) with known DILI liability (amiodarone, nefazodone, troglitazone etc.) and control compounds. Serial dilutions of testing compounds were applied and dose response curves were generated following bioluminescent ATP viability measurements. In comparison to 2D monolayer culture from the same lots of PHHs, 3D PHHs consistently increased the sensitivity (2-10 fold lower IC50 values than those from 2D culture) to the treatment of DILI compounds. Using a margin of safety (MOS) analysis comparing IC50 to clinical Cmax values of tested compounds confirms that 3D hepatotoxicity assays have superior prediction to 2D assay addition, high confidence based 3D assays are being developed with PHH spheroids for mechanistic insights into DILI with model compounds. Together, our results demonstrate 3D PHH spheroids as a superior in vitro model to predict DILI with high throughput capacity.

1359 Liver-Chip Model Identifies Mitochondrial Dysfunction, Oxidative Stress, and Inflammasome Response as Potential Pathways of Toxicity for the GPR40 Agonist TAK-875

Drug-induced liver injury (DILI) is a major reason for safety-related attrition in the pharmaceutical industry. There is continued search for in vitro models that can be used to consistently and reliably select compounds with reduced liability for liver injury. 3D liver models have the potential to revolutionize in vitro liver toxicity testing. We describe a microfluidic Liver-Chip model that contains primary hepatocytes, sinusoidal endothelial cells, stellate, and Kupffer cells in a spatial configuration that mimics their organization in the liver. Albumin secretion and abundant CYP450s activities are maintained in culture for ≥2 weeks. The model can detect diverse liver pathophysiology, including hepatocellular injury, lipid accumulation, and stellate activation. The fluidic component allows for ease of sampling of effluent for detection of cytokines and DILI biomarkers (miR122, α-GST, CK-18, ccCK18, and total HMGB1). These endpoints were characterized with tool compounds including APAP, fialuridine, and methotrexate. The model was also used to investigate the mechanism of DILI by the GPR40 agonist TAK-875 following daily treatment at 3, 10, and 30 μM for ~2 weeks. There was significant turnover of TAK-875 and formation of its reactive acyl glucuronide metabolite (TAK-875AG) on Day 1 (16%) and on Days 7/14 (44%) compared to 1% turnover obtained in human hepatocytes. The oxidative metab-
olite (M-I) was formed at 4% in the Liver-Chip compared to reports of 10% found in humans. Inhibition of MRPI expression, perturbation of mitochondrial membrane potential, and formation of reactive oxygen species and inflammatory cytokines were observed at concentrations bridging the clinical Cmax (10 μM). Interestingly, hepatic lipid accumulation and stellate cell activation were also observed at 10 μM. These data suggest that accumulation of a reactive TAK-875AG, cell stress, and an innate immune response are possible triggers for TAK-875-mediated liver toxicity. In summary, the Liver-Chip is amenable for microscopical, biochemical, and DilI biomarker evaluation and will be useful for prediction and mechanistic investigation of DilI.

Liver fibrosis is the excessive accumulation of extracellular matrix proteins such as collagen, which can lead to cirrhosis, liver failure and transplantation. Anti-fibrotic therapies aim at inhibiting the induction of fibroblasts (HSC), preventing the deposition of extracellular matrix proteins (ECM). Beside the HSC, presence of other liver cell types as opposed to a 2D monolayer greatly decreases the number of cells required per well and likely increases the physiological relevance of the model. In liver spheroids exposed to the Cyp2e1 substrate acetaminophen for 6 days (p<0.05) compared to scrambled control, however, the functional effect of knockdown could not be confirmed as no cytotoxicity was observed in non-transfected PMH spheroids exposed to the Cyp2e1 substrate acacetaminophen for 6 days at concentrations up to 10 mM (p>0.05 for ATP and albumin compared to control). Efforts to quantitate Cyp enzyme activity and transporter levels in PMH spheroids are ongoing. Taken together, our results demonstrate the successful long-term culture of PMH in 3D spheroids—methodology that can now be expanded to CC strains that will enable rapid and cost-effective implementation of this approach at all stages of drug development. The objective of this study was to evaluate 3D spheroid methodology for the long-term culture of primary mouse hepatocytes (PMH) to support an initial focus on drug-induced liver injury (DILI). Cypreserved hepatocytes from C57BL/6 mice were seeded on ultra-low attachment 96-well plates and allowed to spontaneously self-aggregate. PMH spheroids were then cultured in 96-well plates for 5 days. ATP levels were maintained, while albumin production was significantly higher on culture Day 13 compared to Day 6 (p<0.05). Transfection of Cyp2e1-targeting siRNA performed on culture Day 0 resulted in an 85% reduction of Cyp2e1 mRNA levels in PMH spheroids and a 70% reduction of Cyp2e1 mRNA in PMH spheroids at Day 4 (p<0.05 compared to scrambled control). The successful long-term culture of liver fibrosis, suitable for high-throughput efficacy and safety screening of anti-fibrotic drugs.

Drug-induced liver injury (DILI) remains a major cause of drug attrition during drug discovery and development. As there is a significant need for more predictive models for DILI, a microengineered Liver-Chip was developed to include all relevant cell types more representative of the in vivo tissue. To ensure hepatic function, we recapitulate in vivo metabolic capabilities, that has potential for long term maintenance of cell viability to enable repeated drug exposures, has well characterized toxicology endpoints sensitive for cell-specificity over time, and is capable of demonstrating the diverse mechanisms of DILI. Advanced engineering fabrication techniques were applied to achieve a high level of control over the chip microenvironment. The model incorporates relevant cell-ECM interactions, a hepatocyte and liver sinusoidal endothelial cell interface, with relevant cytoarchitecture and physiological flow. In addition to the human Liver-Chip, rat and dog models were developed to enable characterization of species differences with respect to pharmacokinetics, toxicity and mechanism of action. Liver-specific functions were measured for all three species and demonstrated maintenance of in vitro relevant levels of functionality including albumin secretion, CYP450 enzyme activity, and expression of transcriptomic profile that demonstrated functional diversity over conventional 2D models. We evaluated Liver-Chips for each of the three species to evaluate non-clinical to clinical translation of experimental findings with Bosantan, an endothelin receptor antagonist and BSEP transport inhibitor that has clinical to clinical translation of experimental findings with Bosantan, an endothelin receptor antagonist and BSEP transport inhibitor that has been used in clinical trials. We also demonstrated that this model of liver fibrosis, suitable for high-throughput efficacy and safety screening of anti-fibrotic drugs.

Liver fibrosis is a major fibrotic disease that occurs in a variety of organs as a consequence of chronic injury. It is characterized by the excessive accumulation of extracellular matrix proteins (ECM) in liver, which can lead to cirrhosis, liver failure and transplantation. Anti-fibrotic therapies aim at inhibiting the induction of fibroblasts (HSC), preventing the deposition of ECM. Beside the HSC, presence of other liver cell types as opposed to a 2D monolayer greatly decreases the number of cells required per well and likely increases the physiological relevance of the model. We demonstrated the successful long-term culture of PMH in 3D spheroids—methodology that can now be expanded to CC strains that will enable rapid and cost-effective implementation of this approach at all stages of drug development. The objective of this study was to evaluate 3D spheroid methodology for the long-term culture of primary mouse hepatocytes (PMH) to support an initial focus on drug-induced liver injury (DILI). Cypreserved hepatocytes from C57BL/6 mice were seeded on ultra-low attachment 96-well plates and allowed to spontaneously self-aggregate. PMH spheroids were then cultured in 96-well plates for 5 days. ATP levels were maintained, while albumin production was significantly higher on culture Day 13 compared to Day 6 (p<0.05). Transfection of Cyp2e1-targeting siRNA performed on culture Day 0 resulted in an 85% reduction of Cyp2e1 mRNA levels in PMH spheroids and a 70% reduction of Cyp2e1 mRNA in PMH spheroids at Day 4 (p<0.05 compared to scrambled control). However, the functional effect of knockdown could not be confirmed as no cytotoxicity was observed in non-transfected PMH spheroids exposed to the Cyp2e1 substrate acetaminophen for 6 days at concentrations up to 10 mM (p>0.05 for ATP and albumin compared to control). Efforts to quantitate Cyp enzyme activity and transporter levels in PMH spheroids are ongoing. Taken together, our results demonstrate the successful long-term culture of PMH in 3D spheroids—methodology that can now be expanded to CC strains. The use of 3D spheroids as opposed to a 2D monolayer greatly decreases the number of cells required per well and likely increases the physiological relevance of the in vivo model. The ability to perform siRNA knockdown in PMH spheroids will enable rapid validation of candidate risk factor genes identified by our CC platform.
we treated the human hepatocytes in both models with inducers like Omeprazole, Phenobarbital and Rifampycin and examined the metabolic cytochrome P450 (CYP) capabilities. For hepatocytes in RAFT™ Culture System the response was both stronger and more stable over 17 days in culture compared to the sandwich cultures, improved stability of P450s by culturing hepatocytes in 3D could enable long-term and repeat dosing for toxicity analysis using primary human hepatocytes.

1366 Expanded Primary Human Liver Sinusoidal Endothelial Cells as a Tool to Complement Hepatotoxicity Studies
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Liver sinusoidal endothelial cells (LSECs) are highly specialized endothelial cells lining the walls of hepatic sinuses. Their key roles include the transfer of substances between blood and liver parenchyma, rapid internalization of blood-borne macromolecules, liver regeneration and immune tolerance. Despite their substantial contribution to liver homeostasis, LSECs are often overlooked during hepatotoxicity assays due to insufficient cell numbers and a limited proliferation capacity. To address this issue, we employed our previously described upcyte® technology for expansion of primary LSECs by lentiviral transduction of human 3D liver microtissue (hLiMT) assay to understand whether cell viability and overall cell numbers were stable in 2D and 3D culture formats. Future investigations will focus on acute and chronic exposure to hepatic toxic model compounds in both 2D and 3D cultures. Taken together, our data suggest that upcyte® LSECs combine many characteristics of primary LSECs with the advantage of an extended lifespan, facilitating their use in hepatotoxicity assays under reproducible and standardized conditions.

1367 Withdrawn by Author

1368 Co-Culture of Primary Human 3D Hepatic Spheroids with Non-Parenchymal Cells Allows for Detection of Immune-Related Drug-Induced Liver Injury Compounds
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Idiosyncratic drug induced liver injury (IDILI) remains a major cause of pharmaceutical drug failure. As a component of the innate immune system, the liver contains non-hepatocyte cells such as natural killer T cells (NKT) and Kupffer cells (KCs) among others. Here we used an in vitro human 3D liver microtissue (LiMT) assay to understand whether co-culturing non-parenchymal cells (NPCs) could detect known IDILI compounds. When human NPCs were incorporated into LiMTs and treated with 1 µg/mL LPS, increases in cytokines were detected in the media, compared to non-LPS treatment. Additionally, some NPC lots displayed greater levels of CD68+ and KCs among others. Generated upcyte® LSECs expressed typical endothelial cell markers (CD31, von Willebrand factor), LSEC-specific receptors (mannose receptor, LYVE-1 and L-SIGN) and showed uptake of several macromolecule ligands (e.g. serum albumin, aggregated IgG). Since LSECs are involved in drug-induced liver injury, we investigated acetaminophen-induced cytotoxicity. Interestingly, LSECs showed a significant decrease in IC50 when compared to upcyte® hepatocytes, indicating that these cells are a promising tool to complement hepatotoxicity methods. Experiments were conducted with primary human hepatocytes and compared different 2D and 3D culture approaches. Among 8 different medium formulations tested, the most appropriate medium was a mixture of hepatocyte and LSEC medium, as demonstrated by high preservation of macrophages markers (e.g. albumin, CD31). Cell viability and overall cell numbers were stable in 2D and 3D culture formats. Future investigations will focus on acute and chronic exposure to hepatic toxic model compounds in both 2D and 3D cultures. Taken together, our data suggest that upcyte® LSECs combine many characteristics of primary LSECs with the advantage of an extended lifespan, facilitating their use in hepatotoxicity assays under reproducible and standardized conditions.
presence of LPS (NCP-1: 69.0 +/- 0.2 µM versus NCP-3: 319.6 +/- 1.7 µM). DCF showed 2-fold greater cytotoxicity with the NCP-1 lot compared with NCP-3, independent of LPS co-treatment. LPS alone for 72 h stimulated IL-2, IL-6 and IL-8 from NCP-1 compared with NCP-3, however there were no significant differences between TVX treatment with or without LPS in either NCP lot. Our results demonstrate that incorporation of low cultured immune cells into 3D hLiMTs can help to drive in-vitro detection of liver-toxic compounds as measured by cytotoxicity endpoints. Due to donor specific variability, characterization of NPC lots, by identification of markers for the inherent cell types present and their reactivity towards LPS-co-treatment, could enable more sensitive detection of DILI compounds.

1369 A 3D Human Microtissue Model of the Liver

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Drug-Induced Liver Injury (DILI) contributes to drug failures, drug withdrawals and acute liver failures. Moreover, the liver is known to strongly interact with other organ systems and in some instances the metabolites secreted by the liver are responsible for other organs injury. Engineered 3D liver models may increase the physiological relevance of drug toxicity assays by maintaining the expression levels of key cytochrome P450 enzymes and the metabolic activity in liver cells. The objective of this study was to create a three-dimensional (3D) microtissue model of the human liver. A human hepatoma cell line (HepaRG) was used to create the 3D microtissue liver model. These cells express the major xenobiotic sensors, drug transporters, phase I and II drug metabolizing enzymes. A 3-chamber microfluidic device (DAX-1, AIM Biotech) was used to create the liver 3D tissue. The device consists of a single chamber that is connected to two adjacent fluidic channels used to feed the microtissue. Briefly, liver cells at a concentration of 50 million cells per mL were mixed in either a collagen type 1 solution (7 mg/mL concentration) or fibrin solution (10 mg/mL). Then added to the central chamber of the microfluidic device. The gel and cell mixture was subsequently left to polymerize in the central chamber. This microfluidic device facilitates the generation of an interstitial fluid flow through the porous microtissue and thus enables feeding of the microtissue but also allows sampling of metabolites in the low pressure microfluidic channel. To evaluate both the viability and the ability of HepaRG liver cells to respond to compounds in the microfluidic chip, testosterone at 50 µM concentration was added to the central chamber. This microtissue model enabled feeding of the microtissue but also allows sampling of metabolites secreted by liver cells. Moreover, our results also suggest that the seeding matrix is an important factor in the metabolism of HepaRG liver cells. The metabolite concentration was three times higher when liver cells were seeded in collagen type 1 versus fibrin gel. We have therefore developed a microtissue model and demonstrated that this model can be used to assess the functionality of CYP 3A4 in HepaRG cells and quantify their secretion of metabolites. In the future, we plan to use this model system to investigate liver–kidney interactions.

1370 Up-Regulation of Fibroblast Growth Factor (Fgf) 21 by Cisplatin in Mouse Liver and Hepatoma Cells

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Cisplatin (cis-diaminedichloroplatinum, CDDP) is one of most widely used chemotherapeutic drugs. Numerous adverse effects have been noted during cisplatin therapy, which largely limit its clinical applications. Cisplatin has only been sporadically reported that cisplatin causes liver dysfunction. Fibroblast growth factor (Fgf) 21 is an important regulator of glucose, lipid and energy metabolism. Recent studies demonstrated that Fgf21 also plays an important cytoprotective role against chemical-induced toxicities, such as of dioxins, acetaminophen and endotoxins. This study was designed to determine the regulation of Fgf21 by cisplatin in mouse liver and cultured mouse hepatoma cells, as well as the underlying mechanisms. Our data demonstrated that cisplatin increased the mRNA and protein expression of Fgf21 in wild-type (WT) mouse liver and in cultured mouse Hepa1c1c7 hepatoma cells. In addition, both mouse liver and cultured mouse Hepa1c1c7 hepatoma cells, cisplatin increased mRNA expression of manganese superoxide dismutase (MnSOD), which is generally induced in the presence of mitochondrial stress. Seahorse study further demonstrated that cisplatin concentration-dependently induced mitochondrial stress in cultured mouse Hepa1c1c7 cells. Moreover, in mouse liver, cisplatin increased activating transcription factor (ATF) 4 mRNA expression, which is generally activated by mitochondria stress and subsequently up-regulates Fgf21 expression. In conclusion, cisplatin induced Fgf21 expression in mouse liver and cultured mouse hepatoma cells, maybe via activation of mitochondria stress-ATF4 signaling.

1371 Microcystin-Lr Hepatic and Renal Toxicity in Non-Alcoholic Fatty Liver Disease

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Microcystin-Lr (MCLR) is a hepatotoxin produced by blue-green algae and is transported into cells by organic anion transporting polypeptides (Oatp). Non-alcoholic fatty liver disease (NAFLD) is an increasing chronic liver disease in the United States and is a risk factor for chronic kidney disease (CKD). Previous data showed that rodent models of NAFLD decreased expression of hepatic Oatps and caused increased plasma concentrations of Oatp substrates. We hypothesize that MCLR will exacerbate liver disease in NAFLD and, due to increased plasma concentrations, increase kidney toxicity in NAFLD. Three rat diets were used: control (healthy), methionine and choline deficient (MCD) diet (NAFLD), and a high fat/high cholesterol diet (HFFC) (NAFLD). Dose groups included vehicle, 10µg/kg and 30µg/kg MCLR via i.p. injection every other day for 4 weeks. Liver pathological incidence and severity score results indicate increased fibrosis in all high dose MCLR groups, greater inflammation, fibrosis and biliary hyperplasia in the low dose MCLR MCD group, and dose-dependent decreased steatosis in both NAFLD groups, suggesting MCLR drives NAFLD towards lower lipidosis and more fibrosis. Kidney pathology scores showed elevated protein casts and glomerular pathology in MCLR treated healthy and HFFC groups. Biochemical analyses indicate that high dose MCLR increased plasma alanine transaminase (ALT) and urinary kidney injury molecule-1 (KIM-1) in both healthy and HFFC groups. With high dose MCLR, plasma glucose decreased in both healthy and HFFC groups but insulin increased only in the HFFC. At both MCLR doses plasma triglycerides decreased only in healthy animals, while plasma cholesterol increased only in HFFC animals. We also observed decreased hepatic and renal mRNA expression of several Oatp isoforms with MCLR exposure. These data suggest that MCLR exposure exacerbates pathological features of NAFLD in both the liver and kidney and alters key metabolic features of disease. Funded by 4R00ES024455.
mitochondrial fatty acid beta-oxidation. Most non-DILI compounds were not detected in any of the assays. These results indicate that each assay has its own advantages and limitations, thus, a combination of these assays is useful for estimating the DILI potential of compounds. 1) J. Toxicol. Sci., 42, 349-357, 2017

1373 A Sensitive Donor Is Identified in a Multi-Donor Evaluation of the Toxicity of the Fialuridine and Clevudine Nucleoside Analogues in the In Vitro Human Hepatocap® Model

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FIAU is a nucleoside analogue that was under development for Hepatitis B but was discontinued in clinical trials following development of acute liver failure and death hypothesized to be related to mitochondrial dysfunction. Conventional preclinical testing did not predict such findings. FMAU is a nucleoside analogue similar in structure to FIAU, except that the iodo group is replaced by a methyl group. This compound also did not manifest preclinical findings. However, a small percentage of patients developed severe myopathy with mitochondrial changes after multiple months of treatment leading to termination of FMAU development for several countries. We evaluated both FIAU and FMAU for a 12-day treatment period in the in vitro HepatoPac® model in five human donors of varying ages (i.e., pediatric vs. adult), genders, and ethnicities to assess potential for differential donor sensitivity using assays for mitochondrial and nuclear DNA levels, urea production, lactate dehydrogenase (LDH) release, and transcriptional profiling with RNA-seq (FIAU) only. For FIAU, mtDNA declines were similar across donors with IC_{50}s of ~0.1 µM. However, nuclear DNA, reflecting general cytotoxicity, was more significantly impacted for two donors (Donors 4-S, D4-S) with IC_{50}s ~5 µM; whereas the other donors had IC_{50}s >10 µM, including a donor (D3) which manifested no nuclear DNA decline up to the highest concentration tested (30 µM). One of the donors (D4) with greater sensitivity to the nuclear DNA change also manifested the earlier and more robust declines in urea production and increases in LDH release. D4 also had the most robust mtDNA decline (~67%) with 30 µM FMAU treatment (top dose). The other endpoints were insensitive for all donors treated with FMAU. RNA-seq analysis showed that D4 had the most significant mitochondrial fatty acid beta-oxidation. Most non-DILI compounds were not detected in any of the assays. These results indicate that each assay has its own advantages and limitations, thus, a combination of these assays is useful for estimating the DILI potential of compounds. 1) J. Toxicol. Sci., 42, 349-357, 2017

1374 Examination of Drug-Induced Liver Injury in Uptcye Hepatocytes

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Liver toxicity is a major cause of drug failure in clinical trials as well as market withdrawal of approved drugs. In addition, due to species differences in drug metabolism, it is often difficult to extrapolate the results obtained from preclinical animal models to humans. The ucpcyte® cells are proliferating human hepatocytes that retain important drug metabolizing enzymes, such as cytochrome P450 3A4 (CYP3A4), which make them an attractive model system to examine drug-induced liver injury (DILI). In this study, we examined the cytotoxicity in ucpcyte® cells for 10 compounds known to be bioactivated in liver. The potency for inducing toxicity was compared to that of a cell line that has reduced expression of CYP3A4. Several compounds had higher toxicity in ucpcyte® than in the cells with lower CYP3A4 expression. These studies show that using ucpcyte® hepatocytes to study DILI can potentially reduce attrition due to metabolism-mediated toxicity and improve safety of drug candidates.

1375 Cyp Silenced Human Hepatocytes for Metabolite Assessment in a Toxic Response

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It is important to identify the role of a given metabolite and of the corresponding CYP(s) involved in its formation in the toxic response of the human liver to a xenobiotic. The metabolite assessment could anticipate hepatotoxicity, DDIs, and the inter-individual variation in the drug safety. The current approach used for CYP reaction phenotyping consists of i) incubating the hepatocytes with either reversible CYP inhibitors such as ABT or more irreversible inhibitors such as azamulin; and ii) comparing the response to the test compound in the conditions with/without inhibitors. These conventional studies are done ex tremoraneously, and thus our goal is to prepare a performance-controlled, frozen and ready-to-use cell-based assay system for fm measurement and metabolite assessment. Primary human hepatocytes would be evaluated from different donors were pre-incubated separately with three specific inhibitors to generate three targeted major CYP-silenced PHH, in which a single CYP was chemically and irreversibly inactivated. The potent inhibition of fresh and thawed CYP-silenced PHH batches and related controls was evaluated using CYP-specific probe substrates. The results showed the targeted CYP activity was specifically and significantly inhibited in specific CYP-silenced PHH. For example, around 80% of CYP3A4-mediated metabolism of testosterone was inhibited in CYP3A4-silenced PHH inactivated by azamulin even the optimization of MBI conditions could further increase silencing efficiency. It has been reported that bioactivation of aflatoxin B1 and lapatinib to reactive metabolites by CYP3A4 contributed importantly to hepatotoxicity in PHH and HepaRG™ cells. Therefore, both of CYP3A4-silenced PHH and control cells were incubated with aflatoxin B1 or lapatinib at different concentrations, and then the formation of MBI was measured and analyzed by LC-MS/MS. The preliminary results of this study showed that the concentration levels of both aflatoxin B1 and lapatinib metabolites dropped by 60-80% correspondingly, compared to the controls. Those ready to use cytopreserved hepatocytes with specific CYP silencing allow a direct evaluation of metabolic liabilities. The cell-based assay is relevant for conducting the metabolite assessment in an integrated functional environment, taking into account the interplay between transporters and phase I and II metabolism.

1376 Fasiglifam (Tak-875): Investigating Drug-Induced Liver Injury Utilizing In Vitro Mechanistic Assays

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Fasiglifam (TAK875, a GPR40 agonist) developed for the treatment for type 2 diabetes mellitus was withdrawn from Phase III clinical trials due to drug-induced liver injury (DILI). Rodent and human in vitro liver models were used to investigate the potential hepatotoxic mechanism of action. TAK-875 is 99.84% protein bound, with an estimated free plasma concentration of 14 nM. Therefore, exposure conditions with and without serum were used throughout. High-content screening (HCS) revealed effects on mitochondrial membrane potential in HepG2 cells, primary human hepatocytes and 3D human liver microtissues (HLIMT) at a range of time points, implicating mitochondria have a potential role in TAK-875-induced liver injury. The mitochondrial potential was uncoupled at 5.6µM in HepG2 cells in the absence of serum; however, no response in MMP was observed with serum up to a concentration of 100µM. Further using the mitochondrial glutamate (GLU) assay in serum and serum-free conditions showed no significant shift between Glu and Gal conditions up to 400µM (+serum) even though cytotoxicity was observed. In the absence of serum, a positive 3.67-fold shift was shown with a galactose AC_{50} of 27.5µM. Using the Seahorse XF96 analyzer to determine mitochondrial oxygen consumption rates (OCR) and reserve capacity. The OCR AC_{50}s of TAK-875 in HepG2 cells were 16.9, 2.8, and 4.1 µM at 0hr, 1hr, and 24hrs, respectively. The reserve capacity AC_{50}s of TAK-875 in HepG2 cells were 12.2, 4.66, and 0.97µM at 0hr, 1hr, and 24hrs, respectively. In human hepatocytes the OCR and reserve capacity AC_{50}s values at 0hr were determined to be 86.4 and 116 µM. This indicates hepatocytes and HepG2 cells respond comparably to TAK-875 mitochondrial liability would have been flagged as a concern using in vitro models of hepatotoxicity, thus demonstrating the valuable role of in vitro screening for hepatotoxicity in drug discovery strategies.
Acetaminophen (APAP)-induced liver injury after an overdose is the major cause of acute liver failure in the USA. APAP hepatotoxicity is mediated by formation of a highly reactive intermediate, N-acetyl-p-benzoquinone imine (NAPQI), which depletes hepatic GSH and initiates hepatoocyte injury. While treatment with N-acetylcysteine (NAC) to replenish GSH is the current standard of care for APAP overdose, anecdotal evidence suggests that co-administration of NAC with 4-methylpyrazole (4MP) is beneficial in the clinic. The current study examined the influence of 4MP on APAP induced cell signaling and attempts to uncover the basis for this protection. Co-treatment of 4MP at a dose of 50 mg/kg to male C57Bl/6 mice administered APAP (300 mg/kg) showed significant protection against elevations in plasma ALT levels at 6 hours. This protection was confirmed by histology, which showed attenuated centrilobular necrosis and DNA fragmentation. Moreover, APAP-induced activation of JNK, mitochondrial translocation of phospho-JNK and Bax were all prevented by 4MP co-treatment. Release of mitochondrial inter-membrane proteins AIF and Smac into the cytosol were also blocked. Investigation of the mechanism behind these effects revealed significant inhibition of cytochrome P450 activities by 4MP in vitro. In addition, 4MP treated animals showed 95% reduction in APAP protein adducts at 6 h and a 66% attenuation of hepatic GSH depletion at 30 min after APAP treatment. Analysis of APAP metabolites revealed that the APAP-GSH conjugate and its metabolites (APAP-Cys, APAP-NAC) were reduced by 80-98.3% by 4MP treatment while generation of the glucuronide conjugate, which represents 95% of the total metabolites, was not affected. APAP-sulfate levels, which represent 6% of the total metabolites, were reduced by 49%. The relevance of these findings for humans was assessed in primary human hepatocytes. Similar to the findings in mice, co-treatment of 4MP (2 mM) with APAP (10 mM) eliminated cell death in human hepatocytes at 24 h. Together, these findings indicate that 4MP is highly effective in protecting against APAP in both murine and human hepatocytes by blocking the generation of the reactive metabolite through inhibition of cytochrome P450 enzymes. 4MP can complement NAC in preventing mitochondrial dysfunction and the progression of liver injury.

Drug-induced Liver injury (DILI) is one of the major impediments in drug development. Acetaminophen (APAP)-induced liver injury is a classic model of DILI. Flavin-containing monoxygenase 3 (FMO3) is an enzyme involved in metabolism of several xenobiotics, such as amphetamine and its analogs. In a rodent model of APAP autoprotection, dramatic increases in hepatic Fmo3 mRNA and protein expression have been observed. Fmo3 induction is seen in association with highly increased serum levels of methionine sulfoxide (MSO), an endogenous metabolite of this enzyme. In female mice, pharmacological inhibition of Fmo3 results in greater susceptibility to APAP-induced liver injury compared to males. Moreover, in vitro overexpression of Fmo3 confers partial, yet significant protection from APAP cytotoxicity. Although these studies are suggestive of a potential hepatoprotective role of Fmo3 in APAP hepatotoxicity, the molecular mechanism of this protective function is still unknown. In this study, we investigated the capacity of MSO to protect against APAP cytotoxicity using two immortalized hepatocyte cell lines, HepaRG and HC04 cells. Additionally, we evaluated the role of MSO in the hepatic differentiation of HepaRG liver progenitor cells. Our results show that MSO treatment by itself at a wide range of concentrations does not produce any cytotoxicity. As expected, APAP treatment produced cytotoxicity in both cell lines in a dose-dependent manner, as evidenced by LDH release into media. However, MSO treatment (pre-as well as co-treatment) did not afford any protection against APAP cytotoxicity in either cell line. Furthermore, addition of MSO to both growth and differentiation media did not alter the expression of FMO3 in HepaRG cells. Hepatocyte differentiation status was determined by gene expression analysis of prototypic drug metabolizing enzymes such as CYP2E1 and 3A4. Overall, these data indicate that the dramatic increases in serum MSO levels observed in our mouse model of APAP autoprotection, does not appear to be the mechanism by which Fmo3 induction confers protection against APAP toxicity.
1381 Bile Acid Profiling in a Cyp7a1 and Cyp27a1 Double-Knockout Mouse Model

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Bile acids (BAs) are a diverse population of amphipathic molecules that are synthesized through the enzymatic oxidation of cholesterol in the liver. The synthesis of BAs has traditionally been shown to occur via two pathways. Cholesterol 7 alpha-hydroxylase (Cyp7a1) performs the initial and rate-limiting step in the classical pathway while sterol 27-hydroxylase (Cyp27a1) initiates the hydroxylation of cholesterol in the acidic pathway. These two pathways are believed to be responsible for the vast majority of BA synthesis, roughly 75% and 25% of total BA production, respectively. While the role of individual BA species as physiological detergents is relatively ubiquitous, their endocrine function as signaling molecules and role in disease pathogenesis has been found to be BA species specific. In order to better understand the pharmacologic and toxicologic roles of individual BA species in an in vivo model, Cyp7a1 null mice (Cyp7a1-/-) were crossed with Cyp27a1 null mice (Cyp27a1-/-) to create double null mice (Cyp7a1-/-/Cyp27a1-/-) that are deficient in both enzymes. Wild type, Cyp7a1-/-, Cyp27a1-/-, and Cyp7a1-/-/Cyp27a1-/- mice were euthanized at 4 months of age. Serum, gallbladder, liver, and small intestine were collected for BA analysis. BAs were quantified by LC-MS to create a synthesis profile. We found that there was an increase in Cyp27a1 gene expression (11.3 x) in Cyp7a1-/- mice, which may be an attempt to compensate for the loss of Cyp7a1, while there was no change in the mRNA levels of Cyp7a1 in Cyp27a1-/- mice. In the Cyp7a1-/- mice, we observed a reduction of BAs in the gallbladder (86.3%) and in the liver (74.9%) as compared to wild type mice. In the ileum of Cyp7a1-/-/Cyp27a1-/- mice, the gene expression at mRNA levels of fibroblast growth factor 15 was reduced 77.7%, suggesting a decrease in FXR activity in response to low BA levels. It is also important to note that there were no differences in serum biomarkers or gross signs of liver injury among these four strains of mice. In summary, the two genes, Cyp7a1 and Cyp27a1, are critical in mice for BA homeostasis, and generation of the double null mice may serve as a useful model to study the functions of individual BA species. Funding: NIH ES050522, ES007148, R01GM104037.

1382 Comprehensive Examination of Induction of Drug Metabolizing Enzymes in Human Upcyte® Hepatocytes

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Drugs that induce xenobiotic metabolizing enzymes (XMEs) responsible for their own metabolism or that of a co-administered drug are a major source of concern in drug discovery. Human upcyte® hepatocytes are proliferating hepatocytes that retain many characteristics of primary human hepatocytes and are an important model for studying drug-drug interactions (DDI). We conducted a comprehensive examination of altered gene expression in upcyte® cells treated with a selection of reference XME inducers. Cells were treated with prototypical agonists of Pregnan X Receptor (PXR, rifampicin), Constitutive Androstane Receptor (CAR, rifampicin), Aryl Hydrocarbon Receptor (AhR, MeB), Farnesoid X Receptor (FXR, GW4064), Liver X Receptor (T0901317), Peroxisome Proliferator-Activated Receptor Alpha (PPARA, GW509735), Nuclear factor (erythroid-derived 2)-like 2 (Nrf2, Sulforaphane) or Liver Receptor-Homology 1 (ML179). Next-Generation sequencing (NGS) was used to quantify the altered gene expression induced by these drugs with a focus on XMEs that can affect the availability of biomarkers of drug metabolism and the characteristic induction of XMEs makes upcyte® hepatocytes suitable for DDI screening, as well as more in-depth mechanistic investigations.

1383 Utilization of a 3D Bioprinted Liver Tissue Model to Evaluate the Antifibrotic Effects of an ALK5 Inhibitor in a TGFβ-Induced Model of Hepatic Fibrosis


Compound induced chronic liver injury can lead to initiation of profibrotic processes resulting in sustained production of growth factors and profibrotic cytokines where inflammation, tissue remodeling and repair pathways are activated simultaneously to counteract the injury. Evaluation of potential antifibrotic therapies are limited using conventional non-human animal models, due to their inability to accurately reflect complex in vivo human biology, while 2D models lack the multicellular complexity and life span required to study fibrosis progression and regression. Utilization of a human 3D-bioprinted liver tissue model (ExVive® Human Liver Tissue) comprised of primary hepatocytes, hepatic stellate cells (HSCs), and endothelial cells, which can model TGFβ induced fibrosis, enables a mechanistic interrogation with an anti-fibrotic compound. In this study, galunisertib, a small molecule ALK5 (TGFβRII kinase) inhibitor, was used to evaluate pathway-specific blockage of TGFβ-induced fibrogenesis. The co-administration of galunisertib with TGFβ prevented the characteristic features of TGFβ-induced fibrosis, including upregulation of collagen deposition, phosphorylated SMAD2/3, and TIMP-1. Increased HSC activation was observed only in the TGFβ-induced fibrosis model, demonstrated by α-smooth muscle actin (αSMA) labeling and upregulation of ACTA2 transcript. Tissue and hepatocellular health remained stable following treatment with galunisertib, as shown by LDH release, viability, and albumin production which remained similar to vehicle levels, suggesting prevention of TGFβ induced tissue damage. These results demonstrate that a progressive in vitro model of liver fibrosis can be utilized to interrogate disease-associated pathways, and establish proof of concept for application of the model for preclinical evaluation of certain classes of anti-fibrotic drugs.

1384 A Common Genetic Variant Associated with the Risk of Idiosyncratic Liver Injury Due to a Variety of Drugs Is Identified via Hepatic Gene Expression-Based Clustering Analysis

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Genome-wide association studies (GWAS) to identify common risk factors for idiosyncratic drug-induced liver injury (IDILI) have been largely unsuccessful. The objective of this study was to explore the possibility that clustering drugs by underlying mechanisms of toxicity might increase the power to detect gene variants associated with IDILI susceptibility. Terminally differentiated HepaRG cells were treated with a single subtoxic concentration (5X Con) of 30 IDILI drugs or vehicle control for 8 and 48 h. (N=3 triplicates on day 6 and time point). Microarray gene and cellular pathways were used to cluster drugs into groups with similar transcriptional responses. GWAS performed using Caucasian genotype data from IDILI cases enrolled by the Drug-Induced Liver Injury Network and the International Severe Adverse Events Consortium as well as matched population controls identified a significant association in the region of microRNA-4750. It is also associated with cis-eQTLs for poly-nucleotide kinase 3 phosphate and PTOV1 antisense RNA2. PrediXcan, a method to impute gene expression profiles based on genetic data, permitted the possible expression differences in DILI cases for two genes near rs78137368: zinc finger protein 234 and V-set and immunoglobulin domain containing 10 like (p<0.0056 in blood). In conclusion, this study demonstrates a unique approach to improve the power for genetic studies of adverse drug response, which was utilized here to identify a pathway with the risk of IDILI associated with a common genetic variant. Ongoing efforts will explore the biological significance of these associations.

1385 Regulation of Steatosis in Upcyte® Cells by Nuclear Receptor Agonists

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Steatosis, typified by excessive accumulation of intracellular lipids, is a common liver disease triggered by various factors, including certain drugs and environmental chemicals. Nuclear receptors (NRs) are important transcriptional regulators that drive lipid metabolism and homeostasis, amidst other functions. The upcyte® human hepatocyte system has recently emerged as a good alternative to traditional non-human animal models, due to their inability to accurately reflect complex in vivo human biology, while 2D models lack the multicellular complexity and life span required to study fibrosis progression and regression. Utilization of a human 3D-bioprinted liver tissue model (ExVive® Human Liver Tissue) comprised of primary hepatocytes, hepatic stellate cells (HSCs), and endothelial cells, which can model TGFβ induced fibrosis, enables a mechanistic interrogation with an anti-fibrotic compound. In this study, galunisertib, a small molecule ALK5 (TGFβRII kinase) inhibitor, was used to evaluate pathway-specific blockage of TGFβ-induced fibrogenesis. The co-administration of galunisertib with TGFβ prevented the characteristic features of TGFβ-induced fibrosis, including upregulation of collagen deposition, phosphorylated SMAD2/3, and TIMP-1. Increased HSC activation was observed only in the TGFβ-induced fibrosis model, demonstrated by α-smooth muscle actin (αSMA) labeling and upregulation of ACTA2 transcript. Tissue and hepatocellular health remained stable following treatment with galunisertib, as shown by LDH release, viability, and albumin production which remained similar to vehicle levels, suggesting prevention of TGFβ induced tissue damage. These results demonstrate that a progressive in vitro model of liver fibrosis can be utilized to interrogate disease-associated pathways, and establish proof of concept for application of the model for preclinical evaluation of certain classes of anti-fibrotic drugs.
primary hepatocytes to examine liver diseases in vitro, including steatosis. In this study, upcyte cells were used to examine the role of NR to affect lipid accumulation and modulate gene expression. Prototypical activators of the pregnane X receptor (PXR, rifampicin), constitutive androstane receptor (CAR, CITCO), aryl hydrocarbon receptor (Ahr, Mebio), farnesoid X receptor (FXR, GW4064), liver X receptor (T0901317), peroxisome proliferator-activated receptor alpha (PPARA, GW300735) or nuclear factor (erythroid-derived 2)-like 2 (Nrf2, sulforaphane) or liver receptor homology 1 (ML179) were examined. Examination of lipid accumulation and altered gene expression from steatotic upcyte cells would provide insights into potential use of nuclear receptors as therapeutic targets to treat liver disease.

1386 An Optimized Process for Isolation, Culture, and Freezing of Human Kupffer Cells and Hepatic Stellate Cells to Develop Liver Co-Culture Models for Toxicological Studies

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The use of hepatic non parenchymal cells (NPCs) has recently increased for the development of liver co-culture models to study liver toxicity. The NPCs are composed of Kupffer cells (KCs), hepatic stellate cells (HSCs) and sinusoidal endothelial cells. The KCs are the resident macrophages of the liver and play a key role in immune response to injury, and the HSCs are delicate to isolate, to cryopreserve and to retain their functionality after thawing. The aim of the present study was to establish a robust protocol for isolation, culture and cryopreservation of high quality functional human KCs and HSCs. Primary hepatic cells were isolated from liver resection of different donors. The different types of NPCs were recovered and separated with a Percoll gradient. Following specific binding step, the KCs were purified, cultured and frozen by optimized process. In parallel, other fraction of cells were seeded to induce HSCs proliferation. After the second to the fourth passage in culture, HSC were also freezing. Characteristics of fresh and thawed KCs and HSCs were tested 1 to 4 days post-seeding. The results showed that KCs expressed CD68 macrophage marker and were able to phagocytize FITC-latex beads (1 µm). Both fresh and thawed HSCs up to 9 subcultures expressed only αSMA showing an activated state. However, fresh HSCs from rare donors were quiescent with desmin expression and positive red oil staining of αSMA showing an activated state. However, fresh HSCs from rare donors were quiescent with desmin expression and positive red oil staining of retinol storage. However KCs and HSCs are very sensitive to isolation, culture and cryopreservation of high quality functional human KCs and HSCs. The NPCs are composed of Kupffer cells (KCs), hepatic stellate cells (HSCs) and sinusoidal endothelial cells. The KCs are the resident macrophages of the liver and play a key role in immune response to injury, and the HSCs are delicate to isolate, to cryopreserve and to retain their functionality after thawing.

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Diethylglycol (DEG) is an organic compound that can be found in consumer goods, including brake fluid, but can also be an adulterant in medicines by acting as a counterfeit solvent. DEG poisons affect the liver and nervous system, but have been characterized predominantly by acute kidney injury and necrosis of the proximal tubule cells. Diethylglycol is metabolized to two primary metabolites: 2-hydroxyethoxyacetic acid (2-HEAA) and diglycolic acid (DGA). In human proximal tubule (HPT) cells, DGA alone has been shown to be the metabolite that produces concentration-dependent cell death by decreasing the production of ATP and substrates involved in the electron transport chain, downregulating this energy-producing pathway. DGA has also been shown to chelate free calcium in a similar strength as known calcium chelator EGTA. To determine the effects of DGA on intracellular calcium levels in whole cells, a time-point study with subtoxic concentrations of DGA were conducted using primary human proximal tubule cells. DGA-treated cells were harvested and analyzed via spectrophotometry using the ratiometric calcium indicator dye Fura 2-AM. A higher ratio of emission from the dual excitation wavelengths, 340/380, would indicate a higher amount of calcium in the cell. Additionally, HPT cells were treated in a DGA dose response and analyzed for oxygen consumption rate (OCR) and extracellular acidification rate (ECAR), a measurement of glycolysis, using the Seahorse XF24 Analyzer. The measured 340/380 ratios from fresh and DGA incubated cells at timepoints between 2-48 hours showed a significant increase in intracellular calcium at the 25mM DGA concentration at 36 and 48 hours. DGA also decreased OCR and increased ECAR in a dose-dependent manner, lending more support to the effects of DGA on energy production. These data suggest that DGA appears to increase total intracellular calcium levels, but studies using other calcium sensing techniques need to be performed to determine the location of this effect.

1388 Effects of Lead on Calcium Oxalate Crystallization in an Insect Model of Nephrolithiasis

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Calcium oxalate (CaOx) accounts for 75-80% of kidney stone composition, and CaOx-containing stones are highly recurrent. Genetic predisposition, gender, geographic region, diet, and low fluid intake all contribute to disease pathogenesis. However, an important cause of renal CaOx formation that remains insufficiently studied is environmental exposure to pollutants, specifically nephrotoxic metals. Lead (Pb2+) is of particular interest as epidemiological data indicates that low-level Pb2+ exposure and blood Pb2+ levels (0.48µM-3.85µM) in humans increase kidney stone formation. Using our Drosophila melanogaster (fruit fly) model, we sought to determine the effects of exogenous Pb2+ on CaOx crystallization. This physiologically simple, yet evolutionarily conserved model allows us to mimic and easily visualize and quantify CaOx formation within intact Malpighian tubules (MT, insect renal structures). We found that MTs were isolated from wild-type flies (w1118), and treated with oxalate (5 mM) + Pb2+ acetate (2 µM) for 1h. Following exposure, MTs were imaged using differential interference microscopy (DIC), and crystals quantified (crystal number, total crystal area, and average size/crystal) using ImageJ. CaOx crystal number and total crystal area were significantly increased (~50-fold) in Pb2+-oxalate-exposed MTs when compared to oxalate alone treated controls. Furthermore, MTs exposed to Pb2+ alone had no crystallization indicating a role for Pb2+ in the increased formation of CaOx, and not PbOx. Our data indicate that exogenous Pb2+ plays a role in CaOx crystallization, which in turn could correlate to a higher propensity for CaOx stone formation and a worsening of nephrolithiasis pathology. Additionally, this model could be used as a high throughput assay for testing a variety of nephrotoxic agents to determine their effect on crystal formation. Future studies will determine whether flies reared on a Pb2+-containing diet mimic the same increases in CaOx crystallization parameters described above, as well as effects of Pb2+ on MT calcium levels.

1389 Early Screening for Nephrotoxicity Employing Transporter Over expression Cell Lines

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Transporters play a significant role in regulating various physiological pathways. They can also mediate active uptake of drugs resulting in drug sequestration in the cells leading to potential toxicity. The kidney maintains total body homeostasis by conserving essential nutrients and eliminating potentially toxic xenobiotics, xenobiotic metabolites, and metabolic wastes. Proximal tubules contribute to the regulatory and secretory process via multiple membrane transporters in the basolateral and luminal membrane. Many drugs associated with kidney proximal tubule toxicity are hydrophilic - suggesting that active uptake via transporters drive the cell accumulation for such low permeable drugs. However, cells commonly used in vitro toxicology as lack transcellular expression which can lead to false negative results. Here we used OAT1, OAT3 and OCT2 over-expressing HEK293 cells to see whether they could improve our predictivity for nephrotoxic compounds. Transfected cells were treated with Cidofvir, Tenofovir (substrate of OAT1 and OAT3) and Cisplatin (OCT2 substrate) and kinetic impedance was measured as an indicator for cytotoxicity. In the parental HEK293 cell line both Cidofvir and Tenofovir did not cause any impedance change compared to control. However both OAT1-HEK293 and OAT3-HEK293 cells exhibited toxicity with the compound treatment (more severe toxicity observed with OAT1-HEK293). This result correlates with the compound uptake data (much greater uptake in OAT1 over
expression cells compared to that of OAT3 over expression cells). Similar differential toxicity profiles were also demonstrated with cisplatin using OCT2 over expressing cells compared to the parental cells. Inhibition of OAT1 and OAT3 (using probenecid) and OCT2 (using quinidine) reduced the toxicity associated with test compounds indicating that the increased toxicity is associated with increased cell accumulation. Both Cidofovir and Tenofovir were further examined in the microfluidic titer plate OrganoPlate® seeded with ciPTEC or RPTEC provides a promising drug screening platform as it allows multiplexing of biomarker analysis upon exposure to drugs of different classes.

1390 Drug Toxicity Screening in a High-Throughput Kidney-on-a-Chip


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Kidneys-on-a-chip have the potential to reduce animal experimentation and improve predictivity of drug-induced kidney injury during drug development. Here, two human immortalized proximal tubule epithelial cell lines (ciPTEC and RPTEC) cultured in a multi-compartment microfluidic titerplate (Organoplato®) were evaluated as an high-throughput tool to assess drug-induced kidney injury. ciPTEC (Radboudumc) and RPTEC (Sigm Aldrich) were cultured in Organoplato® (MIMETAS B.V.) onto a collagen-I matrix and exposed to twelve compounds known for their nephrotoxic potential (including cisplatin, tenofovir, tobramycin and cyclosporin A) for 24 and 48h. Cell viability was assessed with a WST-8 assay and through biomarkers released into supernatant (LDH, NAG and mRNA's). Barrier integrity of RPTEC monolayers was evaluated by measuring diffusion of labeled dextran from the apical to the basal compartment. Finally, intracellular RNA was extracted from the kidneys-on-a-chip for gene expression analysis. Upon 10 days of culture under flow conditions, ciPTEC and RPTEC formed tubule structures in the Organoplato®. Significantly decreased cell viability and claudin-2 gene expression as well as increased release of LDH and NAG (9 compounds at 48h exposure) were detected in treated ciPTECs. Under the same experimental conditions, secretion of specific mRNAs into the perfusion buffer was increased in 11 out of 12 compounds at 24h exposure. Barrier integrity of RPTEC was reduced for 7 compounds as assessed by dextran diffusion. The microfluidic titerplate Organoplato® seeded with ciPTEC or RPTEC provides a promising drug screening platform as it allows multiplexing of biomarker analysis upon exposure to drugs of different classes.

1391 Cisplatin-Mediated Renal Cytotoxicity: The Protective Effect of Cinnamaldehyde, Kaempferol, and Resveratrol

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The cancer chemotherapeutic agent cisplatin is used clinically in patients with testicular, ovarian and cervical cancer. The primary serious effects include neurotoxicity and nephrotoxicity. Interventions to reduce cisplatin (CIS) cytotoxicity rely on hydration and diuresis. Agents that may reduce the renal cytotoxicity but not impact the cancer chemotherapy would improve cancer cure rate and benefit patient quality of life. This study examined the protective effect of three natural products: cinnamaldehyde (CIN), kaempferol (KAM) and resveratrol (RES) to reduce CIS cytotoxicity. The overall hypothesis of this project is that these agents will reduce cisplatin nephrocytotoxicity by maintaining mitochondrial function. Human proximal tubular cells were plated and allowed to equilibrate for 48h followed by a 1h pretreatment with vehicle (DMSO), 0-40 uM CIS, 0-20 uM KAM and 0-10 uM RES followed by a 24h treatment with 0-50 uM CIS. All studies were conducted as a minimum of 3 independent experiments. Viability was assessed by MTT leakage and by trypan blue exclusion. CIS was cytotoxic within 24h relative to vehicle control. CIN, KAM and RES did not affect MTT or Trypan Blue exclusion when compared to vehicle control (p>0.05). CIS induced a concentration dependent decline in viability after a 24h exposure to 0-50 uM relative to vehicle control. CIN, KAM and RES provided protection for CIS cytotoxicity to HK-2 cells. RES provided the best protection at the lowest concentration. Further studies examined mitochondrial function following a 24h exposure to cisplatin in cells treated with vehicle or RES using a Seahorse Xp analyzer. Optimization of cell number, FCCP and glucose were conducted prior to treatments. Basal and maximal or uncoupled mitochondrial function were assessed using the Mito Stress Kit. A separate series of studies conducted the Glucose Stress test and following a 24h exposure to CIS in the presence of RES or vehicle. Leakage of the mitochondrial protein, cytochrome c was evaluated 24h after CIS exposure in the presence of vehicle or RES. CIS induced cytochrome c leakage as detected by Western analysis. CIN, KAM and RES were protective for CIS cytotoxicity based on viability. RES reduced CIS cytotoxicity at the lowest concentrations compared to CIN and KAM. RES preserved mitochondrial basal and maximal oxygen consumption following CIS exposure which may be part of its protective effects. Supported by NIH Grant 5R01DK110343.

1392 Podocyte Culture-Based Screening Method Provides New Screening Approach for Nephrotoxicity

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Podocyte integrity, an important determinant for the pre selective properties of the glomerular filtration barrier, is impaired in individuals with type 1 and type 2 diabetes as well as in individuals with known or unknown exposure to chemicals / radiation. We have established a primary podocyte culture model that will rapid screening of multiple chemicals for toxicity. We provide data from our podocyte culture method from wild type and mutant animal model (TRPC6-KO mice) that shows differential regulation of cell division and apoptosis. We have used the following assays to establish the differential response of primary podocytes: a) Annexin-PI assay to distinguish apoptosis and necrosis, b) Mitot assay to assess the mitochondrial involvement, c) Caspase-3 assay, d) UV-irradiation assay to identify P-S3 related pathways and f) Serum free growth assay for stress related pathways. We exposed the podocytes to the following chemicals: a) PAN, b) STZ, c) OAG, d) hyperforin, and e) UV-irradiation. In general, the TRPC6-KO mice manifested lower rate of apoptosis and higher level of DNA synthesis / cell division. There are dose dependent changes in the increased DNA synthesis and decreased cell death rates for various chemicals and radiation. This rapid screening approach for multiple chemicals or toxicants provides plenty of data regarding cell death and cell proliferation that can be effectively used for further dissection of distinct pathways of toxicant exposure related kidney dysfunction.

1393 Persistence of Bromate-Induced Epigenetic Changes in Kidney Cells

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Bromate (BrO3) is a water disinfection byproduct we’ve previously shown to induce nephrotoxicity in vitro and in vivo. We also showed that the epigenetic inhibitors 5-aza-2-deoxycytidine (5-Aza) and trichostatin A (TSA) increased BrO3- nephrotoxicity while altering the expression of the cyclin-dependent kinase inhibitor p21 expression. In this study we used a novel approach called targeted genome bisulfite sequencing (TGBS) to determine the percent methylation at the p21 promoter in human embryonic kidney (HEK293) and rat normal kidney (NRK) cells. Treatment of the cells with 5-Aza decreased DNA methylation at 35% at the s5-inducible element (SIE-1), a transcription factor binding site in human p21 promoter, but did not alter methylation at the rat p21 promoter. We also observed differences in the basal promoter methylation between rat and human p21, suggesting that the rat and human p21 are differentially regulated by DNA methyltransferases. In contrast, sub-chronic BrO3- exposure failed to alter this methylation in both human and rat renal cells. In contrast, sub-chronic exposure of NRK cells to similar concentrations of BrO3 altered histone acetylation, as determined by chromatin immunoprecipitation (ChIP) assays. Changes in histone acetylation correlated to changes in p21 protein expression. Changes in histone acetylation were not observed in HEK293 cells. The persistence of these epigenetic changes was assessed by discontinuing exposure to BrO3 or the epigenetic inhibitors. 5-Aza-induced promoter demethylation remained stable after the withdrawal; however, TSA- and BrO3-induced histone hyperacetylation recovered back to basal levels after 3 days of withdrawal. These data suggest that BrO3 regulates the renal expression of p21 using mechanisms that alter histone acetylation, and by not inducing de-methylation of its promoter. The data also show species- and time-dependent difference in the epigenetic regulation of p21. These data suggest that epigenetic changes in rat p21 expression cannot be directly extrapolated to human p21, especially when assessing the risk of renal toxicants in humans.
Evaluation of the Epithelium Lining the Renal Papilla in Rats That Are Normal or with Chronic Progressive Nephropathy: It Is Not Urothelium


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Chronic progressive nephropathy (CPN) is a common disease in rats, especially in the F344 and Sprague-Dawley strains. It is more common and more severe in males than females. One of the manifestations of high grade CPN is proliferation of the lining of the renal papilla, which is currently designated as urothelial hyperplasia in the INHAND classification. However, the true urothelium is the lining of the kidney pelvis that leads into the ureter. The present study was to design to evaluate whether the epithelium lining the renal papilla is truly urothelial and whether the CPN-associated hyperplasia represents urothelial proliferation. The differentiation of the lining of the papilla in aged and young male rats was assessed by immunohistochemical staining of uroplakin, a group of urothelial specific proteins. Kidney tissue from 8 aged male F344 rats and 5 young male Sprague-Dawley rats was used for the study. All of the aged rats had CPN, with 4 of the F344 rats having hyperplasia of the epithelium lining the papilla. Four of the aged rats did not have this proliferation. The kidneys from the 5 young Sprague-Dawley rats were normal. No uroplakin cytoplasmic staining was observed in the cells lining the renal papilla in any of the rats, whether hyperplastic or not, indicating that these cells are not urothelial. Furthermore, mitotic figures were not observed in this epithelium, even with hyperplasia. Based on these findings, we recommend that the epithelium lining the renal papilla not be classified as urothelial, and that the proliferation of this epithelium associated with CPN be designated as hyperplasia of the epithelium lining the papilla. Furthermore, this proliferation should be separated in diagnostic systems from true urothelial hyperplasia of the kidney pelvis.

Microcystin Exposure Modulates NOX-2, miR21 Axis in Ectopic Glomerular Toxicity in Underlying Non-Alcoholic Fatty Liver Disease

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NAFLD often results in cardiovascular, intestinal and renal complications. Previous reports from our laboratory exhibited NAFLD induced ectopic inflammatory manifestations in the kidney that gave rise to glomerular inflammation. Extending our studies, we hypothesized that existing inflammatory conditions in NAFLD could make the kidneys more susceptible to environmental toxins. Our results showed that exposure of Microcystin-LR (MC) in NAFLD mice caused a significant increase in mesangial cell activation as evidenced by increased a-SMA in the extracellular matrix surrounding the glomeruli. Renal tissue surrounding the glomeruli also had increased NOX2 activation as shown by increased co-localization of P47 Phox and its membrane component gp91 that. To show whether NOX2 activation and subsequent NOX2-miR21 axis were key to microcystin exposure and exacerbation of renal toxicity, experiments were designed in vitro with immortalized mesangial cells of mouse origin. The cells incubated with (a) apocynin and (b) nitroven spin trap DMPO and (c) miR21 inhibitor showed significantly decreased a-SMA, miR21 levels and proinflammatory cytokine release in the supernatant. In parallel, mice lacking miR21, a proinflammatory miR, known to be activated by NOX2, when exposed to MC in NAFLD showed decreased mesangial cell activation. Mechanistically, phenyl boronic acid incubated cells that are exposed to MC showed significantly decreased mesangial cell activation showing that peroxynitrite might be the major reactive species involved in mediation the activation process, release of proinflammatory micro RNAs and cytokines that are crucial for renal toxicity. Thus, in conclusion, MC exposure causes NOX2 activation that leads to mesangial cell activation and toxicity via release of peroxynitrite and miR21.

Drug Interaction Screening in a Microfluidic Human Renal Proximal Tubule


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A kidney-on-a-chip resembling in vivo conditions allows screening for functionally relevant compounds at a low cost. A human renal conditionally immortalized proximal tubule epithelial cell line overexpressing organic anion transporter 1 (CIPTET-OAT1) in a microfluidic titerplate (OrganoPlate®). CIPTET-OAT1 was cultured under fluid shear stress in the OrganoPlate®. Gene expression of P-glycoprotein (Pgp), organic cation transporter 1 (OCT1) and organic cation transporter 2 (OCT2) was assessed. Calculation of AUC and CMFDA were used to measure activity of P-gp and MRP2/4, respectively. PSC833 and a mixture of PSC833, MK571 and CO143 were used to inhibit P-gp and MRP2/4, respectively. A blinded screening for drug interactions of 12 potentially nephrotoxic compounds (e.g. cispilatin, tenofovir and cyclosporine A) was performed to evaluate their activity in this human renal epithelial cell line model. Drug treatment with cyclosporine A increased the fluorescent signal. In the blinded screening, five compounds (including cyclosporine A) caused a significant interaction with P-gp. For MRP2/4, four compounds (including cyclosporine A) caused a significant interaction. Comparison with in vitro and in vivo data of these compounds should further validate our model. We demonstrate a kidney-on-a-chip, composed of CIPTET-OAT1 in the OrganoPlate®, suitable for high-throughput screening at the pre-clinical phase in drug development.
Human immunodeficiency virus (HIV)-infected patients have higher prevalence of chronic kidney disease (CKD) than the general population. Both conditions are associated with decreased gluteal availability, which impairs the detoxification of electrophiles through the mercapturative pathway. This route is almost exclusive of kidney tubular cells, generating cysteine-S-conjugates, upon uptake and subsequent acetylation by N-acetyltransferase 8, form mercapturate and coenzyme A (CoA) that are eliminated in urine. The aim of the study was to compare urinary mercapturates of cysteine-S-disulfides (uNAC) and CoA (uCoA) between HIV-infected patients with a decrease in kidney function and those that remained stable during 1-year of follow-up. Urine samples were collected at admission (T0) and 12 months after (T12). Estimated glomerular filtration rate (eGFR) was calculated by CKD-EPI equation using creatinine, expressed in mL/min/1.73m². Patients were stratified according to their eGFR baseline values: normal control (NC)≥90, early kidney dysfunction (EKD): 60≤eGFR<90 and CKD<60; and eGFR evolution: non-progression (NP) with stable eGFR; progression (P) with decreasing kidney function≤10% from T0. uNAC and uCoA were quantified by HPLC-FD, presented as % of T0. HIV-patients (n=105) were 68% men; 75% Caucasian; median [interquartile range, IQR] age 50 [44-58]; 49 were NC, 46 were EKD and 10 were CKD. eGFR was 88 [76-98] at T0 and 81 [67-90] at T12 (p=0.007). At T12, 52% of patients were P. uNAC was lower in P vs NP, particularly for EKD-P (mean±SD 64±26%, p<0.05) and CKD-P (70±18%, p<0.001) (Two-way ANOVA). Among NP group, was lower in P vs NP, particularly for EKD-P (mean±SD 64±26%, p<0.05)

The incidence of type 1 diabetes (T1D), as well as its associated risk of chronic kidney disease (CKD) or end-stage renal disease (ESRD), are on the rise. T1D is an autoimmune disease in which insulin-producing beta cells are destroyed. T1D is manifested as increased expression of inflammatory proteins, elevated glucose concentrations, and decreased insulin levels. Increased incidence of T1D has been suggested to be a result of environmental factors such as exposure to polycyclic aromatic hydrocarbons (PAH). 2-aminoanthracene (2AA) is a PAH that has been associated with the onset of early diabetic symptoms. This study was conducted to identify if 2AA dietary ingestion would induce T1D renal injuries. To accomplish the research objective, three-week-old male Sprague-Dawley rats were assigned into three 2AA dietary ingestion groups (0 mg/kg, 50 mg/kg, and 100 mg/kg) for 12 weeks. Animals were evaluated for their food intake, body weight, total body, and kidney weight after euthanasia. Further experiments involving the analysis of serum glucose, creatinine concentration, albumin-creatinine ratio, and expression of inflammatory and renal injury gene markers were performed. The Sprague-Dawley rats in the 100 mg/kg group lost 5% less weight than the other treatment groups and converted roughly 3% more of their food intake into body mass. The kidney weight per body weight of the 100 mg/kg treatment group was 30.1% greater than the control group. Creatinine concentration of the 100 mg/kg group was 46.2% greater than the control group. FABP1 and SPP1 expression were elevated in treated groups. These results suggest that 2AA may induce the early diabetic renal injuries of hyperfiltration and microalbuminuria; however, further studies utilizing urine analysis, glomerular filtration assessment, greater 2AA concentrations, different delivery methods, longer trials, and ELISA should be conducted to further assess the effect of 2AA on diabetic kidneys.

Ellagic acid-artesunate hybrid molecule on selected kidney function parameters in mice. Recently through rational drug design approach, single hybrid molecules with multiple functionality and molecular targets have been developed as a novel treatment for many diseases, e.g., the ellagic acid-artesunate hybrid molecule, which has been demonstrated to possess high antimalarial activity. However, there are growing concerns about the toxic effects of these synthesized molecules on physiological systems. The aim of this study, therefore, was to investigate the effect of novel ellagic acid-artesunate hybrid molecule on selected kidney function parameters in mice. Thirty mice were randomly distributed into six groups of five mice each (A-F). Group A was orally administered 5% DMSO, while groups B, C, D, E, and F were administered 5, 10, 20, 40, and 80 mg/kg body weight of the hybrid molecule, respectively, for 28 days, after which the kidney function indices were determined. The results revealed that hybrid molecule caused no significant change (p>0.05) in plasma concentrations of chloride, calcium, bicarbonate, and phosphate ions at various doses compared to controls. However, the hybrid molecule significantly (p<0.05) reduced plasma concentrations of sodium ion at doses higher than 10 mg/kg and plasma potassium ion concentration at all doses compared to controls. The hybrid molecule produced no alteration (p>0.05) in plasma creatinine and urea concentration. Renal alkaline phosphatase activity was significantly increased (p<0.05) compared to controls upon treatment with the hybrid molecule. However, plasma alkaline phosphatase activity, and renal and plasma γ-glutamyl transferase activities were not significantly (p>0.05) altered at all doses administered compared with controls. Furthermore, Na⁺, K⁺ - ATPase activity in the kidney was significantly reduced (p<0.05) at doses higher than 5 mg/kg body weight, while Ca⁺⁺, Mg²⁺ -ATPase, and Mg²⁺ -ATPase activities were not altered as a result of administration of the hybrid molecule. The findings suggest that the hybrid molecule may adversely affect reabsorption of sodium ion in the kidney after prolonged administration at higher doses.

Chlorinated benzenes are commonly used as chemical intermediates in the manufacture of a wide range of commercial products and are found as environmental contaminants. Toxicity induced by chlorobenzenes includes neurotoxicity, hepatotoxicity and nephrotoxicity. While nephrotoxicity of some di- and trichlorobenzenes is associated with rat α2u-globulin, it is less clear if these halogenated benzenes have direct effects on the kidney. The purpose of this study was to examine the in vitro nephrotoxic potential of the three trichlorobenzenes (1,2,3-, 1,2,4-; and 1,3,5-trichlorobenzene [TCB]) using isolated renal cortical cells (IRC) from male Fischer 344 rats. IRC (~4 million cells/ml; 3 ml) were incubated with shaking at 37°C under a 95% oxygen/5% carbon dioxide atmosphere with a TCB (0 - 1.0 mM) or vehicle (dimethyl sulfoxide) for up to 60 min. General cytotoxicity was determined by determining trypan blue exclusion of IRC and measuring changes in lactate dehydrogenase (LDH) release. In some experiments, IRC were pretreated with an antioxidant or cytochrome P450 (CYP) inhibitor before 1,2,3-TCB (1.0 mM) or vehicle. 1,2,3-TCB proved to be the most potent nephrotoxicant of the three TCBs, inducing an increase in LDH release at 0.25 mM within 30 min. Both 1,2,4- and 1,3,5-TCB required TCB concentrations of 1.0 mM to induce cytotoxicity at 30 min. 1,2,3-TCB cytotoxicity was accompanied by reduced ATP levels but not increased levels of protein carbonyls. 1,2,3-TCB cytotoxicity was also reduced by both antioxidant and CYP inhibitor pretreatments. These results indicate that 1,2,3-TCB is the most nephrotoxic TCB in IRC and that CYP-mediated oxidation contributes to TCB nephrotoxicity. Mitochondria appear to be targets for TCB (and/or its metabolites), but lipid peroxidation is not the mechanism of cell death. Supported in part by NIH grant P20GM103434.
from a new high content screening assay we developed, with drug exposure information, in order to predict the risk for nephrotoxicity. We developed a high content screening assay, using 5 fluorescent dyes to score parameters associated with nuclei, cell morphology, mitochondrial function, actin cytoskeleton and cell membrane permeability, in conditionally immortalized proximal tubal epithelial cells that were transfected with the drug transporter OAT1 (ciPTEC-OAT1). We scored how 48 hour incubation with 38 nephrotoxic and 24 non-nephrotoxic drugs affected the endpoints in the assay. First, we confirmed that ciPTEC-OAT1 was more sensitive to the nephrotoxic substrates for OAT1 than parent ciPTEC. Next, by manual data analysis, we found that parameters related to cellular and nuclear structure and mitochondrial health were more sensitive than nuclei counts. In order to access the full predictive power of the assay, we built a mathematical model integrating all 250 parameters we derived from the high content image analysis, as well as human exposure information (Cmax). This model was able to correctly classify 35 of 58 nephrotoxic drugs (60% specificity, 92%) and 23 of 24 of non-nephrotoxic drugs (specificity: 96%). As the assay can be performed in a relatively high throughput (3 day assay protocol, 96 well format, cost effective), and has good predictivity, it is compatible with implementation in an early drug discovery setting.

1405 Characterization of the Dose- and Time-Response of miRNA and mRNA Profiles in the Serum and Kidney of F344 Rats Recovering from a Nephrotoxic Co-Exposure to Melamine and Cyanuric Acid


We reported previously that a 28-day exposure to nephrotoxic doses of melamine and cyanuric acid (MEL&CYA) modulated the serum miRNome of male and female F344 rats. To confirm and expand these findings, we have now assessed the changes in the serum miRNome induced by a nephrotoxic dose of MEL&CYA in F344 rats upon 90-days of exposure and during 90-days of recovery. We further assessed molecular changes (miRNA and mRNA) in the kidney of these animals, and compared their dose- and time-responses with kidney histopathology, serum creatinine, and blood urea nitrogen. Serum samples were screened (n = 5 per sex, 0 and 5 mg/kg body weight (bw)/day each MEL&CYA) using quantitative real-time RT-PCR (qPCR). The levels of several serum miRNAs were decreased by treatment, including some that we had previously identified in the 28-day exposure study (e.g., mir-106b-5p and mir-128-3p). In addition, a subset of these miRNAs was found to be modulated in the kidneys of these animals, although the direction of the effect was reversed in most cases. The expression of putative target genes of the modulated kidney miRNAs, as identified using miRWalk 2.0, and of several genes included in a Rat Nephrotoxicity Panel (TaqMan® Array) was also different in treated versus control rats. Characterization of the dose- and time-response of these effects showed that the serum and kidney miRNAs were not affected by non-nephrotoxic doses of MEL&CYA (1.25 and 2.5 mg/kg bw/day each), while the miRNAs modulated by the 5 mg/kg bw/day MEL&CYA dose returned to baseline upon 90 days of recovery from treatment. Taken together, these results suggest that exposure to a nephrotoxic dose of MEL&CYA induces miRNA and miRNA changes at the serum and kidney levels that correlate with the histopathological changes observed in the kidneys of the same animals. This work was sponsored under an interagency agreement between the FDA/NCTR and the NIH/NTD FDA IAG # 224-12-0003/NIEHS IAG # AES12013.

1406 Effect of Juvenile Chlorpyrifos Exposure on Novel Object Recognition in Adolescent Rats


Chlorpyrifos (CPF) is an organophosphate insecticide that is widely used in agricultural regions in the United States. Developmental exposure has been hypothesized to lead to long-term behavioral effects in children, and exposure has been correlated with decreased cognition and attention deficit/hyperactivity disorder (ADHD). In previous studies, we have reported that developmental exposure to CPF inhibits fatty acid amide hydrolase (FAAH), an enzyme which breaks down endocannabinoids in the brain. This inhibition occurs at exposure levels that do not result in the inhibition of acetylcholinesterase, the canonical target for CPF. In this study, the effects of developmental exposure to low levels of CPF on novel object recognition memory were investigated. From postnatal day 10 (PND10) to PND16, rat pups were orally exposed...
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Childhood exposure to chlorpyrifos (CPF), an organophosphorus insecticide, results in negative long-term neurologic effects. We have previously reported that low-level developmental exposure of rats to CPF disrupts endocannabinoid (EC) degradation through fatty acid amide hydrolase (FAAH) inhibition and leads to increased social play behavior once adolescence is reached. Increased activity in the EC system may be responsible for the observed altered behavior. Phosphorylation of the cannabinoid receptor (CB1R) is an indicator of EC activation. This study compared the phosphorylation of the CB1R in brain regions of control and treated rats immediately following behavioral testing. On postnatal day (PND) 10, rats were exposed orally to either corn oil, 0.5 mg/kg CPF, 0.75 mg/kg CPF, 1.0 mg/kg CPF or 0.02 mg/kg PF-04457845 (a specific FAAH inhibitor) daily for 7 days. On PND 36, social behavior was monitored and all treated groups spent more time playing than did controls. Western blotting was used to quantify the amount of CB1R and phosphorylated CB1R proteins in the hippocampus, amygdala, prelimbic cortex, agranular insular area, and nucleus accumbens. There were no significant effects of treatment on the amount of CB1R protein in any of the five brain regions. Increased CB1R phosphorylation occurred in all brain regions following social play, but no effects of CPF treatment were observed. This suggests that increased EC activation is not a causative factor in the increased social play observed in CPF treated rats. It remains unclear whether developmental CPF exposure induces an alteration in EC tone or an alteration in other neurotransmitter systems that could explain the observed changes in social behavior. Funded by NIH R15ES023162.

1408 Increased Expression of c-Fos in Brain Regions following Repeated Developmental Exposure to Low Levels of Chlorpyrifos

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Chlorpyrifos (CPF) is an organophosphate insecticide that acts primarily through the inhibition of acetylcholinesterase (AChE) and the subsequent accumulation of acetylcholine. At higher levels of exposure, this accumulation leads to overstimulation of postsynaptic receptors leading to neuronal excitation. However, neurotoxic effects have been reported following repeated developmental exposure to CPF at levels that do not inhibit AChE. It is not clear if low level repeated exposure to CPF will induce neuronal excitation. c-Fos is a transcription factor that plays a part in many cellular events such as cell proliferation, differentiation, and survival. The induction of c-Fos has been used as a marker of increased neuronal activity. In our study, we focused on determining if exposure to low levels of CPF induces neuronal excitation and, if it does, determining the regions of the brain in which this occurs. For this purpose, juvenile rats were orally exposed daily to either corn oil, 0.5, 0.75, or 1.0 mg/kg CPF from postnatal day 10 (PND10) to PND16. Rats were sacrificed 12 hours after the final treatment and brains were collected and sliced using a microtome to isolate specific regions of the brain. Immunohistochemistry was then performed on these specific regions to determine c-Fos expression. Staining revealed increased expression of c-Fos in the caudate putamen (CPu) and lateral septal nucleus (LSD) regions of the brain following treatment with low levels of CPF. These data suggest that exposure to low levels of CPF can induce neuronal activity resulting in the increased expression of c-Fos. Funded by NIH R15ES023162.

1409 Effects of Alpha-Cypermethrin and Prenatal Stress on Embryonic Brain Development

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Alpha-cypermethrin is a type II pyrethroid that has been recommended for use by pregnant women to prevent mosquito-borne transmission of the Zika virus. As such there is high probability for prenatal exposure to the developing embryo. Previously, we have shown that prenatal stress is a risk factor for disrupted embryonic brain development and delays the migration of GABAergic progenitor cells in the developing forebrain in a mouse model. In addition, several studies have suggested that psychological stress may influence chemical toxicity through alterations in metabolism and tissue distribution. As such, the present study examined the effects of prenatal exposure to alpha-cypermethrin on mouse embryonic brain development, alone and in combination with maternal stress. CD1 dams were administered alpha-cypermethrin at doses of 0 mg/kg, 3 mg/kg, or 10 mg/kg daily throughout the first 14 days of pregnancy (E0-E13). In addition, half of the mice were subjected to restraint stress under bright light (3x45 min) on the 12th and 13th days of gestation (E12-E13). No overt signs of maternal toxicity (decreased body/organ weights) were observed in any dams. Embryonic body and placenta weights were unchanged among all treatments in several cases of abnormal development including exencephaly, growth retardation, and early stage embryonic death were observed in a small fraction of high dose cypermethrin treated embryos but were absent in controls. In mice, an increased incidence of placental fusions was observed in high dose cypermethrin treated litters. Neurodevelopmental endpoints including GABAergic progenitor migration, forebrain volume, and volume of the ganglionic eminence proliferative zone were assessed at E14. High dose cypermethrin resulted in a trend delay in GABAergic progenitor migration. No other endpoint was significantly changed by either prenatal stress or cypermethrin, alone or in combination. This suggests an unintended stress buffering mechanism due to our experimental method, which is currently being addressed in ongoing experiments. In conclusion, although several neurodevelopmental endpoints were not affected, these findings suggest potential adverse outcomes on embryonic development as a consequence of prenatal exposure to cypermethrin at doses below the threshold for maternal toxicity.

1410 Transcripntional Analysis of Alternative Flame Retardant Exposures in hESC-Derived Neural Precursors


Due to their persistence in the environment and suspected ability to cause neurotoxicity in humans, polybrominated diphenyl ether (PBDE) flame retardants (FRs) are being phased out of the marketplace. Alternative FRs, including organophosphorus and polybromobenzene forms, are being introduced in their place and have recently been detected in human maternal/fetal biological matrices. While the developmental health effects associated with alternative FR exposures in utero remain undefined, in vitro assessments suggest similar risks with legacy FRs. Human embryonic stem cells (hESCs) have tremendous potential as a model of in utero xenobiotic exposure. We have previously demonstrated hESC-derived neural precursor cells (NPCs) to be particularly sensitive to PBDEs, which induce significant concentration-dependent cytotoxicity and gene expression changes in pathways related to neurodevelopment and stress. Therefore, in this study we expanded our assessments to include 12 FRs currently being used in consumer products. In a blinded investigation, we discovered 9 of the 12 tested FRs (0-30μM) produced significant cytotoxicity in NPCs, with select alternative FRs showing similar potencies to BDE-47. To identify possible common mechanisms underlying FR toxicity, we profiled NPCs using RNA sequencing. NPCs were exposed to either BDE-47 or alternative FRs: IPP (isopropylated phenyl phosphate), EHPD (2-ethylhexyl diphenyl phosphate), or TBBPA (tetrabromobisphenol A) at 3 or 10 μM. Significant alterations in gene expression were observed for all four compounds (p<0.0001): BDE-47 (277), IPP (1968), EHPD (482), and TBBPA (211). Sixty genes were commonly differentially expressed with all four FRs, including factors suspected of driving neuronal migration.
Autism spectrum disorder (ASD) is considered to be one of the most heritable components of the complex neurodevelopmental disorders (NDD). However, the sharp increase in rates of ASD in recent decades has prompted the search for genetic and environmental interactions that influence individual risk for ASD. PCBs, a class of persistent organic pollutants, have been implicated as environmental risk factors for NDDs, and immune system abnormalities are frequently observed comorbidity in ASD. Therefore, the goal of this study was to examine how genotype and sex influence serum cytokine responses to PCBs. Mice were developmentally exposed to a mixture of PCB congeners (MARBLES mix) present in the serum of a woman who is at high risk for having a child with ASD at four dose groups: low, intermediate, high, and control. In the maternal diet through gestation and lactation. Four genotypes were studied: knock-in mice expressing a human y nanodeoxyribo nucleic acid receptor (RyR) gene of function mutation (T482E-RyR1), the X-linked FMR1 CGG repeat expansion (PCGCG) with 170 repeats) or both mutations (double mutation, DM), and congenic wildtype (WT) mice. At postnatal day 28, serum was collected and analyzed for 23 cytokines and chemokines using a Lumexin multiplex assay. Genotype affects the serum cytokine responses to PCBs, with WT mice exhibiting a non-monotonic dose response for most of the cytokines tested that was not observed for the other genotypes. Sex differences were observed in many cytokine profiles, with data analysis suggesting that circulating serum cytokines in males are more responsive to PCB dose than females. This sex difference is most pronounced in WT and PCGCG animals. These data suggest that male immune responses may be more susceptible to modulation by environmental factors implicated in ASD pathogenesis. Supported by NIEHS (R01 ES014901 to PJL and INP, F32 ES070739 to SS, P01 ES01269 to JVDW and PJL, P01 ES035691 to HUL), NICHD (U54 HD079125 to JVDW, INP and PJL, F32 HD088016 to KPK), and US EPA (RD83542501 to JVDW, INP and PJL).
STX inhibits voltage-gated sodium channels, affecting the propagation of action potentials. Humans are among the species most sensitive to PSP, and neurological symptoms of exposure range from tingling of the extremities to severe paralysis. To protect humans against PSP, there is a ban on harvesting of seafood where the STX levels reach 80 μg/100 g of shellfish tissue. However, shellfish with toxin levels below this regulatory threshold are consumed for consumption. Our objective is to understand the potential health effects of exposure to low levels of STX during sensitive windows of development. Zebrafish embryos were exposed to STX (24 or 48 pg) or vehicle (0.3 mM HCl) at 6 hours post-fertilization (hpf) via microinjection. There was no overt toxicity, but at 10 days post fertilization (10 dpf) there was temporary lack of pigmentation in STX-injected embryos, which resolved by 72 hpf. Using HPLC, we found that STX was retained in embryos up to 72 hpf in a dose-dependent manner. We examined transcriptional profiles in embryos at 24, 36, and 48 hpf. There were no differentially expressed genes (DEGs) in STX-injected embryos at 24 hpf, but at 36 and 48 hpf there were 3547 and 3356 DEGs, respectively, in response to STX. KEGG pathway analysis revealed significant enrichment of genes related to focal adhesion, adhesion junction, and regulation of actin cytoskeleton, suggesting that cell-cell and cell-extracellular matrix interactions were affected by STX. The genes affected are critical for axonal growth and the development of functional neural networks. We also observed differential expression of axon guidance factors (neurins, semaphorins, and ephrins), which can control axon outgrowth. We are currently using immunohistochemistry to confirm these findings. Overall, these results suggest that STX exposure might affect axon outgrowth by modulating cell adhesion molecules. NIH P01ES021923 and NSF OCE-1311462.

The zebrafish clock1a gene regulates Bisphenol A-induced neurodevelopmental toxicity
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Bisphenol A is one of the most important EDCs, possibly inducing the developmental toxicity through multi-mechanisms. The present study had shown that circadian clock gene, clock1a, could be disturbed by BPA. Fluorescein reporter assay confirmed BPA promoted the binding of estrogen receptor to the estrogen-response elements (EREs) of clock1a promoter. Then we used clock1a-/- zebrafish to detect the developmental toxicity after 96 h treatment of BPA in different concentrations of BPA (0, 1, 5, 25 μM). The results revealed the increase of malformation rate and death rate, the decrease of the total distance and total trace with a dose-dependent and the reaction to the dark stimulus was weakened in light and dark alternating experiment, apoptosis has increased after BPA treated. Furthermore, all these changes are more pronounced in clock1a-/- groups. Nrf2 as a clock-controlled gene downstream of clock1a, its promoter contains the E-box sequence, its expression is rhythmic and the rhythm changes after BPA treatment.Taken together, clock1a and Nrf2 are important genes that regulate the BPA induced neurodevelopmental toxicity.

Exploring the role of host-associated microbiota as mediators of Bisphenol A chemical toxicity in zebrafish
Exposure to Bisphenol A (BPA), a widespread environmental contaminant, has been associated with adverse endocrine and neurodevelopmental effects. Growing public concern over the safety of BPA has resulted in swift replacement with a suite of alternatives that uniformly lack adequate testing data. Because the microbiota play important roles in nervous system development and harbor the ability to bioactivate or detoxify xenobiotics, we hypothesized that developmental exposure to BPA compounds may influence microbiota structure leading to colonization-dependent neurotoxicity. To test this, a semi-static system was used to expose conventionally colonized zebrafish to BPA, Bisphenol AF (BPAF), Bisphenol B (BPB), Bisphenol F (BPF), or Bisphenol S (BPS). The classic estrogen receptor agonist 17beta-estradiol (E2) was used as a positive control. At 10 days post fertilization (dpf) larvae were assessed for mortality and a range of phenotypes was observed: BPB > E2 > BPF > BPA > BPAF. To evaluate potential chemical-dependent shifts in microbiota structure in 10 dpf conventionally colonized zebrafish, 16S rRNA gene sequencing was performed. Concentration-dependent disruption of microbiota was observed with exposure to BPA, BPF, and BPS, while exposure to E2, BPB, and BPAF, the most overtly toxic compounds, did not alter microbiota structure. To assess whether neurobehavioral toxicity was mediated by microbiota, we exposed three cohorts of zebrafish to all six compounds: conventionally colonized, axenic (microbe-free), and axenic colonized with zebrafish facility water at 1 dpf. At 10 dpf, neurobehavioral effects were assessed using an established light/dark assay. Hypoactivity was observed in colonized larvae only with E2 exposure. While some BP compounds caused neurobehavioral toxicity in one or more cohorts, the effect was colonization-dependent. Overall, these data demonstrate that the least overtly toxic BP compounds cause the most significant alterations in microbiota structure in 10 dpf zebrafish. These differential chemical effects suggest that current hazard identification strategies have the potential to misestimate risk if chemical-microbiota interactions are not considered. This abstract does not necessarily reflect US EPA policy.

Zebrafish Larvae Require Specific Strains of Bacteria for Neurobehavioral Development
There is an increasing appreciation of the relationship between gut microbiota and nervous system development and function. We previously showed that axenic (microbe-free) larvae are hyperactive at 10 days post fertilization (10 dpf) relative to conventionally colonized larvae. Interestingly, while exposure to heat-killed bacteria or microbe-associated molecular patterns failed to block hyperactivity in axenic larvae, colonization of axenic larvae with Aeromonas veronii or Vibrio choleae produced locomotor hypoactivity relative to colonized controls. These data suggest that there is a developmental requirement for certain types of microbes to modulate host behavior. To address this hypothesis, we exposed three cohorts of zebrafish larvae with 100 cells/ml of each of Actinetobacter, Vibrio, Comamonas, and Comamonadaceae at 1 dpf. Finally, Vibrio-related hypoactivity was found to persist in 10 dpf larvae. These data suggest that specific bacterial taxa are needed to drive normal neurobehavioral development while colonization with other strains may result in behavioral hypoactivity and raise the possibility that environmental chemicals may disrupt neurobehavioral development by selecting for specific classes of host-associated microbes. This abstract does not necessarily reflect US EPA policy.

Sex differences in neurotoxic effects and neurotoxic effects on sex differences
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The most common genetic polymorphism is sex. The most obvious sex differences are in reproduction but other sex differences exist. As with factors like height, sex differences in non-reproductive behaviors are not dichotomous but have overlapping continuous distributions. Most studies have shown that neurotoxicants can have differential effects in females and males. In some cases, these effects diminish or reverse normal sex differences in neurobehavioral function. In rats, we have found that exposure to the organophosphate insecticide chlorpyrifos (CPF) significantly reduces normal sex differences in working memory and activity in a radial-arm maze task. Males typically have fewer working memory errors than females. Low-dose neonatal CPF exposure (1 mg/kg/day, postnatal days 1-4) to rats reduces errors in females and increases them in males, eliminating this normal sex difference. Neurotoxic diminution of normal sex differences is not limited to insecticides. A recently completed study investigated the relevance of sex differences to the effects of two teratogenic components of cigarette smoke: nicotine and polycyclic aromatic hydrocarbons (PAHs). Developmental exposure of rats to a low dose (0.03 mg/kg/day throughout gestation) of the PAH benzo-a-pyrene (BaP) causes a significant reversal of a normal sex difference in locomotor activity in which female rats are normally more active than male rats. Prenatal BaP exposure causes hyperactivity in males but not females, eliminating the normal sex difference in locomotor behavior. Sex-selective effects are also seen with nicotine and BaP exposures in emotional and cognitive tests, with each compound eliminating sex differences in anxiety-like behavior in a novel environment. Intriguingly, BAP exposure...
1420 Brain Growth Spurt Alcohol Exposure Does Not Alter the Dynamics of Microglia, Dendritic Spines, or Their Interactions in Adolescent Mouse Somatosensory Cortex


Patients with fetal alcohol spectrum disorder (FASD) suffer from a wide range of cognitive disabilities throughout life. Auditory, visual, and tactile sensory information processing is often impaired in FASD patients, contributing to learning disabilities and difficulty navigating social scenarios. Rodent studies have shown that alcohol exposure during the brain growth spurt (BGS), when synapses are initially formed, can drive acute neuronal apoptosis and immunological activation of microglia in the somatosensory cortex. It remains unclear to what extent microglia and the excitatory synapses that microglia interact with in the healthy brain still function normally later on in life. We administered 3.6g/kg alcohol subcutaneously during the peak of the BGS (postnatal day 4 through 9 (P4-P9)). Control littersmates were injected with saline vehicle or handled. At P28, we used in vivo two-photon microscopy to image microglia and dendritic spines (structures representing postsynaptic sites of excitatory synapses) in the living, intact somatosensory cortex. We found that microglial process motility was not changed by prior alcohol exposure. While microglia in somatosensory cortices of mice previously exposed to alcohol tended to respond faster and more robustly to tissue injury compared to the response in control mice, the difference failed to reach significance. Neither dendritic spine density nor the proportion of spines falling into distinct morphological categories was affected by prior alcohol exposure. Tracking the same dendritic spines three times over 7 days revealed that spine stability, remodeling, and morphology were also similar among the alcohol and control groups. Quantifying the physical interactions between microglia and dendritic spines in P28 somatosensory cortices, we revealed no alcohol-induced differences in contact frequency, contact duration, or the size of spines more likely to be contacted by microglia. Together, these findings support the surprising conclusion that, without further perturbation, the physiological functions of surviving microglia and excitatory neurons in the mouse somatosensory cortex are intact and not affected long-term by BGS alcohol exposure.

1421 Identifying Sex Differences in Growth Responses to Ethanol in Fetal Neural Stem Cells

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Fetal Alcohol Spectrum Disorders (FASDs) result from prenatal exposure to alcohol, and are the leading non-genetic cause of developmental disabilities. Neural stem cells (NSCs) generate a majority of neurons in the adult brain during the mid-first to second trimester of gestation during which they are particularly susceptible to the teratogenic effects of alcohol. Ethanol exposure loss of NSC number and capacity in NSCs but does not cause increased cell death. One cellular mechanism through which ethanol exerts its effects on NSCs is through its actions on miR-9, a critical regulator of NSC differentiation. Ethanol exposure increases methylation at the miR-9 locus and subsequently reduces its expression in NSCs. Interestingly, previous studies have shown sexual dimorphism in cellular behavior, such as miRNA expression, within endogenous stem cells. Our study is the first to investigate sex-differences in the NSC response to ethanol. Using ex vivo cultures of NSCs derived from gestational day 12.5 C57BL/6j mouse fetal telencephalon and maintained as neurosphere cultures, we assessed the effect of ethanol on NSC sphere formation and sphere size separately in male and female-derived cultures. Preliminary data indicate that ethanol decreases neurosphere formation, and that fetal male-derived cultures are more sensitive to low dose ethanol exposure than female-derived cultures. Further studies characterizing these sex differences in the NSC response to ethanol will help elucidate the pathophysiology of FASD and lead to the development of novel therapeutics. Research supported by grant # R01AA024659.

1422 Identification of Molecular Bioindicators of Thyroid Hormone Action in the Fetal and Neonatal Rat Brain

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Adverse neurodevelopmental consequences remain a primary concern when evaluating the effects of thyroid hormone (TH) disrupting chemicals. Though the developing brain is a known target for TH insufficiency, the relationship between THs in the serum and the central nervous system is not well understood. When and how hypothyroidism exerts a neurotoxic influence on brain development is also unknown due to a lack of described phenotypes induced by mild hypothyroxinemia. To begin to address these outstanding issues we performed a series of dose response experiments in pregnant rats, using low to moderate levels of the goitrogen propylthiouracil (PTU, dose range 0.1 - 10 ppm). THs were quantified in both the serum and brain of offspring at gestational day 20 (GD20) and postnatal day 14 (PN14), two developmental stages recommended in OECD and EPA regulatory guidelines. From this dose response data, quantitative relationships of THs between the serum and brain were described. Next, targeted gene expression analyses that a transcriptional readout could serve as a molecular bioindicator of brain TH status, in lieu of T4/T3 measurements within the tissue. Our results show that the GD20 fetus exhibits a significant reduction of THs in both the serum and brain at low doses of PTU; yet very few of the proposed TH-sensitive candidate genes were significantly altered in the cortex at any dose tested. In the PN14 pup, significant reductions in both serum and brain THs were also observed at several doses of PTU. However, twelve transcriptional targets were identified in the neonate that mirrored the TH insufficiency within the brain. Interestingly, many of these genes encode proteins critical for maintaining future development and organization, which are processes known to cause phenotypic alterations in the brain following developmental hypothyroxinemia. These results inform the quantitative relationship between TH status in the periphery and the developing brain, and offer several target genes that could serve as pragmatic readouts of TH dysfunction in the PN14 rat cortex. This work does not reflect US EPA policy.

1423 Thyroid Hormone Insufficiency and Cortical Heterotopia Induced by Ammonium Perchlorate in Rodent Brain

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A morphological defect, a cortical heterotopia, has been observed in the brains of rat pups exposed to utero to moderate doses of the thyroid hormone (TH) synthesis inhibitor propylthiouracil (PTU). TH insufficiency during late gestation/early postnatal period is required to induce this defect, and its magnitude and incidence are dose-dependent. The present study extends these observations to the environmental contaminant ammonium perchlorate. Pregnant LE rat dams were placed on an iodine-controlled diet on gestational day (GD)2. On GD6, perchlorate (0 1 30 1000 1000 ppm) was administered to the dam via the drinking water. In one group, blood and thyroid glands were collected from dams on GD20, and blood, brain, and thyroid glands were taken from the fetuses. In a second group, pups were sacrificed on PN0, PN2, PN14, and blood and brains were collected. Increases in thyroid gland weights were seen in dams on GD20 and PN14. Expression of gene transcripts (Nis, Tpo) was increased in the thyroid gland of the dam and the fetus. Induction of the thyroid hormone (TH) responsive genes (Camk4, Klf9, Bdnf, Sema7a) was not changed in the fetal brain. Histological analysis of the PN14 brain revealed heterotopia in 0%, 30%, 70% and 100% of animals at 0, 30, 300 and 1000 ppm dose, respectively. Heterotopia were also largest at the highest dose of perchlorate, but consistently smaller in size than the observed hypothyroidism. Preliminary findings are consistent with prenatal TH insufficiency as essential for heterotopia formation, but further suggest that ‘perinatal’ hypothyroxinemia may be necessary for its full expression. Direct pup dosing studies are currently underway to address this hypothesis. These findings support the hypothesis that this morphological defect may serve as a brain-based biomarker of neurodevelopmental insult associated with moderate developmental TH disruption. This abstract does not necessarily reflect US EPA policy.
Human-Based Primary and Induced Pluripotent Stem Cell-Derived Neural Progenitor Cells as Promising In Vitro Models for a Developmental Neurotoxicity Testing Battery


Brain development underlies strictly controlled highly complex mechanisms, which makes the developing brain particularly vulnerable to a chemical insult. However, for the majority of chemicals that are in use the developmental neurotoxic potential is not characterized. Currently, developing brain function and neurotoxicity (DNT) testing is done according to an OECD or EPA in vivo guideline study. These studies are insufficient for large scale DNT testing as they consume high amounts of animals, time and money and bear the issue of species extrapolation. It is therefore generally accepted that an alternative strategy for DNT testing is needed and the use of an in vitro testing battery for compound screening and prioritization is currently under discussion at the OECD level. Our group has developed two in vitro models that represent major processes of embryonic as well as fetal brain development. With high readiness we use human second trimester (fetal) neural stem/progenitor cells growing as 3D cell aggregates called neurospheres. The ‘Neurosphere Assay’ mimics specific neurodevelopmental key events, e.g. proliferation, migration and differentiation into neural effector cells (astrocytes, neurons and oligodendrocytes). We also developed neurospheres from rodents, which can be used for knowledge driven species extrapolation according to the parallelogram approach. In development and with low readiness we use human induced pluripotent stem cells (hiPSCs) which are made from human fibroblasts and reprogrammed to regain a human stem cell-like character. Recently, we established hiPSC-derived neural progenitor cells, cultured as 3D neurospheres, to cover the embryonic development. These hiPSC-derived neurospheres were compared to our primary human neurospheres. We found hiPSC-derived NPC mimic neural development similar to primat NPC. A special property of these hiPSC-derived neurospheres is the ability to differentiate to a functional neuronal network consisting of neurons and glia cells that exhibit spontaneous electrophysiological activity on microelectrode arrays (MEAs). In summary, our assays represent an array of major neurodevelopmental key events and are a promising part of a modular DNT testing battery.

Comprehensive Gene Expression Analysis and Neurotoxicity Testing of Human iPSC-Derived Neural Progenitor Cells and Neurons

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Human induced pluripotent stem cells (iPSC)-derived neural progenitor cells (NPCs) and neurons are an attractive in vitro model to study neurodevelopmental, neurotoxicity and to model diseases. However, there is a lack of validated NPCs and media that support differentiation into multiple types of neurons for disease modeling, drug screening, and toxicity screening. Here, we investigated the expression of genes associated with the differentiation of NPCs during three weeks in dopaminergic differentiation media. Known early neuron markers, MAP2 and TuJ1 genes reached peak expression at two weeks while expression of dopaminergic neuronal genes (TH, Nurr1, VMAT2, AADC) was significantly increased in a time-dependent manner (p < 0.05) in two types of normal NPCs. Furthermore, expression of genes associated with GABAergic (GABRB3) and glutamatergic (VGLUT1, vGLUT2, GLS2) neurons was also induced and peaked at the end of three weeks. This suggests that our NPCs and dopaminergic differentiation media are capable of producing GABAergic and glutamatergic neurons, in addition to dopaminergic neurons. To validate that our NPCs and dopaminergic neuron differentiation media are suitable for drug screening, we conducted neurotoxicity screenings in three types of NPCs (non-reporter NPCs, MANo-NanocL-HaloTag reporter NPCs, and Parkinson’s disease NPCs) and NPCs-derived neurons using Reliable™ cell viability reagent assay and high content imaging analysis. We found that paclitaxel, a microtubule-stabilizing chemotherapeutic agent, significantly induced neurotoxicity (p < 0.001) in the three types of NPCs evaluated, but not in NPC-derived neurons. Vincristine, amiodarone, and chlorhexidine significantly decreased viability of both NPCs and neurons, whereas piperoxane, cisplatin, and hydroxyurea did not induce any significant neurotoxicity in either NPCs or neurons. This study demonstrates that our iPSC-derived NPCs and dopaminergic differentiation media are suitable for studying neurological development and neurotoxicity screening.

Development of a Dysmyelination Test to Study Developmental Neurotoxicity of Environmental Chemicals in a Human Brain Microphysiological System

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Neurodevelopment is highly sensitive to perturbations caused by environmental exposure. Disruption of myelin formation is a key neurodevelopmental event. Dysmyelination, or the failure to form or maintain myelin, can disrupt neuronal signal transmission, trigger degradation of axons, and lead to impaired cognitive function. Chemical disruption of myelin formation has been demonstrated in animal studies, but there are very few in vitro human cell-based models that have shown de novo myelination or classified chemicals using endpoints associated with myelination. Thus, there is a need to develop new methods that have the ability to evaluate myelination impairment as an endpoint for in vitro developmental neurotoxicity (DNT) testing. Our center has recently developed a reproducible human brain microphysiological system (BMPS) derived from induced pluripotent stem cells (iPSCs) that models key neurodevelopmental events, and the wrapping of axonal structures by oligodendrocyte processes was confirmed with confocal and electron microscopy. The objective of this study is to develop a test method which utilizes myelination as an endpoint for classification of developmental neurotoxicants in the BMPS. Using immunocytochemistry, we visualize the co-localization of myelin basic protein (MBP) and neurofilament (NF) and computer-assisted evaluation of myelin formation, we have been able to quantify axon myelination in BMPS. Preliminary data has also demonstrated that the organophosphate flame retardant DNT (1,3-dichloro-2-propyl) phosphate (TDCPP), Bisphenol A (BPA), and Cuprizone (CPZ), which have been demonstrated to cause dysmyelination in animal studies, cause dose-dependent reductions in myelin formation in BMPS at non-cytotoxic concentrations, indicating that the model has potential as a novel DNT test system to study dysmyelination.
We established a test system (finger maze test) for evaluating learning and memory ability in infant cynomolgus monkeys. The system using a puzzle as enrichment is an experimental method that evaluates learning and long-term memory in a relatively short time (5-week training phase, 2-day learning test, and 2-day memory test which is conducted 2 months after the learning test). In this study, we investigated 1) long-term memory retention capacity, 2) spatial cognitive ability, and 3) sex and age differences to expand the scope of finger maze test for pre-clinical learning and memorization researches. In Exp. 1, a memory test was conducted once every 2 months after the learning test to assess long-term memory retention capacity in 1-year-old infants. The success rate at the 2nd memory test (93.6% ± 4.0% mobility after learning test) was comparable to the learning test and 1st memory test (2 months after the learning test), suggesting the infants could memorize what they had learned 4 months ago and that long-term memory of over 4 months was assessable. In Exp. 2, an inverted version of the maze was used to examine if they had simply memorized the correct pattern or were using spatial cognitive ability to recognize the structure of the apparatus. Two months after the learning test, a 2-day memory test with the previously learned task (positive task) was conducted, and a memory test opposite to the previous learned (opposite task) was then conducted for 4 days. The time taken for the test was longer in the opposite task than the positive task (194 vs 274 sec) in Day 1 but the success rate was comparable (86.4% vs 80.0%). The duration for each test decreased over 4 days (274 to 101 sec) and the high success rate continued on Days 2-4 (92.1% to 95.0%). These results indicated that animals recognized the 3D structure of the maze and had the ability to carry out new tasks based on learned memories. In Exp. 3, the finger maze test was given to male and female monkeys of various ages (<1 year, <3 years, >5 years) to examine age and sex differences. Although all generations completed the learning and memory task without sex differences, young adults (<3 years) performed the best and had the shortest training periods. These results show that the finger maze test is useful for assessing short- and long-term memory in cynomolgus monkeys, since they have advanced spatial cognitive ability. The test could be applied to pre-clinical learning and memorization assessment.

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Organophosphorus insecticides (OPs) are the most widely used class of insecticides. Developmental exposure to OPs has long lasting negative impacts, including abnormal emotional behavior. These negative impacts of OPs are observed at levels that cause only minimum inhibiting effects of acetylcholinesterase, the canonical target of OPs. However, exposure to these levels results in the inhibition of endocannabinoid metabolizing enzyme fatty acid amide hydrolase (FAAH) but it is not clear what the long term effects of this inhibition are. In an study, the male rat pups were exposed orally to either corn oil, 0.75 mg/kg chlorpyrifos (CPF), or 0.9 mg/kg PF-04457845 (PF; a specific inhibitor of FAAH) daily from postnatal day 10 (PND10). The brain levels of FAAH were determined using a label free shotgun proteomic approach. The analysis determined FAAH levels against neurofilaments (NF), glial fibrillary acidic protein (GFAP), and myelin basic protein (MBP) were determined NAb (IgM and IgG) levels against neurofilaments (NF), glial fibrillary acidic protein (GFAP), and myelin basic protein (MBP) were determined. Multiregression modeling indicated anti-NF IgG were positively associated with indoor levels of MTBE, which is a byproduct of burning diesel fuel. Organic air pollutants are linked to the nervous system. The Mechanistic Indicators of Childhood Asthma (MICA) study in Detroit, Michigan used a participant-based approach for collection of indoor and outdoor residential air sampling data. This MICA-Air subset of participants conducted a residential sampling of VOCs which included: MTBE, hexane, styrene and BTEX (benzene, toluene, ethylbenzene, and m/p- and o-xylene) using Perkin-Elmer tubes packed with Supelco Carpack B adsorbent. Indoor samples were collected in the bedroom of the participating child. From these samples, blood of children (n=39; 9-13 years old) was collected on the day of their clinical visit, 24 hours after the sampling period. Serum NAb (IgM and IgG) levels against neurofilaments (NF), glial fibrillary acidic protein (GFAP), and myelin basic protein (MBP) were determined by ELISA. Results were analyzed non-parametrically for association with measured levels of a pollutant sources. IgM levels for NAb were inversely and significantly correlated with levels of VOCs. However, anti-NF IgG were positively associated with indoor levels of MTBE, Styrene and BTEX (r=0.25, p<0.02). Multiregression modeling indicated that indoor toluene was the major positive determinant of anti-NF, anti-GFAP and anti-MBP IgG titers (r²=0.2; p<0.05), while two or more BTEX constituents were significant determinants of anti-NF, anti-GFAP and anti-MBP IgG titers (r²=0.2-0.4; p<0.05). The discrepancy between nega-
tive individual pollutant associations with IgM and positive associations with IgG suggests Ig class switching and the development of immunological memory. However, it should be noted that children in this study also had heavy metal exposure, which may be a contributing factor in the NAB response, as previously reported. It should be noted that these associations were independent of the children’s asthma status, the focus of this study, and gender. While collected air samples do not reflect historical conditions, or internal dose, they do indicate exposure risk. These results further indicate that ambient indoor air pollutants impact on the nervous system, as indicated by NAB, in environmentally vulnerable populations. This abstract does not necessarily reflect US EPA policy.

**1432 Developmental Neurotoxicity Resulting from Pharmacotherapies Used in Preterm Labor, Modeled In Vitro**

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Preterm labor is frequently treated with terbutaline (to inhibit uterine contractions) and dexamethasone (to promote fetal lung maturation), drugs that are suspected to have an adverse impact on fetal brain development. We evaluated these two agents, separately or together, for effects on critical stages of neurodevelopment in two cell models that simulate key decision points in neural development: the emergence of neurons and glia from embryonic neural stem cells (NSCs), and the selection of neurotransmitter phenotype in neurotypic PC12 cells undergoing neurodifferentiation triggered by nerve growth factor. In NSCs, terbutaline reduced cell numbers and diverted neurodifferentiation in favor of the formation of glia; these effects reflected actions at β-adrenergic receptors, since they were blocked by propranolol. The in vivo effects were developmental. We injected pregnant mice with terbutaline in vivo, which produces glial activation. Dexamethasone likewise reduced NSC cell numbers but in contrast, suppressed the formation of glia; dexamethasone also suppressed NSC differentiation into neurons, to a greater extent than its effect on glia. The combination of terbutaline + dexamethasone produced outcomes similar to those of dexamethasone alone. In the PC12 model, terbutaline suppressed the emergence of both dopamine and acetylcholine phenotypes; dexamethasone, on the other hand, enhanced the dopaminergic phenotype at the expense of the cholinergic phenotype. Again, treatment with both drugs produced an outcome resembling that of dexamethasone alone. Our results provide clear evidence that the drugs used in preterm labor have a direct, adverse effect on multiple stages of neurodifferentiation, but that the effects of dexamethasone are more profound than those of terbutaline and can override those of the β-agonist. Since both of these agents have been implicated in neurodevelopmental teratology, the present findings provide a potential underlying mechanism for epidemiological associations of these therapies with increased risk of learning disabilities and autism spectrum disorders. Supported by NIH ES010356.

**1433 Role of the Gut Microbiome on the Ontogenesis of the Enteric Nervous System**

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The enteric nervous system (ENS) is a complex network of neurons and glial cells organized in two plexuses that function to coordinate the gastrointestinal tract development occurring within a constantly changing environment, which, after birth, ends in the establishment of the gut microbiome. The gut microbiome has been linked with the development of the ENS and the physiology of the gut. However, how altering the microbiome affects the development of the ENS is not clear. We hypothesized that depleting the microbiome by exposure to antibiotics during prenatal and postnatal development impairs the maturation of the ENS. We will test our hypothesis by assessing the effects of antibiotics on the activity and anatomy of the ENS in mice expressing the genetically encoded calcium indicator GCaMP5g and the fluorescent reporter tdTomato in glia. Gial expression will be driven by crossing reporter mice Sox10CreERT2 mice. Pregnant females will receive an antibiotic cocktail in drinking water after breeding and throughout pregnancy. Control females will receive normal drinking water. Samples of the colon and ileum will be harvested from pups at postnatal days 1, 7, and 14 to image spontaneous activity of glial cells using GCaMP5g fluorescence. We will analyze the architecture of the ENS by imaging tdTomato fluorescence in neurons and glia. We anticipate that the results from our study will provide important insight into the effects of antibiotics on the neural control of the digestive tract.

**1434 Alterations in Developmental Serum Testosterone Concentrations and Adult Social Deficits Produced by Early-Life Exposure to Ultraviolet Particulate Matter**


Air pollution is a worldwide environmental health concern. Recent epidemiological evidence suggests that poor air quality may contribute to the etiology of sex-biased neurobehavioral disorders such as Autism Spectrum Disorders (ASDs). Our previous studies indicate that male, but not female, mice exposed during the neonatal period to concentrated ambient ultrafine particles (<100 nm diameter; CAPS) had significantly enlarged lateral ventricles, a characteristic of ASD and schizophrenia. Additionally, CAPS reduced corpus callosum size and corresponding myelin basic protein morphology in males, but not in females, with preliminary evidence suggestive of decreases in serum testosterone concentrations. To expand on these previous findings, neonatal mice were exposed to terbutaline (10-20 fold) ambient ultrafine particles using the Harvard Ultralfine Concentrated Ambient Particle System (HUCAPS). Twenty-four hours following the final exposure, P14 mice showed CAPS-induced reductions in serum testosterone, while no significant effects were seen in females. Furthermore, there were significant social behavior deficits in adulthood, with CAPS-exposed males showing significantly fewer nose-to-nose interactions with novel male mice, identified using the social novelty paradigm. Again, females showed no significant changes. Following another independent breeding and CAPS exposure, animals were tested for deficits in social behavior utilizing the social conditioned place preference paradigm. Again, CAPS-exposed males, but not females, showed deficits. CAPS males had significantly fewer entries into the social conditioned compartment, with increases in the isolation compartment. These data suggest that early development exposure to CAPS alters early endocrine signaling pathways, leading to lifelong social behavior, including social preference and communication. Taken together, these data represent three experimental studies that indicate CAPS produces developmental neuropathology, preferentially in males, that is consistent with features of sex-specific neurodevelopmental disorders like ASDs. Supported by R21 ES019105.

**1435 Influence of Genetic Background on Polychlorinated Biphenyl (PCB) Developmental Neurotoxicity**

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The prevalence of many neurodevelopmental disorders (NDDs) is on the rise, and while there is strong genetic predisposition for many NDDs, there is evidence that environmental factors contribute to individual risk. PCBs have recently been identified as possible NDD risk factors. Experimental studies have shown that a subset of environmentally relevant PCBs may alter neuronal function by sensitizing receptor tyrosine kinases (RyRs), thereby modifying calcium-dependent signaling pathways important in activity-dependent dendritic growth. This study tested the hypothesis that neurodevelopmental outcomes following developmental exposure to PCBs are influenced by heritable mutations that alter the fidelity of Ca2+ signals. Transgenic mice expressing a human RyR gain-of-function mutation (T4826I-RyR1), the X-linked FMR1 CGG repeat expansion (170-200 repeats; CGG), or both mutations (DM), and congeneric wildtype (WT) mice were exposed to MARBLES PCB Mix at 0, 0.1, 1.0, and 6.0 mg/kg/day in the maternal diet throughout gestation and lactation. The MARBLES PCB Mix mimics the proportions of the top 12 PCB congeners found in the serum of mothers in high risk for having a child with a NDD. One male and female pup from each litter was examined at postnatal day (PND) 26-29 for grooming as a measure of repetitive behavior, and at PND 28-31 for sociability using an automated social approach assay. Genotype alone influenced outcome: Relative to sex-matched WT controls, T4826I-RyR1 and DM females spent significantly more time grooming, and the DM males and females exhibited decreased sociability as evidenced by failure to spend more time with a novel mouse compared to a novel object. PCBs significantly increased grooming in the 0.1 mg/kg group and increased social behavior in the 1.0 mg/kg group to the same extent as observed in DM mice. These findings suggest that (1) PCBs can cause NDD-relevant phenotypes but that the outcome is modulated by genetic background; and (2) sex differences in the prevalence and/or severity of NDDs may be due to sex-specific responses to environmental factors. Supported by NIEHS R01 ES014901, P50 and INP; T32 ES007059, SS; P01 ES013661, HUL; and NICHD (F32 HD088016, KPK).

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Evolution of Autism Research: The Role of Environmental Health Sciences

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The phenotypic and genetic heterogeneity of Autism Spectrum Disorder (ASD) suggests both genetic and environmental factors are involved in its etiology. Xenobiotic contributors may impact gene expression via GxE interactions and epigenetic modifications, or even modify metabolic or immune pathways. To understand the current state of the science and evolution of environmental health science (EHS) research in ASD, a network analysis of the exposures, genes, and mechanisms investigated in over 20 years of grants funded by NIEHS—a major supporter of EHS research—was conducted. Temporal analysis reveals a diverse portfolio comprised, in the beginning, of projects researching a few metals (e.g., lead and mercury) as well as brominated (e.g., PBDEs) and chlorinated compounds (e.g., PCBs). Over time, the portfolio expanded to include a wide variety of xenobiotic exposures and endocrine disruptors as well as more recent categories like air pollutants. Many projects also considered the varied contexts of and pathways linked to health-related endpoints that include neurodevelopment and, eventually, also the immune system, metabolic disease, and the microbiome. Of about 140 genes, 29% are included in the SFARI gene list—a curated database of autism susceptibility genes. GO enrichment analysis of these genes reveals categories related to synapse assembly/potentiation, brain development, and social behavior. Further network analysis shows non-SFARI gene-connectedness to identified autism genes and is representative of researchers’ interest in genes not previously associated with autism. Many known response genes to environmental factors that interact with biological processes (e.g., oxidative stress, immune response, cell signaling). Furthermore, immune-related genes appear central in a co-occurrence network and connected to gene categories involved in metabolic disease, cell signaling, and synaptic function. Bipartite network analysis displays how these groups of genes most associate with studies on exposures such as pesticides, brominated compounds, metals, air pollutants, and hormonal mimics, several of which have produced key advances in environmental risk research on ASD. Finally, the results reflect the increasing understanding of the complexity of the genetic etiology of ASD, but also increased awareness of the toxicology community regarding a broad range of exposures that may impact neurodevelopment.

Building a Community: An Application of Social Network Analysis to Collaboration in NIEHS-Funded Environmental Autism Research

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Collaborative and interdisciplinary research is a vital strategy for translating environmental health research from observations in humans to mechanistic toxicology and impetus to future studies, as well as to health interventions and public health impacts. One way to measure and visualize collaboration is through co-author networks, which allow for the calculation of metrics that reflect the structure and dynamics of the network. In this analysis, environmental autism research is used as a rich case study in an area that has been prioritized for funding by NIEHS. This field includes multiple disciplines such as toxicology, epidemiology, and neuroscience as well as multiple posited exposures that may contribute to autism development, among them air pollution, heavy metals, and persistent organic pollutants. Using autism-related publications linked to NIEHS grants and co-author networks generated over time to demonstrate the growth of research in the field and integration of new groups within the network. We reviewed 208 autism-related articles published between 2004 and August 2017, with 540 unique authors. The analysis shows significant growth in both the number of publications and authors in this field since 2004. However, the network’s density, a measure of the overall connectedness of the network, has remained quite low over time. By 2016, the network showed nine weakly connected components: one large sub-network that is institutionally and regionally based, supported by multiple PIs, and eight smaller sub-networks headed by single PIs. It is clear that the network size and density have increased, and that these groups are publishing independently of each other, even in cases where they are working with similar models or on related toxicants. Specific individuals remain key gatekeepers of communication and act as bridges within and between groups, however, new authors and fields are beginning to play a larger role in the network. The incorporation of new authors and fields in the network reflects the active engagement by NIEHS and other funding organizations, e.g., targeted workshops, but the continued low density of the network may indicate that additional steps are warranted. This type of analysis could be used in other areas of toxicology to understand the growth of and opportunities for interdisciplinary collaboration.

Neonatal Hyperoxia Followed by Concentrated Ambient Ultrafine Particle Exposure Leads to Cumulative Learning Deficits in C57Bl/6J Mice


Advances in neonatal care have drastically increased the survival of preterm infants, who are exposed to a unique set of environmental conditions during a vulnerable stage of neurodevelopment. The etiology of neurodevelopmental disorders is multifactorial and includes genetic and environmental factors. The polycomb gene complex, which includes Rbfox3, Dnmt1, and Dnmt3a, is critical for neurodevelopment in the central nervous system of animals and may also affect neurodevelopment in humans. A previous study showed that mice exposed to a single episode of hyperoxia and CAPS (cerebellar peduncle-ablated pups) exhibited deficits in multidimensional learning compared to WT mice exposed to hyperoxia followed by CAPS. This study aimed to determine the cumulative effects of hyperoxia followed by CAPS at different time points, as well as the effects of exposure to ambient ultrafine particles (CAPS). Hyperoxia exposure impaired multidimensional learning as early as 2 days of age, and CAPS exposure at 3 days of age. The combination of hyperoxia and CAPS exposure led to cumulative learning deficits in C57Bl/6J mice. These results suggest that exposure to ambient ultrafine particles may impact neurodevelopment, and that further research is needed to understand the mechanisms underlying these effects.
deficits on an extinction paradigm in which reinforcement was subsequently withheld. Further elucidation of specific behavioral and pathological underpinnings of the unique effects of the combined insults will provide important insights into the potential for enhanced adverse outcomes in premature infants exposed to air pollution.

**1440 Combined Neurodevelopmental Pyrrolizidine Pesticide and Stress Hormone Exposure in Mice Affects the Dopaminergic Pathway in ADHD**

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Attention-Deficit Hyperactivity Disorder (ADHD) is one of the most common neurodevelopmental disorders, with the dopamine circuit playing a critical role in disease etiopathogenesis. While genetic variation contributes to incidence of ADHD, more recent work identifies exposure to environmental toxicants, specifically pyrethroid insecticides, as a neurodevelopmental risk factor. These findings are in line with our previous work, which shows neurodevelopmental exposure to deltamethrin (DM) is associated with changes in pre- and postsynaptic dopaminergic signaling and ADHD-like hyperactivity and inattention behaviors in a mouse model. In addition to elevated pesticide exposure, children of low socioeconomic status have higher levels of stress hormones, which can affect the dopamine circuit. ADHD is also more prevalent in low socioeconomic status groups. While these exogenous risk factors intersect at the dopamine circuit, their potential interaction has not been evaluated. Thus, we hypothesized that combined exposure to DM and the stress hormone corticosterone (CORT) would result in additive molecular, behavioral, and epigenetic effects in the dopamine system compared to each individual exposure. In our study, C57/Bl6 pregnant dams were exposed to 3 mg/kg DM during gestation and lactation until weaning of offspring at postnatal day (PND) 21. Thereafter, offspring were exposed to 25 mg/mL CORT in their drinking water until adulthood at PND 60. Controls included vehicle-only, DM-only, and CORT-only groups. Alterations to dopamine signaling in male and female offspring were evaluated by neurobehavioral, neurochemical, and epigenetic assessments focused on elucidating dysfunction in pre- and postsynaptic elements of the dopamine circuit. Results show that dual exposure to DM and CORT during neurodevelopment is associated with alterations in expression and function of critical dopaminergic synaptic proteins in the striatum accompanied by deficits in ADHD-relevant neurobehaviors. This is the first study to a) examine the combined effects of two environmental exposures that affect the dopamine system in the context of ADHD and b) integrate both epigenetic and molecular alterations with neurobehavioral assessments in a mouse model. Results from this study indicate that it is imperative to study multiple neurodevelopmental exposures at once to elucidate pathophysiological mechanisms of ADHD.

**1441 Gene Environment Interaction in 3D iPSC-Derived Autism Model**


Autism is a main concern of developmental neurotoxicity. The recent dramatic increase of autism cannot be explained solely by genetics or exposure to environmental chemicals, but rather as interplay between environmental toxicants, specifically pyrethroid insecticides, as a neurodevelopmental risk factor. These findings are in line with our previous work, which shows neurodevelopmental exposure to deltamethrin (DM) is associated with changes in pre- and postsynaptic dopaminergic signaling and ADHD-like hyperactivity and inattention behaviors in a mouse model. In addition to elevated pesticide exposure, children of low socioeconomic status have higher levels of stress hormones, which can affect the dopamine circuit. ADHD is also more prevalent in low socioeconomic status groups. While these exogenous risk factors intersect at the dopamine circuit, their potential interaction has not been evaluated. Thus, we hypothesized that combined exposure to DM and the stress hormone corticosterone (CORT) would result in additive molecular, behavioral, and epigenetic effects in the dopamine system compared to each individual exposure. In our study, C57/Bl6 pregnant dams were exposed to 3 mg/kg DM during gestation and lactation until weaning of offspring at postnatal day (PND) 21. Thereafter, offspring were exposed to 25 mg/mL CORT in their drinking water until adulthood at PND 60. Controls included vehicle-only, DM-only, and CORT-only groups. Alterations to dopamine signaling in male and female offspring were evaluated by neurobehavioral, neurochemical, and epigenetic assessments focused on elucidating dysfunction in pre- and postsynaptic elements of the dopamine circuit. Results show that dual exposure to DM and CORT during neurodevelopment is associated with alterations in expression and function of critical dopaminergic synaptic proteins in the striatum accompanied by deficits in ADHD-relevant neurobehaviors. This is the first study to a) examine the combined effects of two environmental exposures that affect the dopamine system in the context of ADHD and b) integrate both epigenetic and molecular alterations with neurobehavioral assessments in a mouse model. Results from this study indicate that it is imperative to study multiple neurodevelopmental exposures at once to elucidate pathophysiological mechanisms of ADHD.

**1442 Role of Benzyl Salicylate in the Development of Autism**

A. G. Ransom, and O. Bagasra. Claflin University, Orangeburg, SC. Sponsor: A. Baines

Benzyl salicylate is a naturally occurring compound found in plants and plant extracts. It is often a major ingredient in numerous cosmetic products and serves as a fragrance/tick agent in food. It is commonly used as a fragrance additive/solvent and UV light absorber. The International Fragrance Association has placed a restriction on its use in consumer goods due to risk of causing sensitization and allergic reactions. Benzyl salicylate is an endocrine disruptor and is suspected to be an environmental toxin. Overall, studies confirm it is a known human immune system toxicant or allergen. It is hypothesized that if found present in the blood-stream of a pregnant adult female, benzyl salicylate could potentially cause damage to the developing brain of a fetus. This investigation examined the effects of various concentrations of benzyl salicylate on the neurons of two neuroblastoma cell lines, CRL-2266 and CRL-2267, which served to represent a developing fetal brain. The cell lines were extensively analyzed for any morphological abnormalities in comparison to a control group. These abnormalities included deviation in axonal length, central chromatolysis, synctia formation, and axonal degeneration. Analyses indicated that neurons from both male and female cell lines that were exposed to benzyl salicylate underwent substantial modifications, specifically synctia formation (P<0.0248) and chromatolysis (P<0.0444). It was concluded that exposure to benzyl salicylate could potentially cause serious neurological damage to a developing fetal brain and therefore possibly lead to autism development.

**1443 Does Raising Perinatal Serotonin Availability Rescue Sociability via Oxytocin Signaling in the BTBR Mouse Model of Autism?**


Autism is a lifelong disorder with prominent social behavior deficits and exaggerated restrictive-repetitive habits that confers staggering costs for families and to society. Clinical and basic research evidence suggests that serotonin (5-HT) system dysfunction or low 5-HT availability in brain contribute to the etiology of some forms of autism, which may underlie social behavior impairments. Recent studies have shown that 5-HT availability in fetal brain depends more on available maternal tryptophan (TRP), the precursor to serotonin. Given this, we hypothesized that either maternal dietary TRP supplementation or administration of a selective serotonin reuptake inhibitor such as Prozac (fluoxetine) that inhibits 5-HT transporters (5-HT Transporter) during gestation/lactation could potentially protect a developing fetal brain otherwise at risk of developing autism. To test this, we utilized black and tan brachyury tufted (BTBR Itpr3T+/tf) mice, as the strain exhibits dominant and reliably hereditary impaired social interactions and restricted behavior phenotypes that persist across their lifespan; impaired serotonin system development and function is also evident in this strain. BTBR mouse dams were administered diets either with control TRP levels (2.1 g/kg, daily requirement for mic) or 5X enhanced TRP (12.6 g/kg) throughout pregnancy/lactation. While it is still early in the study and data collection is strongly perturbed by CPF in CHD8+/− organoids vs. CHD8+/+ control. Perturbation of WNT/beta-Catenin pathway was previously shown to be associated with CHD8 mutation. Beta-Catenin level was induced by both CHD8 mutation and CPF exposure, while GSK3β was similar in both lines. Oxidative stress was higher in CHD8−/− organoids and was further induced by CPF exposure. Thus, we identified a potential synergy of CHD8 mutation and the pesticide CPF in perturbation of neural development and functions. Further experiments are being conducted to identify molecular mechanisms of potential synergy between CHD8 genetic background and chlorpyrifos exposure.

KO organoids. In addition synaptogenesis and neurite outgrowth were strongly perturbed by CPF in CHD8−/− organoids vs. CHD8+/− control. Perturbation of WNT/beta-Catenin pathway was previously shown to be associated with CHD8 mutation. Beta-Catenin level was induced by both CHD8 mutation and CPF exposure, while GSK3β was similar in both lines. Oxidative stress was higher in CHD8−/− organoids and was further induced by CPF exposure. Thus, we identified a potential synergy of CHD8 mutation and the pesticide CPF in perturbation of neural development and functions. Further experiments are being conducted to identify molecular mechanisms of potential synergy between CHD8 genetic background and chlorpyrifos exposure.
spring of fluoxetine treated dams have significantly greater preference for social interaction than saline treated dams (p<0.05, N=3-5). Behavior data from male offspring of fluoxetine treated dams haven’t been analyzed yet. Male burying data showed no significant changes in restrictive repetitive behaviors among groups.

1445 Quantitative Measurement of Neural Autoantibodies to Map-2, Tau, Tubulin, and Gfap Proteins by Elisa in the Seria of Patients with Gulf War Illness (GWI)

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For Gulf War Veterans (GWW), the effects of war continued long after they returned home. In addition to the psychological ramifications, veterans and civilian workers showed exacerbated effects of medically unexplained chronic multi-system disorders. The causality of illness may be due to the compounds they were exposed to, which include acetylcholine (ACh) or modulate the pharmacokinetics of substances that control the metabolic activation or breakdown of ACh inhibitors. Such compounds include chloropyrifos, sarin, sulfur sarin, sulfur mustard, pyridostigmine bromide, DEET, opiates as therapeutics and the enzymes responsible for drug metabolism such as cytochrome P450 reductases, liver microsomal oxidases, etc. Inaccessibility of the nervous system has impeded the evaluation of cellular and molecular changes that result in neurodegeneration. Discharges of neural proteins during this process can induce autoimmune response that can be measured by novel biomarkers.

When we screened for novel nervous system biomarkers in the sera of GWVs from our pilot study, we found 2 to 9 fold increase of autoantibodies to the neuronal specific proteins. We have now developed ELISA to determine and quantitate serum autoantibodies against microtubule associated proteins (MAP-2), microtubule associated protein tau (Tau), tubulin and glial fibrillary acidic protein (GFAP). This method quantitatively distinguishes IgG levels of the autoantibody titers at 0.1 microgram level of the specific neuronal proteins. Determination of specificity was achieved by absorption studies to estimate the threshold level. We performed dose response to determine the optimum concentration of each protein. Supported in part by DOD Contract No. W81XWH-15-1-0641.

1444 Manganese (Mn) Exposure Differentially Alters Expression of SLC30A10 and S100A9 in a Mouse Model of Huntington’s Disease

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Huntington’s Disease (HD) is an autosomal dominant neurodegenerative disorder resulting from an expanded CAG repeat in the Huntingtin (Htt) gene. Despite its monogenetic etiology environmental factors strongly modify age-of-onset and progression. Mn is an essential nutrient and co-factor for several enzymes regulating antioxidant status, urea cycle metabolism, and neurotransmitter synthesis. In vitro and in vivo models of HD have demonstrated reduced Mn bioavailability in the brain suggesting significant perturbations in Mn biology. To understand differences in the transcriptional response to Mn exposure, male and female YAC128Q (HD) mice as well as wild-type (WT) littermates were given a single subcutaneous injection of 50 mg/kg MnCl2•(H2O)4 and sacrificed immediately, 1 hour (h), 4 h, 12 h, or 24 h after. Select brain regions (cortex and striatum) and spleen were collected at each time point, and mRNA expression of Mn responsive genes by qRT-PCR and oxidative stress markers (WT only). The Mn-transporter SLC30A10 and S100 calcium binding protein 9 (S100A9) were differentially expressed after Mn exposure in WT animals only. In the cortex, SLC30A10 mRNA was downregulated 15% 12 h post injection (p = .0032) while there was no change for HD mice. Interestingly, basal cortical SLC30A10 mRNA was significantly lower in HD compared to WT (p = .048), S100A9 was upregulated 16% by 4 h post injection (p = .043) and 36% by 12 h post injection (p = .0002) in WT cortical tissue. However, in HD cortical tissue, S100A9 increased 8.18% only at 1 h post injection (p = .032). In the striatum, S100A9 increased 30% in WT mice 12 h post injection (p = .0032) while there was no change for HD mice. Similarly, hepatic S100A9 increased 24% 1 h post injection (p = .026) and sustained a 18% increase 4 h post injection (p = .0231) in WT mice with no significant changes in HD mice. These Mn-induced transcriptional alterations occurred without inducing oxidative stress (measured by thiobarbituric acid reactive substances, total glutathione, and vitamin C levels). The differential transcriptional response to Mn in HD compared to WT provides further evidence that Mn biology plays a critical role in the pathophysiology of HD. Supported by NIH T32 ES7028 (JMW, ACP, ABB) and RO1 ES016931 (ABB).

1446 Biomarkers for Chronic Fatigue Syndrome (CFS) and Irritable Bowel Syndrome (IBS) Compared to Gulf War Illness (GWI) in Gulf War Veterans

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Approximately one third of the American military personnel who served in the Gulf War from August 1990 to June 1991 experienced chronic symptoms of GWI. Other Gulf War Veterans’ illnesses included CFS and IBS. CFS is characterized by debilitating fatigue, unrestful sleep, neuropsychological decrements and chronic pain. IBS is characterized by recurrent abdominal pain and bowel difficulties. In the present study, we used our newly developed biomarkers using western blot assay to screen serum for the presence of autoantibodies (AA) against the following neural proteins: neurofilament triplet proteins (NFNP), tubulin, microtubule associated tau protein (tau), microtubule associated protein-2 (MAP-2), myelin basic protein (MBP), myelin associated glycoprotein (MAG), calcium-calmodulin kinase II (CaM-II) and glial S100 protein. Serum reactivity was measured as arbitrary chemiluminescence units. The study included 50 CFS, 50 IBS, 68 GWI and 26 non-veteran asymptomatic served as controls. None of the patients showed any significant increase in AA against S100B. Patients with CFS showed statistically significant increase in AA only against four proteins over controls, presented in descending order: MAP-2 (2.90) > GFAP (2.88) > Tubulin (2.05) > CaMkII (1.51) > MBP (1.48) > SNCA (1.42) > NFP (1.21) > MAG (1.176) > Tau (1.04). IBS patients exhibited the following pattern: MAG (3.20) > MBP (3.19) > S100CL (1.39). GWI patients showed the following pattern: MAP-2 (4.81) > MBP (3.93) > NSF (3.63) > Tubulin (3.48) > Tau (3.17) > MAG (3.14) > S100CL (3.03) > GFAP (2.68) > CaMkII (2.48). These results showed that patients with CFS and IBS have less levels of AA against fewer neural proteins, indicating that the levels of AA against neural proteins in these patients are at the threshold levels that accompany brain injury in contrast to patients with GWI and in agreement with absence of neurological symptom complaints in these patients. Supported in part by DOD Contract No. W81XWH-15-1-0641.

1447 Copper-Induced Upregulation of miRNA Directs the Suppression of Endothelial LRPL1 in Alzheimer’s Disease


Reduced expression of low density lipoprotein receptor-related protein 1 (LRP1) in brain capillary with age is one of putative risk factors for the pathological buildup of Aβ in the brain and development of Alzheimer disease (AD). The exact mechanism of accelerated loss of LRPL1 in the brain capillary, however, remains unanswered. We and others have previously reported that copper-mediated downregulation of LRPL1 was proteasome-dependent, while the pro-inflammatory cytokine-induced loss of LRPL1 was proteosome- or lysosome-independent. In this study, we extended our investigation to examine whether microRNA, a small non-coding RNA involved in post-transcriptional modifications, mediates the repression of endothelial LRPL1. Three putative microRNAs, miR-205-5p, miR-200b-3p and miR-200c-3p, targeting LRPL1 mRNA were found to significantly increase in human primary microvascular endothelial cells (MVECs) after copper or IL-1β exposure. Application of these microRNA mimics to MVECs alone significantly reduced LRPL1 levels, and pharmacological inhibition of these microRNAs effectively reversed the loss of LRPL1 by copper or IL-1β. In J20 AD mouse model of AD, we found that 1.3 ppm copper drinking water for 9 months developed a marked reduction of LRPL1 in brain capillaries and increased amyloid-β burdens when compared to control J20 mice. Taken together, our findings support the pathological impact of copper-induced inflammatory responses and Aβ clearance in the brain, at least in part, by upregulating microRNAs miR-205-5p, miR-200b-3p and miR-200c-3p and further down-regulating endothelial LRPL1. Our findings provide a possible underlying mechanism by which environmental risk factors contribute to the development of AD neuropathology.
Alzheimer's disease (AD), the most common neurodegenerative disorder and disproportionately affects females, with >60% of those diagnosed with AD being female. Pathologically, AD is characterized by amyloid-beta (Aβ) plaques and neurofibrillary tangles (NFTs). Aβ plaques are comprised of extracellular aggregates of peptide fragments (Aβ38, Aβ40, Aβ42), sequentially cleaved by secretases from the amyloid precursor protein (APP). NFTs are formed by the aberrant phosphorylation and folding of the microtubule associated protein tau (MAPT). Though aging, family history, and the APOE ε4 genotype have been shown to play prominent roles in AD risk, little is known about environmental contributions to disease etiology. As such, a multitude of environmental and biological factors are likely important in astrogliosis of late-onset AD. Previously, we identified that serum levels of dichlorodiphenylchloroethylene (DDE), a metabolite of the pesticide dichlorodiphenyltrichloroethane (DDT), was significantly higher in the serum of patients compared to age-matched controls. These results suggested that DDT may be a potential risk factor for AD. Early acute studies indicated a high concentration of DDT decreased oxidative respiration in isolated rat heart and liver mitochondria. However, little is known about the effects of DDT on mitochondrial bioenergetics in the brain. Thus, the aims of the present study were to determine the role of DDT in the brain and to delineate the potential impact of increased Aβ burden on mitochondrial bioenergetics. Mitochondria exert a wide variety of cellular functions including generation of ATP by oxidative phosphorylation, secretion of cytokines, and detoxification of reactive oxygen species. Mitochondrial dysfunction has been widely implicated in neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease. Previously, we reported significantly higher levels of dichlorodiphenylchloroethylene (DDE), a persistent metabolite of the pesticide dichlorodiphenyltrichloroethane (DDT), in the sera of AD patients compared to age-matched controls. This work provides insight into potential mechanisms that may contribute to the association between DDT exposure and increased risk of AD. Supported in part by NIH R01ES026057.

**1448 Sex-Specific Effects on Alzheimer's Disease-Related Proteins after Exposure to DDT in 3xTG Mice**

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Alzheimer’s disease (AD), one of the most debilitating neurological diseases that impair sensory, motor, as well as cognitive functions. An early detection method for AD has gained tremendous importance recently. An exploration of olfactory pathway alterations at sequential stages of plaque deposition in rodent model potentially describes a spatiotemporal pattern of Aβ deposition related to early olfactory loss. In the present studies a styrylbenzene derivative, fluoro-styrylbenzene (FSB) was used to detect amyloid plaques and Fluoro Jade C (FJC) was used to detect degenerating neurons in the olfactory bulb (OB) of Tg AD rats ranging from age 4-20 months. FSB has the affinity to stain both amyloid plaques and tangles in the human AD brain. Non-Tg rats did not show any amyloid plaques or FJC degenerating neurons. At 4 months of age, very few nonfibrillar amyloid plaques were seen in the olfactory bulb and 1-2 amyloid plaques were observed in the piriform cortex in a 25 um coronal section, however, at 6 months of age, moderate amount of plaques in the Tg AD rats were confined to the olfactory nerve layer (ONL). Neurons were more involved in the ONL compared to the olfactory bulb and piriform cortex. Axonal degeneration was seen in the glomerular layer of the OB, but not in the hippocampal CA1 or CA3 regions. In aged Tg AD rats, FSB stained fewer amyloid plaques and tangles. At 20 months, FSB stained fewer amyloid plaques and tangles. At 20 months, FSB stained fewer amyloid plaques and tangles. Thus, these results demonstrate that DTT causes mitochondrial dysfunction in cultured primary astrocytes at relatively low concentrations. These data further suggest that greater cognitive deficits may be observed in the aged as compared to adult mice. Supported by NIH R01ES026057, R01ES027481, and R01ES021800.

**1449 Age-Related Hippocampal Vulnerability to Adult Neurogenesis following Pyrethroid Exposure**

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Over 16 million people in the United States are living with cognitive impairment (CDC, 2009). Environmental factors, including pesticide exposure, are thought to be significant contributors to neurodegeneration and cognitive dysfunction in some populations. There is growing evidence that adult neurogenesis in the hippocampus is important for learning and memory. Recent studies demonstrate that adult born neurons in the dentate gyrus (DG) in the hippocampus can functionally integrate into the existing neuronal circuitry and contribute to hippocampal-dependent learning and memory. Furthermore, disruption of adult neurogenesis can lead to deficits in hippocampal-dependent learning and memory. We previously reported that repeated exposure to deltamethrin resulted in reduction of progenitor cell proliferation in the DG of hippocampus and learning deficits in young mice. Here, we examined the impact of age on hippocampal neurogenesis in mice of different ages following deltamethrin exposure compared to age-matched control. Data revealed that deltamethrin decreased bromodeoxyuridine (BrdU)-positive cells in the DG of the hippocampus by 39% in young (3 month-old), 45% in adult (6 month-old), and 70% in aged (13 month-old) mice, while Ki67+ cells was reduced by 47% in young, 45% in adult and 67% in aged mice, indicating potential impairment of cellular proliferation. In addition, deltamethrin exposure decreased the number of nestin-expressing neural progenitor cells by 44% in young, 49% in adult, and 62% aged mice. Together, these findings suggest that deltamethrin-induced disruption of hippocampal neurogenesis is a significant contributor to the magnitude of deltamethrin-induced disruption of hippocampal neurogenesis. Based on the importance of hippocampal neurogenesis in the regulation of cognitive function these data further suggest that greater cognitive deficits may be observed in the aged as compared to adult mice. Supported by NIH R01ES026057, R01ES027481, and R01ES021800.
1452 Downregulation of 14-3-3 Protein Levels in an Animal Model of Neurodegeneration and in Human Alzheimer’s Brains


The 14-3-3 proteins are among the most abundant proteins expressed in the brain, comprising about 1% of its total soluble proteins. They bind to specific phospho-serine and phospho-threonine-containing motifs found on a variety of signaling proteins (kinases and transcription factors, among others) to regulate a wide array of cellular processes including cell cycling, apoptosis, and autophagy. We have examined the expression of 14-3-3 and its different isoforms in frontal cortex of kainic acid-treated and control rats, as well as in frontal cortex of post-mortal Alzheimer’s disease (AD) patients and age-matched control subjects. Among the different 14-3-3 isoforms in control rats, the relative abundance of expression is in the following order: 14-3-3-eta > tau > a > gamma > b > d > e > delta; whereas in human control samples the sequence of relative abundance is 14-3-3-eta > gamma > tau > epsilon > sigma > zeta/delta > alpha/beta. While 14-3-3-eta is the most abundant isoform in both rat and human frontal cortex, its relative proportion among all the isoforms is less than 50% in rat but nearly 90% in human, suggesting a more predominant role for it than all other 14-3-3 isoforms in human frontal cortex. Twenty-four hours following a kainic acid injection (10 mg/kg i.p.), there was a significant decrease in the total protein levels of 14-3-3 as well as the isoforms eta, tau, epsilon and gamma, when compared to those in control animals. AD samples, there was a significant decrease in total 14-3-3 levels and the eta and gamma isoforms, and no difference in 14-3-3-tau levels between AD and control brains. Together, these results demonstrate an abundance of several 14-3-3 isoforms in the frontal cortex and that a downregulation of total 14-3-3 protein levels and specific 14-3-3 isoforms is associated with neurodegeneration. Given the known function of 14-3-3 proteins as inhibitors of apoptosis, the observed decreased levels of 14-3-3 proteins could be early molecular events leading to increased activities in cell death signaling and eventually neurodegeneration. Based on these results and available evidence from other neurodegenerative diseases and animal models, 14-3-3 proteins may serve as an early biomarker of neurodegeneration and a potential target of therapeutic intervention for neurodegeneration.

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1453 Effects of the mGluR Agonist CHPG in a Mouse Model of Alzheimer’s Disease

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Previous work in our laboratory indicated that stimulation of metabotropic glutamate receptors (mGluRs) by injection of the agonist ACPD or 2-Chloro-5-hydroxyphenylglycine (CHPG) elicits an increase in myelin proteins and BDNF (brain derived neurotrophic factor) following a cuprizone elicited demyelinating lesion (Fulmer et al., 2014 and personal communication). More recently, we found that this effect of CHPG can also be elicited by an intraperitoneal (i.p.) injection to a cuprizone treated mouse. Based on the neuroprotective effects of CHPG observed in the demyelination model, we sought to determine if mGluR agonists could have similar protective effects in an Alzheimer’s Disease (AD) mouse model, in which pathologies include neuronal and oligodendrocyte degeneration. Levels of BDNF and myelin proteins were measured in triple transgenic (3xTg) AD and control brains at several stages of development. Examination of the basal forebrain (BF) demonstrated that at 12 months 3xTg-AD mice exhibit a significant decrease in BDNF compared to control mice as well as a decrease in the myelin proteins myelin basic protein (MBP) and myelin associated glycoprotein (MAG). These deficits were reversible after administration of exogenous BDNF (0.5ug/ul) into the lateral ventricle. To determine if the myelin proteins and BDNF would also be reversed by an i.p. injection of CHPG, we injected CHPG (40 mg/kg) three times a week for one week. Statistically significant increases in BDNF (257%), MAG (172%) and MBP (167%) were detected in the BF of 3xTg-AD mice suggesting that a similar mechanism is present in mice with an ongoing demyelinating lesion in pituitary; however, in the presence of an ongoing demyelinating lesion, it is possible that the effects of CHPG are not as pronounced. These studies suggest that CHPG may have a protective effect on demyelination in multiple models of neurodegeneration. Future experiments will assess the role played by BDNF and provide an understanding of the cellular mechanisms underlying these effects. Furthermore, these results may shed light on a feasible therapeutic approach to control the progression of AD. Mice were provided by F. LaFerla (UC Irvine); Supp. NIH HD23315 and NS036647.

1454 The Metabotropic Glutamate Receptor Agonist 2-Chloro-5-Hydroxyphenylglycine (CHPG) Increases BDNF and Myelin Proteins after Cuprizone-Induced Demyelination

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Treatment with the demyelinating agent cuprizone causes a decrease in brain-derived neurotrophic factor (BDNF) and myelin proteins with an upregulation of metabotropic glutamate receptors (mGluRs) within the lesion site. Similarly, many patients with multiple sclerosis not only have a decrease in myelin proteins, but also have enhanced expression of mGluRs within active chronic lesions and decreases in BDNF. Therefore, an intriguing therapeutic approach for these types of demyelinating diseases may be to enhance the endogenous source of BDNF. The aim of this study was to enhance endogenous BDNF through the selective mGluR Group I agonist CHPG by using the clinically relevant approach of a peripheral injection. Cuprizone or identically processed control feed was fed to mice for 4 or 6 weeks prior to intraperitoneal (ip) injections of saline or CHPG (20 or 40 mg/kg). Western blot was used to analyze the levels of BDNF, myelin proteins, and oligodendrocyte progenitor cell (OPC) markers after cuprizone treatment. CHPG increased levels of BDNF and myelin proteins 24 hours after injection. Cuprizone also caused an increase in OPC markers NG2 and PDGF-Rα, which were reduced by 40 but not 20 mg/kg CHPG. Interestingly, CHPG did not alter these proteins or myelin proteins in control-fed mice. Furthermore, preliminary data suggests that BDNF and myelin proteins remain elevated following ip injections of CHPG every other day for 2 weeks, suggesting that CHPG’s effects are maintained over time. To begin to elucidate the receptor responsible for CHPG’s actions, the selective mGluR5 antagonist 2-methyl-6-(phenylethynyl)pyridine (MPEP) was injected directly into the lesion site prior to ip injection of CHPG. When MPEP was injected with ip CHPG myelin proteins or BDNF were not enhanced. Preliminary data indicates that these effects are reduced when MPEP is administered before CHPG, suggesting both a role of mGluR5 in mediating the actions of CHPG and that CHPG injected peripherally acts within the lesion site. Taken together, these data suggest that selective mGluR Group I agonists such as CHPG may be a therapeutic approach for treating demyelinating diseases by increasing the levels of BDNF and myelin proteins. Supported by NIH NS0336647, T32ES0507148, F31NS098642 and NMSS RG 425784/1.

1455 Med64-Quad II System Accelerates the Studies of the Roles of NMDA Receptor in Synaptic Plasticity and Epileptogenesis in Acute Mouse Hippocampal Slice

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Micro-electrode arrays (MEAs) have been widely utilized to measure neuronal activities in vitro. The MEA technology offers many unique advantages to investigate neuronal circuitry, interaction, models of learning and memory, development, aging, disease and neurotoxicity. While several high-throughput platforms have been utilized for drug screening with cultured cell applications in recent years, there have been limited platforms designed for acute slice applications. Here we present the capabilities of the highly sensitive Med64-Quad II system, a novel medium-throughput MEA designed specifically for acute or cultured slice applications. We demonstrate the reliability and reproducibility of inducing LTP and spontaneous spike recording simultaneously in 4 acute hippocampal slices from 6-7 weeks old male ICR strain mice. Following theta burst stimulation, amplitude and slope were monitored for an additional 60 minutes. Pretreatment of NMDA receptor antagonist APV at 50uM in bath solution blocked LTP development. Bath application of NMDA at 10uM produced transient depression of fEPSP rather than enhancement of fEPSP. NMDA induced synchronized burst. The synchronized burst was inhibited by APV. NMDA induced synchronized burst can be useful as a cellular epilepsy model to study epileptogenesis and NMDA receptor can be a therapeutic target for epilepsy treatment. The results indicate that Med64-Quad II system can accelerate LTP mechanism study, studies for epilepsy disease model and therapeutic drug discovery. This system provides a useful tool for disease model research, drug discovery, target validation, compound screening for antiepileptic drug targets and pharmacological studies in acute brain slice applications in vitro.

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Glutamate is one of the major neurotransmitters in the central nervous system where it binds and activates ligand-gated ion channels and metabotropic glutamate receptors. Glutamate-evoked excitotoxicity plays a role in neuropathological conditions including ischemic stroke (IS) and traumatic brain injuries (TBI), and has been implicated in the etiology of degenerative diseases such as Alzheimer’s disease (AD), and Parkinson’s disease (PD). There are currently no cures for these conditions. Therefore, drugs with the potential to cure these diseases or delay their onset are highly desirable. In this study, we developed an automated high throughput screen (HTS) cell based assay to screen a collection of highly diverse chemical molecules to identify compounds which would inhibit HT22 cells from glutamate toxicity. Cells were treated with cell-permeable compound (HT22) to convert to resazurin, a non-fluorescent molecule to resorufin, a highly red fluorescent compound in a variety of cells, including HT22. We validated the assay by screening accleration of 1280 compounds in the Prestwick library of pharmacologically active compounds. A total of 25 compounds were identified as actives in the primary screen. These compounds are currently undergoing confirmation. In addition, we screened a library of Kinase inhibitors consisting of 32 chemical molecules in dose response. Of the 32 compounds, 10 molecules exhibited inhibitory activities against glutamate toxicity, with IC 50 ranging from 500 nM to 5 uM. These confirmed compounds from the kinase set are currently under study to identify the mechanism of neuroprotection and the potential to repurpose these compounds as medicines for glutamate induced neuropathological conditions. In the future, we plan to screen a subset of 450,000 compounds from a proprietary library of chemical molecules in our collection to discover novel chemical structures with the potential to be developed as treatment of neurodegenerative diseases.

**A Serum-Free Human iPSC-Motoneuron Schwann Cell Myelination Model**

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Schwann cell myelination is a critical step during development of the peripheral nervous system (PNS). Myelin protects the axons of motor and sensory neurons from damage, and greatly increases the efficiency and rate of action potential conduction along the Nodes of Ranvier that form between myelin segments. Damage to myelin segments by auto-antibodies is common etiology in peripheral demyelinating diseases that affect millions of people in the U.S. Current human cell-based models fail to recapitulate myelination of motoneurons by Schwann cells under serum-free conditions which is especially important for studying auto-immune diseases where patient sera must be present to mimic pathology. Here we describe a serum-free, human-based myelinating co-culture model composed of iPSC-derived motoneurons (hMNs) and Schwann cells (hSCs). We used immunocytochemistry and confocal microscopy to determine myelination based on myelin basic protein (MBP), neurofilament heavy chain (NF-H), and S100b protein marker co-localization. Myelin segments were quantified by counting the number of myelin expressing hSCs (myelin segments) relative to the total number of aligned Schwann cells. This model is the first human-based, serum-free myelinating system, and represents a milestone in tissue engineering that has utility for basic research into the molecular pathways that influence axon myelination and screening of drug candidates to treat demyelinating diseases. This serum-free system is especially relevant for studying auto-immune demyelinating diseases where added patient serum is critical for mimicking the disease phenotype.

**An In Vitro NMJ Model for Studying the Pathology and Treatment of ALS**


Amyotrophic lateral sclerosis (ALS) is a fatal neural disease that is characterized by the dysfunction of the neuromuscular junction (NMJ). Pathological hallmarks of ALS include motoneuron degeneration and impaired energy metabolism which has been linked to mitochondrial dysfunction and glutamate toxicity. Although most ALS cases are sporadic, familial ALS accounts for up to 10% of cases with 20% of such cases identifying mutations in the superoxide dismutase (SOD) 1 gene. Despite extensive research, no consensus on the main toxicity of these mutants has emerged. *In vitro* modeling of the NMJ would represent a powerful tool to delineate events leading up to the formation and subsequent disruption of the NMJ. We have developed a serum-free, compartmentalized in vitro NMJ model system capable of isolated stimulation of either muscle or motoneuron (MN), and selective treatment of either muscle, motoneurons or both. Induced skeletal muscle contraction was monitored by pixel subtraction video recordings. This system has been validated with 3 different neurotransmitters, for which not only dose response curves were generated, but also different mechanisms were revealed. This analysis provided a physiologically relevant tool to assess the pharmacology of neuromuscular blocking drugs. This functional system was extended to a disease model by using induced pluripotent stem cell (iPSC) derived SOD1 motoneurons co-cultured with human skeletal myoblasts to investigate MN-induced ALS pathology. From this study, we have identified structural and functional perturbations in SOD1-MN NMJs including decreased synchronous contractions, reduced NMJ stability and increased motoneuron varicosities compared to wild-type NMJ systems to develop a specialized “fingerprint” associated with several SOD1 mutations. We later treated these systems with compounds to alleviate the SOD1-induced deficits. Specifically, we observed increases in synchronous contraction and NMJ stability. This functional system can be used to study other ALS therapeutics including efficacy and toxicity, and through simple housing or cellular modifications, study other diseases of the NMJ including myasthenia gravis and muscular dystrophy and be used as a toxicological screen for toxic side effects.

**Use of Peptides to Disrupt NO Synthase Recognition by the Hsp90/Hsp70 Chaperone Machinery**

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The Hsp90/Hsp70 chaperones are upregulated during stress and regulate the quality control of proteins during toxicant injury. Although it is clear that chaperones specifically recognize damaged proteins in a sea of proteins in their native conformations, it remains unknown exactly how this occurs. We utilized neuronal NO synthase (nNOS) as a model Hsp90/Hsp70 chaperone client to study this process. Specifically, we utilized synthetic peptides (8-12 mer) that correspond to important domains on nNOS to block recognition by chaperones. Such an approach successfully identified a domain recognized by chaperones on the neuronal growth factor receptor that is exposed only on the monomeric protein. We chose sites near the heme domain that are thought to become exposed upon damage and subsequent monomerization of nNOS. To assess the extent to which peptides interfered with chaperone action, we utilized a convenien assay that measures the chaperone-mediated insertion of heme into the heme-deficient monomeric form of the nNOS. Thus, by simple measurement of reduced nNOS and SH-SY5Y human neuroblastoma cells supplemented with galactose-supplemented media to mimic the cell-type and cellular processes, we could readily assess chaperone recognition of nNOS. We tested 20 peptides and found that targeting three regions within the nNOS dimerization interface (the BH4 cofactor binding site, the Zn2+ binding loop, and the active site cleft opening) interfered with chaperone action on nNOS. As a control, peptides with novel chemistries derived from regions outside the dimerization interface did not affect the heme insertion. We are currently testing the peptides to see if they directly block association of Hsp90 and Hsp70 to nNOS. Identifying the site of binding will greatly aid in understanding how chaperones recognize damaged nNOS and may provide a useful method to pharmacologically control levels of nNOS.

**Heterocyclic Amine-Induced Bioenergetics Alterations**

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Environmental factors have been repeatedly implicated in Parkinson’s disease (PD) etiology. Pesticides have been extensively studied. Emerging data also suggests that dietary toxins should be considered. Heterocyclic amines (HCAs) are primarily produced during high temperature meat cooking. Our group has extensive data showing that several HCAs cause dopaminergic neurotoxicity in primary midbrain cultures. To date, we tested 1100 compounds and found numerous HCA subclasses. Since some of these HCAs are structurally very similar to known mitochondrial complex I inhibitors, we hypothesized that HCAs of dietary relevance will inhibit mitochondrial function. To test our hypothesis, we used SH-SY5Y human neuroblastoma cells supplemented with galactose-supplemented cells, more closely mimicking in vivo physiology. We tested the effect HCA exposure [12-amino-9H-pyrido[2,3-b]indole}
**1461 Novel Keap1 Inhibitors Protect against Neuroinflammation In Vitro**

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NF-E2-related factor 2 (Nrf2) is a critical cytoprotective transcription factor. Under basal conditions, Nrf2 is bound to a Nrf2-interacting protein Kelch-like ECH-associated protein 1 (Keap1) and targeted for proteasomal degradation. Keap1 can be covalently modified allowing the release and translocation of Nrf2. The activation of Nrf2 can induce the transcription of cellular defense genes such as heme oxygenase 1 (Ho-1) and NAD(P)H quinone dehydrogenase 1 (Nqo1). Thus, specific inhibitors of the Keap1-Nrf2 protein-protein interaction may represent potential therapeutic agents to treat neurodegenerative diseases. Here, we sought to evaluate whether novel direct Keap1-Nrf2 inhibitors (designated LHs) for their ability to (1) activate Nrf2 signaling, (2) up-regulate Ho-1 and Nqo1 mRNA and proteins and (3) protect against inflammation caused by lipopolysaccharide (LPS) in mouse microglia BV-2 cells. BV-2 cells were treated with LH compounds (10 µM) or the indirect Nrf2 activator DMF. At 6 h, DMF induced Ho-1 and Nqo1 mRNAs by 2- and 5-fold, respectively. The LH compounds significantly increased Nqo1 mRNA levels in microlglial cells between 2- and 20-fold at 24 h. LH385 was the most potent Keap1 inhibitor that significantly induced Nqo1 mRNA expression (up-regulation of Nqo1 proteins in BV-2 cells. In addition, LH385 significantly decreased LPS-induced proinflammatory cytokines IL-1 beta, TNF alpha and nitric oxide synthase (iNOS) mRNAs by 70 to 90%. LH385 also attenuated LPS-induced increases in IL-1 beta and TNF alpha proteins at 6 h. Of note, all eight LH compounds had no effect on cell viability when used at high concentrations (up to 50 µM) in BV-2 microglial cells. Based on these data, LH385 may be a potential therapeutic agent to reduce neuroinflammation by activating the Keap1-Nrf2-ARE pathway. Supported by NIH ES021800, ES005022 and Liberty Science Center Partners Program.

**1462 Sex-Specific Effects of Lead and Prenatal Stress on miRNA Expression in Mouse Frontal Cortex and Hippocampus**

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Environmental lead (Pb) and prenatal stress (PS) are co-occurring environmental and neurotoxicants, known to cause cognitive/behavioral disturbances and to impair learning and memory. Here, we sought to determine how subtle structural difference influence HCA mechanisms of neurotoxicity.

**1463 Enriched Environment Alters Hippocampal, Epigenetic, and Gene Expression Profiles and Mitigates Lead-Induced Memory Deficits**

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Being raised in an enriched environment is a potential social intervention strategy to mitigate cognitive/behavioral deficits associated with exposure to developmental neurotoxicants. However, molecular mechanisms related to environmental enrichment that may underlie structural/functional effects on the brain remain poorly understood. Developmental exposure to lead (Pb) results in behavioral/cognitive impairments and no current therapies have been effective in reversing the deficits. Epigenetic alterations induced by Pb exposure provide a window into possible epigenetic mechanisms underlying these deficits. The purpose of this study was to investigate the extent to which environmental enrichment could mitigate memory consolidation/recall deficits and further modify epigenetic marks and gene expression profiles altered by developmental Pb exposure. Following an early postnatal exposure (EPN) to Pb (birth through weaning), female Long Evans rats were randomly housed under non-enriched (standard housing, 3 rats/cage) or enriched (large enclosures, 6 rats/cage, equipped with variety of toys, running wheels etc., changed 3 times/week) conditions. Non-Pb-exposed controls were housed under similar conditions. At PDN55, the animals were tested for associative memory deficits using a trace fear conditioning paradigm. Memory deficits were observed only in non-enriched EPN females. Enriched EPN females performed similar to non-Pb controls suggesting that living in the enriched environment mitigated the cognitive deficit induced by Pb exposure. To gain insight into possible epigenetic mechanisms underlying these results, we examined global DNA methylation (mC) and hydroxymethylation (hmC) levels in the hippocampus (CA1). Non-enriched EPN females had globally reduced mC and hmC levels. Enriched EPN females and controls had similar mC and hmC levels. Exposure to Pb differentially altered expression level of DNA methylation-related genes in non-enriched animals and environmental enrichment normalized these levels. RNA-seq analysis showed transcriptional profiles in non-enriched EPN females to be significantly different from that in enriched females. The interaction of Pb and enriched environment influenced numerous biological processes related to DNA methylation and chromatin modification. Studies aim to further understand gene level function and networks/molecular mechanisms modified by Pb exposure and early life experiences are in progress. Supported by R01 ES015295.

**1464 Early-Life Lead Exposure Produces Sensorimotor Gating Deficits Consistent with a Schizophrenia Phenotype**


Epidemiological studies have demonstrated detrimental effects of chronic early life Pb exposure (CELE) on children’s intelligence, learning ability and neuropsychological development. Additional studies indicate CELE is associated with the onset and progression of neurological disorders including major depression, anxiety, delinquent behavior, and schizophrenia (SZ). Previous studies from our lab have shown that rats developmentally exposed to Pb express key features observed in SZ including: (1) loss of parvalbumin-positive GABAergic interneurons in relevant brain regions, (2) hyperdopaminergic hyperactivity and (3) increased dopamine receptors in the striatum (Biansfield et al., 2015).
Pre-pulse inhibition (PPI) is widely used to assess sensorimotor gating and deficits are well described in SZ and other neuropsychiatric disorders. In the present study we are investigating sensorimotor gating in Pb2+ exposed rats using a life course approach. Long Evans rats were chronically exposed to control diet or diet containing 1500ppm PbAc from gestation until late adolescence (postnatal day 50, PN50) or adulthood (PN120). This exposure results in blood Pb2+ levels of 0.7 ± 0.6 (control) or 17.9 ± 1.8 μg/dL (Pb2+-exposed). At PN50 and PN120, acoustic startle reactivity (120 dB bursts) and PPI (5 prepulses) were tested in control and Pb2+-exposed male and female rats. In PN50 male rats (Control n=21; Pb2+-exposed n=17), there was a significant effect of prepulse dB, F(3,117.3) = 59.1, p<0.0001, and an overall effect of Pb2+ treatment, F(1,36) = 11.7, p=0.002. In PN50 female rats (Control n=13; Pb2+-exposed n=9), there was a significant effect of prepulse dB, F(2.4,48.3) = 41.7, p<0.0001, and a near significant effect of Pb2+ treatment, F(1,20) = 4.02, p=0.059. In PN120 male rats (Control n=7; Pb2+-exposed n=7), there was a significant effect of prepulse dB, F(1,31,5.3) = 15.7, p=0.001, and an effect of Pb2+ treatment, F(1,12) = 5.0, p=0.045. No significant effects of Pb2+ were observed on acoustic startle response in male or female rats at either age. Taken together our data suggest a Pb2+-dependent impairment on PPI, reproducing behavioral changes present in SZ. Our findings combined with previous studies support the hypothesis that exposure to environmental toxins may contribute to the expression of mental disease later in life. Supported by NIEHS ES006189 to TRG.

1465 Cortical-Hippocampal Circuit Dysfunction Bridging the Cellular to Cognitive-Behavioral Effects of Chronic Early-Life Lead Exposure in Rats


Chronic early-life lead exposure (CELLE) causes neuronal and synaptic dysfunctions in the developing brain leading to learning deficiencies and behavioral abnormalities; but the circuit-level consequences of CELLE that tie cellular effects with cognitive impairments are not well understood. CELLE is known to disrupt the subunit composition of NMDA receptors, undermining synaptic plasticity, and leads to loss of parvalbumin (PV)-positive GABAergic interneurons (PVGI) in the hippocampus (HC) and medial prefrontal cortex (mPFC). These effects are thought to impair the excitatory-inhibitory (E-I) interactions of principal neurons in HC and mPFC within interneuronal networks and consequently disrupt gamma oscillations (30-150 Hz) that depend on E-I coupling as a mechanism for local computation. It remains unknown how lead’s neurotoxic effects actually impact circuit rhythms underlying cognitive functions. Local-field potential (LFP) recordings, reflecting local synaptic and neuronal synchrony, provide a powerful means for evaluating rhythmic coordination within and between brain regions. We recorded LFP simultaneously from HC and mPFC while adult rats (~100 days old) navigated an open environment. In addition to gamma, we observed the theta rhythm (4-12 Hz) that is prominent in HC and mediates interactions throughout the distributed cortical circuitry for navigation and decision making. Whereas theta recorded from control rats showed the same frequency in HC and mPFC, lead-exposed (1500ppm in diet) rats exhibited a theta frequency elevation in mPFC relative to HC consistent across running speeds (1-100 cm/sec). Both theta and gamma coherence in local HC-HC and mPFC-mPFC electrode pairs, as well as distal HC-mPFC electrode pairs, were disrupted in lead-exposed rats reflecting disrupted processing both within and between regions. Notably, gamma-band coherence was diminished both within mPFC and between HC and mPFC. In lead-exposed rats, we also observed a weakening of the relationship between HC theta frequency and animals’ running speeds indicating abnormal coupling of neural circuit dynamics to behavior. These initial observations provide a foundation for bridging the cellular and cognitive-behavioral effects of CELLE. Supported by NIH ES006189 to TRG.

1467 Developmental Manganese, Lead, and Barren Cage Exposure Have Adverse Long-Term Neurocognitive, Behavioral, and Monoamine Effects in Sprague-Dawley Rats


Developmental stress can induce dysregulation of the hypothalamic-pituitary-adrenal axis. Stressors such as low socioeconomic status (SES) may result in adverse long-term neurocognitive and behavioral effects. Children in lower SES households experience more stress and are more likely to be exposed to environmental pollutants (EPA 2008). Lead (Pb) and manganese (Mn), exposure to stress, Pb, and Mn during development may increase the risk of CNS dysfunction. Sprague-Dawley rats were used with one male/female pair within each litter assigned as follows: 0 (vehicle), 10 mg/kg Pb, 100 mg/kg Mn, or 10 mg/kg Pb + 100 mg/kg Mn (Pb-Mn). Treatment was from postnatal day (P) 4-28 with half the litters reared in cages with standard bedding and half with no bedding (Barren). Offspring were tested as adults. Mn and Pb-Mn groups had decreased anxiety, reduced acoustic startle, transient hypoaactivity, increased activity following (+)-methamphetamine, egocentric learning and memory deficits in Cincinnati water maze (CWM), and deficits in latent inhibition. Pb increased anxiety and reduced open-field activity. Barren-reared rats had decreased anxiety, CWM deficits, increased startle, and transient hyperactivity. The Mn, Pb-Mn, Barren-reared groups also had impaired Morris water maze performance. Pb altered corticotropin releasing hormone (CRH) and norepinephrine (NE) S-HT in males + Barren-reared increased noradrenergic S-HT, and Barren-reared decreased noradrenergic dopamine in males. The most pronounced effects were in the Mn and Pb-Mn-exposed groups, indicating that Mn exposure drove most of the adverse effects on behavior and neurotransmitters. Few interactions between Mn and Pb with barren cage rearing stress were found.

1468 Neuroprotective Effect of EGCG on Aluminum Altered Hippocampal NMDA Receptor 1 and 2A/2B Expression in Rats

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Aluminum (Al) is a neurotoxic metal and has been associated with chronic neurodegenerative diseases. N-methyl-D-aspartate receptors (NMDARs) contribute to neuronal development. Chronic Al exposure can affect the glutaminergic system through the NMDARs. Changes caused by Al also affect the development of chronic neurodegenerative diseases. Epigallocatechin gallate (EGCG) has a well-known antioxidant effect and is the most important component of green tea; it is also a subject of research interest in recent years. The purpose of this study was to investigate the effect of Al on hippocampal neuronal
development through the NMDAR 1 and 2A/B expressions and the protec-
tive role of EGCG in Al-induced changes through the NMDARs on
the hippocampus, which is known to be the most affected region in
chronic Al toxicity. In this study; 120 male Sprague-Dawley rats were
randomly divided into 10 groups (n=12). Control (C) was given 1 mL/
day saline orally; 100 mg/kg bw aluminum chloride (AlCl3) was admin-
istered for 8 weeks to AlCl3 group; 20 mg/kg EGCG was given for 4 and
8 weeks as EGCG C groups. AlCl3 was administered with two different
doses of EGCG (10mg/kg and 20mg/kg) and 0.15mmol/kg/bw deferox-
amine as study groups. For the other two groups, after 4 weeks pre-ad-
ministration of AlCl3, two different doses of EGCG (10mg/kg and 20mg/
kg) were added to the administration for the last 4 weeks. NMDAR 1 and
2A/B mRNA levels of these parameters were assessed with real-time
PCR and also evaluated immunohistochemically. Al downregulated
NMDAR subunits’ mRNA expression, which is apparent and statistically
significant in NMDAR1 and 2B subunits (p<0.05). Both doses of EGCG
treatment upregulated these changed mRNA expression levels dose-
dependently, but the simultaneous administration is more effective.
These changes are also confirmed with h-score analysis of immunore-
activity. The present study showed Al can induce neurotoxicity through
the NMDARs, and the EGCG has a protective role. In the light of this
study, EGCG application or consumption will be a new approach for the
prevention of neurodegenerative diseases probably induced by Al. This
work was supported by TUBITAK (project number 1155333).

1469 Lead-Induced Auditory Dysfunction and Potentiation of Noise-Induced Hearing Loss

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Environmental exposure to lead, a neurotoxicant, is unavoidable in
urban communities with older infrastructure. Though exposures at low
levels are often overlooked because their adverse health effects are
not obvious immediately, their interaction with other environmental stresses
cannot be ignored. A potent risk factor for lead neurotoxic manifestation,
such as acquired hearing loss, which significantly affects the quality of
life and productivity in 15% of American adults. Lead exposure disrupts
the structure as well as the function of the auditory system. Despite
the overwhelming evidences indicating the ototoxic effects of lead, the
underlying mechanism and its interaction with other ototoxicants
are yet to be fully understood. The aim of this study is to delineate
the mechanism underlying lead-induced auditory dysfunction and its
potential interaction with noise exposure. Young-adult C57BL/6 mice
were exposed to: 1) control conditions; 2) 2 mM lead acetate in drinking
water for 28 days; 3) 90 dB broadband noise 2 hours/day for two weeks;
and 4) both lead and noise. Blood lead levels were measured by induc-
tively coupled plasma mass spectrometry analysis (ICP-MS), lead-in-
duced cochlear oxidative stress signaling was assessed using targeted
gene arrays, and the hearing thresholds were assessed by recording auditory brainstem responses. Chronic lead exposure downregulated
cochlear Sod1, Gpx1, and Gstk1, which encode critical antioxidant
enzymes, and upregulated ApoE, Hspa1a, Ercc2, Prnp, Ccl5, and Sqstm1,
which are indicative of cellular apoptosis. Isolated exposure to lead or
noise induced 8-12 dB and 11-25 dB shifts in hearing thresholds, respec-
tively. Combined exposure induced 18-25 dB shifts, which was signif-
cantly higher than that observed with isolated exposures. This study suggests
that chronic exposure to lead induces cochlear oxidative stress and
additively potentiates noise-induced hearing impairment, possibly
through parallel pathways.

1470 Role of Aluminum-Induced Cytotoxicity in Neurodegeneration of Human Neuroblastoma Cells

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Sponsor: A. Ishaque

Aluminum (Al) has gained considerable attention due to its neurotoxic
effects. The widespread and extensive use of products made from or
containing aluminum provides easy and continuous exposure to humans.
Several pieces of epidemiological and clinical evidence have linked Al
with neurodegenerative disorders, including Alzheimer’s disease (AD).
The neuropathological characteristics associated with AD include the
accumulation of amyloid plaques and neurofibrillary tangles (NFTs).
The cleavage of amyloid precursor protein (APP) by the proteases
β-secretase (BACE1) and γ-secretase, respectively, generates the peptide amy-
loid-beta (Aβ), which then progresses to oligomerization and forms the
final product amyloid plaques. In addition, there are a large number of
studies providing evidence for the presence of Aβ within neurons on post-
mitotic and transgenic mouse brains. Our aims were to test the
effect of aluminum on β-secretase activity and to examine the differen-
tial effects of Aβ1-40 or Aβ1-42 peptides on cellular toxicity in human
neuroblastoma cells. Also, we examined the interactions of Al and Aβ
peptide subtypes with cells. SH-SYSY cells were exposed to different
exposure periods to various concentrations of aluminum chloride in the
presence and absence of Aβ peptides. Cell proliferation, viability and
β-secretase activity were assessed. Exposure to Al at a concentration
range of 0.001 mM to 1 mM did not show marked effect on SH-SYSY cell
proliferation whereas a significant reduction in cell proliferation and via-
bility was observed in a dose dependent manner after exposure to more
than 1 mM of aluminum. Significant increase in the β-secretase activity
was observed after exposure to Al at a concentration range of 0.01μM to
100μM. Treatment of SH-SYSY cells for three days with Aβ1-40 or Aβ1-42
even at micro molar quantities of aluminum concentrations did not
suggest that exposure to AI may contribute to neurodegeneration and AD pathogenesis. Supported by Title III.

1471 Arsenic Exposure Induces Apoptosis in Hippocampal Neurons and Cognitive Impairment in Rats via Upregulated Bmp-2-Dependent Bdnf/ptrkb Signaling Pathway


Arsenic stimulates apoptosis in the brain cells and induces cognitive
deficits. However, mechanism promoting arsenic-mediated neuronal
apoptosis and cognitive impairment is less investigated. Bone morpho-
genetic proteins (BMP) are expressed in the hippocampus, which controls
cognitive performances, and we hypothesized that a deregulated BMP
signaling might affect the hippocampal neuronal apoptosis and cognitive
functions. We first validated an arsenic-mediated dose-dependent
loss in the hippocampal neurons, through Western blotting (WB) and
Nissl’s staining. Increased Terminal deoxynucleotidyl transferase deUTP
nick end labeling (TUNEL)-reactivity in the NeuN cells and an enhanced
cleaved Poly ADP-ribose polymerase (c-PARP) and c-caspase-3, detected
through Immunofluorescence (IF) and WB, verified As-induced neuronal
apoptosis. Investigating the mechanism through in vivo and in vitro studies revealed that arsenic promoted Bone Morphogenetic protein-2
(BMP2) expression, and a downstream BMP Receptor2 (BMPR2) level
and p-SMAD1/5 signaling in the hippocampal neurons. Interestingly,
a BMP antagonist, noggin, reduced the arsenic-induced TUNEL reac-
tivity and neuronal loss, proving the participation of increased BMP2
signaling suppressed Brain-Derived Neurotrophic Factor (BDNF) expres-
sion levels and BDNF/Tbrk signaling in the arsenic-treated hippocampal
neurons. This decreased BDNF/Tbrk pathway appeared essential for
neuronal apoptosis, as evident from a TrkB inhibitor (K252a)-mediated
abrogation of noggin-induced protection to the hippocampal neurons.
Ultimately, we verified cognitive impairments in the arsenic-treated rats
through Passive avoidance test and Y-Maze test and proved a resto-
rative following noggin treatment. The overall present study confirms
that arsenic induces apoptosis in the hippocampal neurons through a
BMP2/p-SMA1/5-dependent BDNF/Tbrk pathway, affecting normal
cognitive performances.

1472 Protective Effect of Mir-124 Against As-Induced Endoplasmic Reticulum Stress and Implications in Neurodevelopmental Outcomes in Children

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As a pervasive environmental contaminant, Arsenic (As) poses a great
public health concern attributing to a myriad of diseases including adverse neuronal outcomes. Exposure to As during the early stages of
brain development has disastrous lasting effects on the hippocampal
functions. Although epidemiological studies report that prenatal As exposure is associated with impaired neurodevelopment in children, the
molecular mechanisms underlying the susceptibility of early brain develop-
ment to As exposure remain poorly understood. Endoplasmic reticulum
(ER) is the organelle where membrane and secreted proteins are synthe-
sized. Challenges to ER function lead to protein misfolding and a state
of irreversible ER stress, which significantly affects the structure as well as the function of the neuronal cells. Despite the overwhelming evidences
indicating the ototoxic effects of lead, as a known neurotoxicant, we
investigated the mechanism underlying lead-induced auditory dysfunction and its interaction with other ototoxicants such as lead acetate and noise.
Young-adult C57BL/6 mice were exposed to: 1) control conditions; 2) 2 mM lead acetate in drinking
water for 28 days; 3) 90 dB broadband noise 2 hours/day for two weeks; and 4) both lead and noise. Blood lead levels were measured by induc-
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duced cochlear oxidative stress signaling was assessed using targeted
gene arrays, and the hearing thresholds were assessed by recording auditory brainstem responses. Chronic lead exposure downregulated
cochlear Sod1, Gpx1, and Gstk1, which encode critical antioxidant
enzymes, and upregulated ApoE, Hspa1a, Ercc2, Prnp, Ccl5, and Sqstm1,
which are indicative of cellular apoptosis. Isolated exposure to lead or
noise induced 8-12 dB and 11-25 dB shifts in hearing thresholds, respec-
tively. Combined exposure induced 18-25 dB shifts, which was signif-
cantly higher than that observed with isolated exposures. This study suggests
that chronic exposure to lead induces cochlear oxidative stress and
additively potentiates noise-induced hearing impairment, possibly
through parallel pathways.
early brain development. Our genome-wide CRISPR screen using an ER stress reporter cell line identified novel suppressors of As-induced ER stress including microRNA(miR)-124. We showed that miR-124 directly targets IRE1 pathway, one branch of UPR signaling via direct binding to the 3′UTR of ERN1 (gene for IRE1). We further demonstrated that miR-124 overexpression reduces As-induced ER stress/UPR signaling.

In addition, As treatment inhibited cell proliferation in human NSCs or neuroblastoma cells with concomitant activation of ER stress/UPR signaling. Furthermore, miR-124 overexpression reduced As-induced growth inhibition in SH-SY5Y cells. Using data from existing genome-wide association studies of an environmental epidemiological cohort, we found that miR-124 polymorphisms were associated with neurocognitive outcomes, though the mechanisms involved are unknown. In a previous study, we hypothesized that miR-124 is a potential target for preventative and therapeutic interventions against detrimental effects of As exposure in children.

**1473** Heterozygous Huntingtin Promotes Cadmium Neurotoxicity via Caspase Activation, Impaired Metal Transport, and Erk- and PKC-δ-Dependent Oxidative Signaling Mechanisms: Relevance to Pathogenesis of Huntington’s Disease

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Expression of Huntingtin gene (Htt) encoding Huntingtin protein (Htt) leads to striatal neurodegeneration in Huntington’s disease (HD). HD is functionally linked to environmental factors, including metal dysmetabolism and diet use. Interestingly, one of the most abundant heavy metals in cigarettes is cadmium (Cd), which also accumulates in the striatum and causes neurotoxicity upon exposure. Thus, we hypothesized that heterozygous Htt, found in most HD patients, in combination with Cd exposure may exhibit disease-toxicant interactions revealing cellular pathways that mediate neurodegeneration. We report that heterozygous Htt significantly increased Cd cytotoxicity as compared to wild-type cells upon 48 h exposure. The heterozygous Htt x Cd-induced cytotoxicity led to a NADPH oxidase (NOX) mediated oxidative stress that was attenuated by ascorbic acid and apocynin, a NOX inhibitor. Heterozygous Htt in tandem with Cd exposure caused increased expression of protein kinase C (PKC) and other key oxidative stress proteins without altering Akt protein levels. Additionally, heterozygous Htt caused increased caspase-9 and caspase-3 activation and blocked the overexpression of estrogen receptor kinase (Erk) upon Cd exposure. Inductively coupled plasma-mass spectrometry (ICP-MS) analysis revealed an increase in Cd uptake and intracellular accumulation in heterozygous Htt-expressing cells upon Cd exposure and no significant changes in the levels of other essential metals. Moreover, Cd exposure decreased expression of divalent metal transporter 1 (DMT1) protein in the striatal cells. We demonstrate that the enhanced Cd cytotoxicity phenotype in heterozygous Htt can be ameliorated with non-toxic levels of zinc, manganese and iron. Collectively, these results demonstrate that heterozygous Htt exhibits neurotoxic properties upon Cd exposure to cause cell death via caspase activation, impaired metal transport, and regulation of Erk and PKC-δ dependent oxidative signaling mechanisms.

**1474** Metastatic M1 Receptor Partially Modulates Higher Sensitivity to Cadmium-Induced Cell Death in Primary Basal Forebrain Cholinergic Neurons: A Cholinesterase Variants-Dependent Mechanism


Cadmium is a toxic compound reported to produce cognitive dysfunctions, though the mechanisms involved are unknown. In a previous work we described how cadmium blocks cholinergic transmission and induces greater cell death in primary cholinergic neurons from the basal forebrain. It also induces cell death in SN56 cholinergic neurons from the basal forebrain through M1R blockage, alterations in the expression of AChE variants and GSK-3b, and an increase in Ab and total and phosphorylated Tau protein levels. It was observed that the silencing or blockage of M1R altered ChAT activity, GSK-3b, AChE splice variants gene expression, and Ab and Tau protein formation. Furthermore, AChE-S variants were associated with the same actions modulated by M1R.

Accordingly, we hypothesized that cholinergic transmission blockage and higher sensitivity to cadmium-induced cell death of primary basal forebrain cholinergic neurons is mediated by M1R blockage, which triggers this effect through alteration of the expression of AChE variants. To prove this hypothesis, we evaluated, in primary culture from the basal forebrain region, whether M1R silencing induces greater cell death in cadmium does, and whether the native AChE variants are involved in the mechanisms described so as to play a part in the cadmium induction of cholinergic transmission blockage and cell death in this cell line through alteration of the expression of AChE variants. Our results prove that M1R silencing by cadmium partially mediates the greater cell death observed in basal forebrain cholinergic neurons. Moreover, all previously described mechanisms for blocking cholinergic transmission and inducing cell death on SN56 cells after cadmium exposure are partially mediated by M1R through the alteration of AChE expression. Thus, our results may explain cognitive dysfunctions observed in cadmium toxicity.
oxidative stress in fish brain, after exposure to mercuric chloride. The present study suggests the toxicity of mercuric chloride by inducing the oxidative stress and adverse alterations in the brain metabolism and possible interference in brain coordination processes and the protective effect of lotus over the mercury induced changes.

1477 Methylymercury Modulates the Autophagic Response in Mice

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Methylmercury (MeHg) is a global, highly lipophilic environmental pollutant that causes damage to various organs specially on the central nervous system (CNS). It has been known that MeHg induces oxidative stress through an increase in intracellular reactive oxygen species (ROS) and a particular affinity for thiol groups of proteins. In this context, autophagy prevents the accumulation of misfolded or damaged proteins and organelles in the cytoplasm and is regulated by cellular stress responses. Since, the molecular mechanisms underlying MeHg toxicity are not clear, the understanding of autophagy implication in MeHg toxicity could prove to be very useful for the understanding and developing of detoxication strategies. Thus, the aim of this study was to evaluate the autophagic response induced by systemic MeHg exposure in cerebellum and cortex of adult mice. Adult male C57BL mice (3 months old) were exposed to MeHg (0.5 mg/L and 5 mg/L; diluted in drinking water; ad libitum) during 30 days. After 24 hours the animals were killed by decapitation, the brain was removed, and the cerebellum and cerebral cortex were isolated to posterior analysis. Western blot analysis showed a significant decrease in the levels of the autophagic-related proteins p62, ATG4A and ULK-1 in cerebellum and cortex of MeHg-exposed mice compared to control mice (5 mg/L). The levels of other important markers for autophagy pathway, such as, mTOR, LC3-II, Beclin-1 are under analysis. Importantly, this is the first study to evaluate the effects of MeHg in an autophagic response in an animal model. In summary, MeHg seems to modulate/impair the levels of autophagic proteins probably via ROS production.

1478 Recovery Effect of Fasudil, a ROCK Inhibitor, on Axonal Degeneration in Spineoneural System of Methylmercury-Intoxicated Rats


Methylmercury (MeHg) is an environmental neurotoxicant that induces neuropathological changes in the spineoneural system as well as the cranial nerve system, such cerebral cortex and cerebellum. Our previous study demonstrated that MeHg exposure (20 ppm MeHg in drinking water for 4 weeks) induced neuropathological changes in both spinoneural and cranial nerve systems in rats. Furthermore, pretreatment of Fasudil, a ROCK inhibitor, prevented axonal degeneration in the spineoneural system, including dorsal column of spinal cord and dorsal root nerve. However, our understanding in the recovery effect of Fasudil on MeHg-induced axonal degeneration is unknown due to the difficulties in maintenance for a long-term survival of MeHg-intoxicated animals. In the present study, we established a new MeHg-intoxicated rat model. Short-term exposure to MeHg (20 ppm MeHg in drinking water for 3 weeks) induced sufficient axonal degeneration in the spineoneural system, but not in the cranial nerve system. Six weeks after MeHg withdrawal, MeHg-intoxicated animals had still maintained those axonal degenerations and the expression of infiltrated microglia without inducing individual death. Moreover, the expression of ROCK was observed in infiltrated microglia of spinal cord. Post-treatment of Fasudil for 6 weeks restored those axonal degenerations and hind limb crossing sign, a characteristic MeHg-intoxicated behavior. Fasudil also suppressed the expression of inflammatory factors such as TNFα and iNOS, which come from infiltrated microglia. These results suggest that Fasudil, a ROCK inhibitor, recovers MeHg-induced axonal degeneration in the spineoneural system through the suppression of inflammatory factors, and is effective for the cure of MeHg-induced neural dysfunction.

1479 Autophagy and Sequestosome1/p62 Play a Protective Role against Low-Dose Methylmercury-Induced Cytotoxicity


Methylmercury (MeHg) is a widespread environmental pollutant and causes serious health problem all over the world. MeHg inhibits learning and memory and causes a serious hazard to health worldwide. However, molecular mechanisms underlying MeHg toxicity remain elusive. Autophagy plays a major and highly conserved degradative pathway in eukaryotes, prevents the accumulation of misfolded or damaged protein aggregates and damaged organelles in the cytoplasm. Here we focus on the role of autophagy against low- dose MeHg exposure. We show that MeHg induced mouse embryonic fibroblasts (MEFs) viability in a dose-dependent manner. Furthermore, MeHg treatment increased levels of autophagy marker LC3-II and SQSTM1/p62. MeHg exposure elevated the number of LC3 puncta in stable GFP-LC3 MEFs and the number of autophagic vacuoles. The accumulation of LC3-II and p62 was much increased by co-treatment of MeHg with autophagy inhibitors. This additive effect of MeHg on LC3-II accumulation in the presence of inhibitors suggests that MeHg stress activates autophagy and promotes autophagosome/LC3-II accumulation. To establish the impact of autophagy in alleviating MeHg toxicity, we assessed MeHg sensitivity in autophagy-related gene 5 deficient (Atg5KO) MEFs. Atg5KO significantly decreased viability of the cell following 24 h of MeHg treatment. The enhanced MeHg-induced cytotoxicity in Atg5KO cells suggests that autophagy protects cells from MeHg-induced cell death. This finding could reveal that autophagy is necessary for protective mechanisms in eliminating intracellular MeHg-induced damaged materials. Moreover, we examined MeHg sensitivity in p62-deficient (p62KO) MEFs. p62KO MEFs exhibited higher sensitivity to MeHg exposure compared to their wild-type (WT) counterparts. Interestingly, an earlier and higher level of accumulation of ubiquitinated proteins was observed in MeHg-treated p62KO MEFs when compared with WT MEFs. Taken together these results suggest that, in addition to autophagy, p62 is crucial for cytoprotection against MeHg and required for MeHg-induced ubiquitinated proteins clearance.

1480 Acute Methylmercury Exposure Alters Immunofluorescence of the Renshaw Cell Area in C57BL/6J Mice

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Methylmercury (MeHg) affects a number of membrane targets and synaptic processes, including excitatory and inhibitory neurotransmission; the latter is affected earlier than the former. Disrupting the ratio of inhibition/excitation can cause excitotoxicity, an effect we reported for acute exposure to MeHg in cerebellum and chronic exposure in a mouse model of amyotrophic lateral sclerosis (ALS). For ALS, a region of particular interest for MeHg neurotoxicity is the spinal cord, especially motor neurons (MN). In this study, we targeted the spinal and their recurrent inhibitory partners, the Renshaw Cells (RCs) of C57BL/6J adult mice. MeHg-induced toxicity was compared using immunohistochemistry after acute exposure (1 h) of lumbar spinal slices to MeHg (5, 10, 20 or 50 μM), or untreated as control. The RC and MN areas were identified by the presence of gephyrin clusters, calbindin d28k, d28k 2B2K immunoreactivity and the presence of cholinergic contacts. Primary antibodies against gephyrin, which anchors inhibitory transmitter receptors including glycine and GABAa to the cytoskeleton of the postsynaptic membrane, calbindin d28k a Ca2+-binding protein expressed in Renshaw and choline acetyl transferase (ChAT), the biosynthetic enzyme for ACh, were found in MNs, respectively. In summary, for all three antibodies underwent a similar MeHg concentration-based pattern of activity. At 20 μM MeHg, immunofluorescence for all markers was markedly increased from control (calbindin 0.11, ChAT 0.41, gephrin 0.18; relative increase) but at 50 μM MeHg immunofluorescence decreased. Pretreatment with strychnine or mecamylamine (MEC), glycine and nicotinic receptor antagonists alone, respectively, decreased immunofluorescence (calbindin 0.17, 0.20 and gephrin 0.29 0.41: relative reduction, respectively), suggesting a block of excitatory nicotinic cholinergic or inhibitory glycnergic transmission. The combination of 20 μM MeHg with strychnine or MEC, markedly decreased immunofluorescence for all markers from the MeHg alone group (calbindin 0.11, ChAT 0.41, gephrin 0.18; relative change). Results (N=6-8) suggest that these proteins are affected during MeHg toxicity through inhibitory and excitatory pathways. Understanding how MeHg-mediated neurotoxicity affects spinal MN pathways, especially inhibitory pathways, is important in assessing its role in MN toxicity in a mouse model of ALS.
Studies have demonstrated that methylmercury (MeHg) produces motor neuron cell death at the lumbar spinal cord region. In many cell types, a key MeHg neurotoxicity marker is dysregulation of intracellular calcium (Ca^{2+}) homeostasis. Spinal cord alpha motor neurons (aMNs) send excitatory signaling onto Renshaw interneurons, which in turn send inhibitory neurotransmitter back to the same aMNs, ultimately modulating their signaling. The effects of Ca^{2+} concentration ([Ca^{2+}]_{i}) during MeHg exposure at the lumbar ventral spinal recurrent inhibition have never been studied. The aim of this project is to determine the effects of acute MeHg-mediated ([Ca^{2+}]_{i}) during the excitatory and inhibitory signaling of recurrent inhibition between aMNs and Renshaw cells. Lumbar sections of adult C57BL6J mice were exposed to 20 μM MeHg during 15 min through a real-time perfusion system. Ca^{2+} changes were recorded using Fluo4-AM at 15 min of MeHg exposure. The role of the acetylcholine (ACh), glycine and GABA receptors, all cytoine-containing ligand gated channels, mediators of recurrent inhibition, was determined using a pharmacological approach. ACh receptor antagonists: mecamylamine (MEC), dihydro-ß-erythroidine hydrobromide (DHIE) and glycine and GABA receptors antagonists: strychnine and bicuculline (BCC), were used as a pretreatment and during MeHg treatment. It is hypothesized that MeHg neurotoxicity produces a differential disruption of Ca^{2+} signaling in these cell types that could potentially lead to hyperexcitability of the Renshaw area. Results show (N = 11) that MeHg treatment alone significantly increases ([Ca^{2+}]_{i}) at 15 min and 1 hr (0.27 and 0.21 relative fluorescence, respectively). Presence of MEC, DHIE, strychnine and BCC during MeHg treatment significantly decreases ([Ca^{2+}]_{i}) at (0.21, 0.18, 0.19 and 0.14, relative fluorescence, respectively) from baseline. Determining the role of MeHg-induced disruption in Ca^{2+} homeostasis through these receptors could potentially elucidate the possible mechanisms of MeHg-mediated ([Ca^{2+}]_{i}) neurotoxicity during recurrent inhibition. Supported by NIH grant R01ES024064.

**Methylmercury Causes Intracellular Calcium Dysregulation in Spinal Cord Slices from SOD1^{G93A} Mice That Is Reduced by Riluzole Treatment**

B. A. Brauer, J. M. Bailey, and W. D. Atchison, Michigan State University, East Lansing, MI.

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease that affects upper and lower motor neurons (MNs). Mice that overexpress the humanized superoxide dismutase-1 gene mutation (SOD1^{G93A}) are used to model ALS; they reliably exhibit an ALS-like phenotype as they age. Previously we showed that methylmercury (MeHg), an environmental neurotoxicant, hastens the onset of the ALS-like phenotype in these mice. Overexpression of Ca^{2+} signaling in aMN neurons is a possible mechanism by which riluzole treatment produces its beneficial effects in the clinical population. These data also demonstrate a possible mechanism by which riluzole treatment produces its beneficial effects in the clinical population.

1481 Effects of Acute Methylmercury-Induced Calcium Changes in the Ventral Lumbar Spinal Cord Region of the C57BL6J Mouse

M. Rios-Cabanillas, and W. Atchison, Michigan State University, East Lansing, MI.

Astrocytes provide neuronal protection from excitotoxicity by eliminating glutamate from the synapses through the excitatory amino acid transporter 1 and 2 (EAAT1 and EAAT2). Mercury and reactive oxygen species reportedly inhibit EAAT1 and EAAT2 re-uptake of extracellular glutamate. In the spinal cord, where motor neuron degeneration occurs, the role of spinal cord astrocytes (SCA) in MeHg toxicity has not been fully characterized. We hypothesized that MeHg could induce down-regulation of EAAT1 and EAAT2 mRNA expression in mouse SCAs. Mouse SCA cultures were exposed to 0.5μM MeHg for 30 min, or 1-12h. RNA samples from SCA were collected at 30 min. Increase EAAT3 mRNA levels were increased at 3h of MeHg exposure ~ 2.8- and 2.2-fold relative to control respectively. Both subsequently declined back to control levels. The increase and later decrease of EAAT1 and EAAT2 mRNA, thus SCAs may initially attempt to buffer the glutamate-mediated excitotoxicity; the subsequent reduction of these mRNAs expression could exacerbate MeHg-induced excitotoxicity. The potential effect of MeHg on EAAT3 mRNA expression was examined in the motor neuron cell line (NSC-34). The predominant role of EAAT3 in neurons is not for glutamate uptake, but for intracellular glutathione synthesis, to protect neurons from oxidative stress. Expression levels of EAAT3 mRNA following 2 μM MeHg exposure was determined from 30 min, 1h and from 3-24h at 3h intervals. EAAT3 mRNA levels were 2.4-fold higher than control mRNA at 30 min. Increase EAAT3 mRNA levels gradually declined after 1h of MeHg exposure; they were significantly lower than NSC34 cells controls with more than a 2-fold decrease at 24h. Thus, MeHg affects EAAT1-2 mRNA expression in SCAs but also affects glutathione-precursor transporter mRNA expression in NSC-34 motor neuron-like cells. Thus, degeneration of spinal cord cells by MeHg exposure could be partially due to alteration of synaptic glutamate clearance-transporter expression in astrocytes and loss of antioxidant precursor transporter expression in neurons. This research was supported by NIH grant R01ES024064, and R25NS090988; ENDURE Program.

1483 Expression of Excitatory Amino Acid Transporter-1 and Glutathione Synthetase in Primary Spinal Cord Astrocytes during Methylmercury Exposure

K. A. Rivera-Caraballo, D. Wiwatratana, and W. D. Atchison, Michigan State University, East Lansing, MI.

Astrocytes provide neuronal protection from excitotoxicity by eliminating glutamate from the synapses through the excitatory amino acid transporter 1 and 2 (EAAT1 and EAAT2). Mercury and reactive oxygen species reportedly inhibit EAAT1 and EAAT2 re-uptake of extracellular glutamate. In the spinal cord, where motor neuron degeneration occurs, the role of spinal cord astrocytes (SCA) in MeHg toxicity has not been fully characterized. We hypothesized that MeHg could induce down-regulation of EAAT1 and EAAT2 mRNA expression in mouse SCAs. Mouse SCA cultures were exposed to 0.5μM MeHg for 30 min, or 1-12h. RNA samples from SCA were collected at 30 min. Increase EAAT3 mRNA levels were increased at 3h of MeHg exposure ~ 2.8- and 2.2-fold relative to control respectively. Both subsequently declined back to control levels. The increase and later decrease of EAAT1 and EAAT2 mRNA, thus SCAs may initially attempt to buffer the glutamate-mediated excitotoxicity; the subsequent reduction of these mRNAs expression could exacerbate MeHg-induced excitotoxicity. The potential effect of MeHg on EAAT3 mRNA expression was examined in the motor neuron cell line (NSC-34). The predominant role of EAAT3 in neurons is not for glutamate uptake, but for intracellular glutathione synthesis, to protect neurons from oxidative stress. Expression levels of EAAT3 mRNA following 2 μM MeHg exposure was determined from 30 min, 1h and from 3-24h at 3h intervals. EAAT3 mRNA levels were 2.4-fold higher than control mRNA at 30 min. Increase EAAT3 mRNA levels gradually declined after 1h of MeHg exposure; they were significantly lower than NSC34 cells controls with more than a 2-fold decrease at 24h. Thus, MeHg affects EAAT1-2 mRNA expression in SCAs but also affects glutathione-precursor transporter mRNA expression in NSC-34 motor neuron-like cells. Thus, degeneration of spinal cord cells by MeHg exposure could be partially due to alteration of synaptic glutamate clearance-transporter expression in astrocytes and loss of antioxidant precursor transporter expression in neurons. This research was supported by NIH grant R01ES024064, and R25NS090988; ENDURE Program.

1484 The Expression of Glutamate Transporter-1 and Glutamine Synthetase in Primary Spinal Cord Astrocytes during Methylmercury Exposure

K. A. Rivera-Caraballo, D. Wiwatratana, and W. D. Atchison, Michigan State University, East Lansing, MI.

Expression of excitatory amino acid transporter (GLT-1) and glutaminase synthetase (GS) plays an important role in cytoplasmic glutamate and the glutamine cycle between astrocytes and neurons. The expression of GLUT1-2 mRNA, thus SCAs contribute both to neuronal survival and degeneration. For MeHg, astrocytes have been demonstrated to be a target of toxicity, with increased oxidative stress and impaired glutamate uptake, yet astrocytes also protect neurons from MeHg-induced generation of oxidative damage through production of glutathione. Astrocytes protect over-extracellular glutamate through re-uptake of the glutamate by way of excitatory amino acid transporter (EAAT). The EAAT type 2 or glutamate transporter 1 (GLT-1) plays a pivotal role in maintaining non-toxic levels of extracellular glutamate while glutamine synthetase (GS) plays an important role in cytoplasmic glutamate and the glutamine cycle between astrocytes and neurons. GS in astrocytes converts glutamate into glutamine, then recycles back to the synapses as a neuronal glutamate precursor. In this study, the MeHg toxicity to primary spinal cord astrocytes (SCA) were examined as a function of time and concentration of MeHg exposure. The degree of MeHg toxicity to SCA was greater in the highest range of MeHg concentration in our study, using 0.1, 0.5, 1, 2 and 5 μM MeHg. The concentration response curve indicated a decrease in IC50 with longer times of exposure to MeHg. The time function of MeHg toxicity indicated a left shift of the concentration response curve in which IC50 decreased for longer MeHg exposure and increased hillslope value. The high limit threshold is 12 h of MeHg exposure with a concentration of 0.3 μM MeHg would cause 50% of SCA to degenerate. The degree of susceptibility of SCA to MeHg also depends upon SCA structure and size. The large flat astrocytes were more resistant, whereas small process bearing astrocytes are more susceptible to MeHg. The mechanism involved in MeHg induced SCA degeneration may be alteration of oxidative stress and impaired glutamate uptake, yet astrocytes also protect neurons from MeHg-induced generation of oxidative damage through production of glutathione.
exposure, there was an increase of population of SCA expressing GLT-1 greater than the SCA control. The level of G5 appeared to decrease for longer exposures to MeHg and this alteration appears to be associated with the GLT-1 expression level. This research is supported by NIH grant R01 ES024064.

**1485** Methylmercury In Vivo Preferentially Potentiates AMPA-Mediated Currents in Brainstem-Hypoglossal-Motorneurons from Mice Expressing the Human Superoxide Dismutase 1 (hSOD1) G93a Gene Mutation

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Gene-environment interactions are believed to contribute to development of Amyotrophic Lateral Sclerosis (ALS), a progressive and fatal disease of motor neurons (MNs). We previously showed that chronic exposure of mice overexpressing the human superoxide dismutase 1 (hSOD1) gene mutation (hSOD1G93a) A mouse model for ALS, to methylmercury (MeHg), accelerated the onset of ALS-like phenotype. In vitro, we also showed that MeHg altered AMPA receptor expression when present with our previous in vitro results: [Ca2+]i at 15 min and 1 hr (0.52, 0.66 relative fluorescence, respectively) in MNs from G93A mice. Conversely, neither AMPA- nor GABA-A receptor-evoked currents were examined in whole cell recording techniques. Untreated littermates were sacrificed at the same time points to serve as controls. AMPA and GABA-evoked currents were examined at two holding potentials. At -50 mV, AMPA and GABA evoked inward and outward currents respectively. However, at a holding potential of -80 mV, which approximates the reversal potential for GABA under our conditions, only AMPA-meditated currents were evoked. MeHg exposure significantly increased amplitude of AMPA-meditated currents, simultaneously reducing GABA-mediated currents in hypoglossal MNs from G93A mice. Conversely, neither AMPA- nor GABA-evoked currents in MNs of SOD1 WT or WT were affected. Thus, consistent with our previous in vitro studies, gene-MeHg interactions appear to contribute to MN excitotoxicity, thereby facilitating development of ALS-like phenotype. Supported by NIH/NIH grant ES024064 and NIH/NIH T32 ES0075250.

**1486** Role of Inhibitory and Excitatory Receptors in Mediating Calcium Changes during Acute Methylmercury Exposure on the C57BL6j Mouse

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Lumbar spinal cord excitatory motor neurons (MNs) and inhibitory Renshaw cells (RCs) interact with each other in a negative feedback mechanism called recurrent inhibition. Interruption of this mechanism by MeHg can result in epileptiform activity. Neurons use glutamate (excitatory) and GABA (inhibitory) neurotransmitters to communicate with each other in either excitatory or inhibitory processes. The effects of MeHg on the Renshaw cells of rats have been studied extensively. However, MeHg alters recurrent inhibition in the absence or presence of strychnine, bicuculline, mecamylamine (MEC) or DhβE. Results show (N=11) that MeHg treatment alone significantly increases [Ca2+]i at 15 min and 1 hr (0.52, 0.66 relative fluorescence, respectively) from baseline. MEC, an excitatory nAChR antagonist, pretreatment fol-

**1486a** Short-Term Effects of Gulf War Illness-Related Chemical Exposure on Brain Monoamines: Modulation by the Neoglycoconjugate LNFPIII

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Of the 700,000 veterans from the 1990-1991 Gulf War (GW), 25-30% suffer from Gulf War Illness (GWI), a complex disease that includes neurological symptoms, such as disruption in movement, memory and mood that might originate from GW-related chemical exposures. Neuroinflammation is a prominent GWI feature. A neoglycoconjugate of Lacto-N-fucopentaose III (LNFPIII), a human breast milk component, has potent immunomodulatory properties. Using a mouse (male C57BL/6) model of GWI (Pyridostigmine bromide + Permethrin) and LNFPIII treatment (both for 10 days), this study sought to determine the short-term effects of the GWI treatment on brain monoamines and the potential protection LNFPIII affords. Six or 48 hr post treatment, brain monoamines were analyzed using high-performance liquid chromatography with electrochemical detection (HPLC-EC) in the prefrontal cortex (PFC), nucleus accumbens (NA), striatum (STR), amygdala (AMY), dorsal hippocampus (dHipp), ventral hippocampus (vHipp), and cerebellum (CER). The GWI chemical treatment resulted in fairly wide spread perturbation of serotonin (5HT) homeostasis that was most notable by 48 hrs and it manifested itself with increased 5-HIAA (a 5-HT metabolite) and trends for decreased 5-HT. LNFPIII treatment maintained 5-HT levels in the NA, STR, and CER, thus, counteracting the GWI chemicals effects. In GWI treated groups, dopamine (DA; 6 h) and its metabolite HVA (6 and 48 h) increased in the PFC and vHipp, with this effect not seen in the mice that were also treated with LNFPIII. Overall, the data indicate that, in the short term, this GWI treatment causes a more widespread serotonergic perturbation and a more focused dopaminergic dysfunction in brain areas that include the ones associated with locomotion, cognition, and mood. At multiple instances, chemical effects were not seen if animals were given LNFPIII, suggesting that this is a treatment option worth further pursuing. Supported by a grant number W81XWH-16-1-0586 (DoD).

**1486b** Comparative Effects of Methylmercury on Nrf2-Keap1 mRNA Expression in Primary Spinal Cord Astrocyte Cultures

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Loss of redox homeostasis is involved in methylmercury (MeHg)-induced cell death. In the central nervous system, astrocytes are the first cells exposed to MeHg and accumulate mercury more than neurons. Neurons, however, are more susceptible to MeHg than astrocytes. Astrocytes play an important role in neuronal protection by supplying antioxidant glutathione (GSH) to neurons, especially during oxidative stress. A master regulator of antioxidants in cells, called nuclear factor erythroid 2-related factor 2 (Nrf2), controls the expression of several antioxidant genes including GSH precursors. Nrf2 is negatively regulated by Kelch-like ECH-associated protein 1 (Keap1) by undergoing ubiquitin proteosome degradation during normal physiology. Once oxidative stress occurs, these cytoprotective proteins are dissociated. The disso-

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1487 Application of Multiple Methods for GFP Detection in Histological Sections in Animal Toxicity Studies

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Green Fluorescent protein (GFP) is widely used as a gene expression reporter and a molecular and cellular tag in biomedical research. Detection of GFP in histological sections is critically important for the visualization of tagged proteins and transfected cells in animal toxicity and efficacy studies. Analysis of GFP reporter gene expression in tissue sections is currently a leading method for evaluation of vector system effectiveness in gene therapy in target tissues such as the eye. Multiple sensitive methods for GFP detection within formalin-fixed paraffin-embedded (FFPE) tissue utilizing direct fluorescent (DF), immunofluorescent (IF), immunohistochemical (IHC) and in situ hybridization (ISH) techniques were established. FFPE sections of known positive (GFP transfected) and negative (naïve) tissue and cell lines were used as controls and to evaluate the specificity of the methods. While IHC and IF staining with commercially available antibodies are the primary methods used for revealing the presence of GFP in cells and tissues, DF detection of GFP in unstained FFPE sections allowed preliminary screening of study slides to find samples with visible signal. Our data showed a direct correlation of signal intensity between DF and IHC/IF staining, although some reports suggest that GFP may lose fluorescence during tissue fixation and processing. The relative simplicity and reliability of the DF visualization significantly decreased the time required for method validation/optimization and enabled rapid identification of samples with low positive signal. Establishing IHC/IF with different species of anti-GFP antibodies (e.g. mouse monoclonal and rabbit polyclonal) permitted the use of varied detection techniques on tissue from a wide variety of animal models used in toxicity studies (mouse, rat, rabbit, primate etc.) in single and multiplex (double) staining protocols. Chromogenic ISH utilizing an RNAscope® protocol designed to detect GFP (enhanced GFP) mRNA expression patterns in FFPE tissue is invaluable in cases in which GFP signal is below the limit of detection for IHC/IF.

1488 Oral Cyclosporine A Immunosuppressive Therapy Leads to Systemic Toxicity in the Royal College of Surgeons Rats


The Royal College of Surgeons (RCS) rats are a widely used model of reces-

18 hrs, it had been reduced to ~ 25% of control levels. Despite reduc-

tion of Keap1 expression, and loss of negative control of Nrf2, there was no concomitant increase in Nrf2 expression in spinal cord astrocytes in response to MeHg. This research is supported by NIH grant R01 ES024064, and R25 Summer Undergraduate Research Program R25 ESI5025060.

1489 The Dual-Effect of Melatonin on Type I Diabetic Retinopathy

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Diabetes is a fast-growing global problem, and diabetic retinopathy (DR) is the leading cause of blindness among Americans over 40 years old. Nearly all patients with type I diabetes and more than 60% of those with type II diabetes will develop DR. Diabetic retinopathy is a dual disorder with microvascular complications and retinal degeneration. Oxidative stress is one of the major culprits in development of DR vascular lesions and photoreceptors death. Scavenging reactive oxygen species may be a potential strategy to delay the DR progression. Here we use melatonin as a potential treatment based on its antioxidative properties for DR complication and screen its effect on microvascular and retinal neuron separately. STZ-induced type I diabetic mouse model was used in this study. STZ mice were separated into two groups, and one of them were given daily melatonin injections while the other group were monitored regularly. Retinal light responses by electoretinography (ERG) measurement and fluorescein angiography (FA) were used to assess changes in retinal neurons and retinal vasculature. Ocular tissues were harvested and analyzed for molecular changes by immunofluorescent staining. There is no significant difference in the body weight among the control, control treated with melatonin, STZ, and STZ treated with melatonin mice, but melatonin treatment seems to increase systemic glucose levels. Melatonin has no significant effect on retinal light responses on control mice. But the STZ mice given daily melatonin had compromised retinal light responses, which was worsened over time compared to the STZ mice without melatonin. However, melatonin treatments reversed STZ-induced changes in mitochondrial dynamics and neovascularization in the retina. Melatonin treatment for three months worsens the retinal light responses, but it might improve diabetes-induced changes in mitochondrial dynamics and retinal neovascularization.

1490 Evaluation of Streptozotocin-Induced Lens Opacities by Using Retro-Illumination Camera in Rats


In the ophthalmologic examination of non-clinical toxicity study, slit lamp is usually used to evaluate the optic media including lens. To monitor and evaluate drug-induced cataract, objective and reproducible method for documenting the cataract formation is important. Photograph taken by slit lamp has the limitation of producing a single section through the lens. Even if the photos of multiple sections are taken by slit lamp, it still gives an incomplete statement of the total amount of lens opacity. Retro-illumination photography can be used in visualization of lens opacity and the use of the retro-illumination camera in documenting cataract is well established in human clinical setting, but not in experimental animals. In this study, the development of cataract in streptozotocin-induced diabetic rats (n = 15) was documented with a retro-illumination camera (Lovex Co., Ltd.) once a week after intravenous injection of streptozotocin (50 mg/kg) during a 13-week period and the applicability of this camera system in rats was evaluated. As a result, lens opacity was noted in all the streptozotocin-induced diabetic rats between 1 and 4 weeks after the injection of streptozotocin. As the early morphological changes, small vacuoles or short radial opacities appeared at the lens equator, or featherly or net-shaped opacities, which are difficult to take photographs by slit lamp, were observed under the retro-illumination camera clearly documented by the retro-illumination camera. Several courses of cataract development were successfully and easily documented and it was suggested that the retro-illumination camera is a useful tool to visualize cataract formation.

1491 Cadmium-Induced Changes in Gene Expression in Human Lens Epithelial Cells

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Vision is a crucial aspect of life for humans and animals, and cataracts are a leading cause of impaired vision and blindness. Although cataracts may form as a process of aging, several environmental and life-style factors increase the risk of this disease, such as cigarette smoking, oxidative stress and steroid treatments. The toxic metal cadmium (Cd), a major contributor to cigarette-related cataract formation, and other related risk factors are known to activate the metal regulatory transcription factor 1 (MRTF-1). Previous research [O’Sheilds et al., Biochim Biophys Acta 2017] showed that the early expression of MTF-1 in human lens epithelial cells (HLE) was induced by the toxic metal cadmium. Here, we used a microarray approach to investigate the gene expression profile of HLE cells exposed to CdCl2. Using this approach, we identified a number of genes that were differentially expressed following CdCl2 exposure. These genes were primarily involved in the processes of apoptosis, cell cycle regulation, and cellular response to stress. The results of this study suggest that cadmium exposure may induce changes in the expression of genes involved in the development and progression of cataracts.
An Approach for Assessing Mild Irritants with the Bovine Corneal Opacity and Permeability Test Method

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The bovine corneal opacity and permeability (BCOP) test method (OECD TG 437) is an in vitro test method that uses isolated bovine cornea from cattle slaughtered for commercial purposes to identify test chemicals that are possibly and severe irritants (UN GHS Category 1). At present, neither the BCOP test method nor any other alternative eye irritation test method is capable of identifying test chemicals as UN GHS Categories 2A or 2B, and efforts to develop such a test are continuing. As part of this effort, we investigated whether or not test chemicals could be identified as GHS Categories 2A or 2B using a modified version of the BCOP test method that includes a histopathological examination. We tested 85 substances to determine an in vitro irritancy score (IVS) and histopathological findings for corneal changes in the corneal epithelium and stroma. IVS values were calculated based on the corneal opacity and permeability values following exposure to a test chemical per OECD TG 437. The corneas were then fixed in 10% buffered formalin solution, stained with hematoxylin and eosin, and subjected to a histopathological examination. Fifteen of the 85 test chemicals are known to be UN GHS Category 2B, and for thirty-one of these, high IVIS values were calculated based on the corneal opacity and permeability values following exposure to a test chemical per OECD TG 437. The corneas were then fixed in 10% buffered formalin solution, stained with hematoxylin and eosin, and subjected to a histopathological examination. Fifteen of the 85 test chemicals are known to be UN GHS Category 2B, and for thirty-one of these, high IVIS values were calculated based on the corneal opacity and permeability values following exposure to a test chemical per OECD TG 437.

Laser-Induced Choroidal Neovascularization as a Model of Age-Related Macular Degeneration in the Minipig


Choroidal Neovascularization (CNV) is a known cause of Age-Related Macular Degeneration (AMD). Lucentis’ (ranibuzumab) is a marketed treatment for AMD, and is hypothesized to function by binding to vascular endothelial growth factors (VEGF) and preventing aberrant vascular growth. Ocular similarities between humans and miniature swine make it a useful large animal model ocular therapies, and this study aimed to validate the miniature swine model of laser induced CNV. In this study, Choroidal Neovascularization was generated in Sinclair minipig using retinal photoocoagulation. After laser induction of CNV, animals received a single 25 µL intravitreal injection of either Lucentis' (n=4), or vehicle (n=4). Eyes examinations and fluorescein angiography were performed to assess hemorrhaging and formation of CNV lesions. CNV lesions developed as expected, as hypofluorescent spots and hemorrhages caused by laser tissue damage were observed during weeks 1 and 2. Grade 1 through 3 CNV lesions were present in weeks 1 and 2. Ocular opacities prevented fluorescein imaging in 2/4 vehicle treated animals compared to 1/4 Lucentis’ treated animals. By week 6 all hypofluorescent spots and hemorrhages had resolved and clinically significant Grade 4 CNV lesions were present, defined by bright hyperfluorescence at the mid-time point and late leakage beyond the treated area during fluorescein angiography. Grade 4 lesions were present at a lower prevalence (25% of observed lesions) in Lucentis’ treated animals compared to vehicle treated animals (33% of observed lesions). Based on vitreous opacities, and anterior segment inflammation, vehicle treated animals developed more severe ocular changes than Lucentis’ treated animals. Lucentis’ treatment demonstrated an anti-angiogenic effect, validating the Sinclair minipig as a model of wet AMD.

Validating a Scoring Methodology for Drug-Induced Anterior Segment Inflammation in Non-Human Primate Eyes

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The eye of the African green monkey (AGM; Chlorocebus sabaeus) shares significant anatomic and physiologic similarity to the human eye. As such, this Old World nonhuman primate species has been increasingly employed as a preclinical ophthalmic test system for safety and efficacy studies. In this study, an ophthalmic inflammation scoring system, adapted from reported clinical and preclinical scales, was validated in eyes receiving intracameral concanavalin A (con A), a mitogen that induces anterior uveitis when introduced into the aqueous chamber. Six adult male and 6 adult female AGMs were evaluated. Following baseline slit lamp exam, laser flare photometry, tonometry, and pachymetry, monkeys received intracameral injection of con A (20 µl of 2.5 µg/µl; a dose titrated to induce mild reversible inflammation). Follow up ocular exams were performed 1, 2, 4, 6 and 8 days later. Anterior chamber flare and cell was scored and clinical pathology recorded by direct external inspection and slit lamp biomicroscopy. Exams consisted of evaluation of the eyelids, palpebral and bulbar conjunctiva, cornea, anterior chamber cell and flare, cell deposits on the corneal endothelium, lens and vitreous, IOP, pupillary diameter, intraocular pressure, and 90 diopter lens with scoring of inflammatory changes within discrete anatomical regions. The scoring regimen was established to cover the full spectrum of potential ocular inflammatory changes, though the con A dose employed to model moderate anterior uveitis the lower end of each scale was primarily applied. Additionally, an evaluation of visual acuity was also conducted prior to sedation for clinical exams to confirm retention of visual function throughout the study and laser flare photometry was also performed. Topical dexamethasone (Dex) was used as a positive control. Intracameral con A induced ocular changes consistent with the clinical spectrum of pathology observed in anterior uveitis and potential test article-associated inflammatory changes, including conjunctival swelling and discharge, corneal clouding, keratic precipitates, anterior chamber cell and flare, cell deposits on the corneal endothelium and lens capsule, fibrin strands, iris hyperemia and edema. Laser flare and clinical ocular evaluations demonstrated a robust and significant anti-inflammatory effect of Dex when compared to vehicle-treated eyes, which validated the applied scoring methodology for the evaluation of anterior chamber inflammation in ocular safety studies.

The Porcine Corneal Opacity and Reversibility Assay (PorCORA) and Assessment of the Drivers of Classification with Regard to Ocular Damage


The Draize rabbit eye test assesses damage to a number of different eye structures, which are scored and weighted based on their anatomic and physiologic importance. The structures are: Cornea (CO), Conjunctiva (Conj), and Iris (IR). Corneal irritation is assessed by opacities on the cornea, Conj irritation is assessed by increased vascularization, and IR damage by function of the iris (ability to constrict or dilate pupil) and deepening of the pupil. The heaviest weighting is on corneal damage, which is 20 points, and finally IR is only 10 points. Since the CO scores have the heaviest weight, and most often are the drivers of eye irritation, we developed an ex vivo corneal model to assess these effects. The PorCORA is an ex vivo corneal assay, which can distinguish between a material's potential to cause severe (reversible) versus
corrosive (irreversible) damage. We tested a total of 56 chemicals and dilutions of chemicals ranging from corrosive (GS category 1) to non-irritating (GS not categorized). Using Cooper Statistics, we arrived at an accuracy of 89% with a positive and negative predictivity of 85% (cat. 1) and 93% (not cat. 1), respectively. To determine if these Cooper Statistics could be improved, we used the drivers of classification concept based on Barros et al., 2016. Upon re-examination of our data based on this published database and methodology, we found that four chemicals (1,2,4-Triazole, N-Butanol, 2,5-Dimethyl-2,5-Hexanediol, and Potassium Cyanate) had invalid tests (animals euthanized prior to day 21) or produced irritation not driven by corneal opacities. These chemicals were removed from our dataset. Without these four chemicals the accuracy improved to 92%. Moreover, the major change was in our positive predictivity, which increased to 91%. The negative predictivity for this subset of chemicals remained the same. Lastly, PorCORA’s predictivity was assessed based on CO persistency; i.e., materials that produced low CO scores (from Draize Rabbit Eye Tests). Of the 56 chemicals tested, 40 had low severity (i.e., CO mean scores < 3). The accuracy for persistence of low CO scores was 88% with positive and negative predictivity of 79%, and 92%, respectively. Based on these data, the PorCORA was proven to be a valid test to assess a material’s potential to distinguish reversible versus irreversible eye damage.

**1496 Sex- and Age-Related Differences in Cadmium-Induced Global Transcriptomic Profiles in Adult Zebrafish Eye Tissues**

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Although cataracts and macular degeneration are disease pathologies related to aging, environmental factors, such as heavy metal exposure, may also increase the risk of disease. Cigarette smoking contributes to the development of these pathologies, with the toxic metal cadmium (Cd) considered to be a major contributor to cigarette-related eye pathologies. Using the zebrafish model, we exposed 6- and 10-month adults to 50 μM Cd for four hours prior to assessing changes in global gene expression by RNA sequencing (RNA-seq) in whole eye tissue. To determine sex-specific changes in gene expression, transcriptomic profiles between 10-month male and female zebrafish were compared. A total of 431 genes (174 up-regulated and 257 down-regulated) were differentially expressed in 10-month males, while 461 genes were differentially expressed in 10-month females (105 up-regulated and 362 down-regulated) in response to Cd treatment. For further characterization of differences in the response to Cd exposure, age-dependent comparisons were performed between 6- and 10-month males. A total of 311 genes (183 up-regulated and 128 down-regulated) were differentially expressed in 6-month males, while 375 genes were differentially expressed in 10-month males (195 up-regulated and 180 down-regulated) in response to Cd treatment. Only 38% of differentially expressed genes in 10-month old zebrafish were shared between males and females, while 32% of differentially expressed genes in male zebrafish were shared between 6- and 10-month old fish. Based on gene ontology (GO) enrichment analysis, the 10-month male Cd exposure (up-regulated genes) was the only treatment with any eye-specific enriched terms (eye development, eye morphogenesis and camera-type eye morphogenesis), while terms related to cellular defense and inflammation were enriched in most treatments. Overall the 6- and 10-month male zebrafish only shared 24% of the enriched GO terms, while 10-month males and females only shared 14% of the enriched terms. These results highlight significant differences in sex and age-dependent differential gene expression in response to Cd exposure, providing greater insight into mechanisms of metal-induced stress which may contribute to the development of eye pathologies (1821ES0263910).

**1497 Cosmetics Europe Analysis: Towards Development of an Ocular Toxicity-Defined Approach Using In Vitro Test Methods and Physicochemical Properties as Components within an IATA**

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In 2017, OECD published a guidance document on an Integrated Approach on Testing and Assessment (IATA) for serious eye damage and eye irritation. This provides guidance on how to integrate existing and / or newly generated information for decision making, including those on further testing or on classification & labelling for potential eye hazard of chemicals. In this context, physico-chemical properties may be used to identify chemicals not likely to cause serious eye damage / eye irritation and to help orient towards a Top-Down or Bottom-Up approach. Overall aim of Cosmetics Europe (CE) eye program is to gain better recognition by regulators / scientific organizations of safety assessments using strategies based on alternative approaches. A core focus area is data integration / evaluation of testing strategies (TS) / approaches. CE has created a comprehensive database of chemicals for which in vitro data are available with corresponding historical in vivo data. Physico-chemical properties are also being integrated. We used ~130 chemicals from this database and built on initial TS developed from the CEFIC CON4EI project, to evaluate robustness of such TS and identify areas for refinement. A key outcome is that combining in vitro test in combination would improve correct identification of Cat. 1 and Cat. 2 but not the specificity beyond that of a single test method. Preliminary analysis of physico-chemical properties using PCA identifies that the specificity of a TS is improved by integrating selected physico-chemical properties without majorly affecting the sensitivity. For example, for liquid chemicals (n=62) combining 2 in vitro test methods (RhCE and BCOP) in a Bottom-Up approach correctly predicts 81.3% Cat. 1, 71.4% Cat. 2 and 75.0% No Cat. Incorporating the physico-chemical properties LogP and vapour pressure increased correct identification of No Cat to 96.8% without major impact on correct identification of Cat. 1 and Cat. 2.

**1498 ROS Generation and JNK Activation Contribute to 4-Methoxy-TEMPO-Induced DNA Damage in HepG2 Cells**


4-Methoxy-TEMPO, a derivative of 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO), is a stable nitroxide radical and is generally used in organic and pharmaceutical syntheses for the oxidation of alcohols. Previously, we reported the involvement of reactive oxygen species (ROS) and c-Jun N-terminal kinases (JNK) in TEMPO-induced apoptosis in mouse LS174Y cells. In this study, we investigated 4-methoxy-TEMPO induced toxicity in human HepG2 hepatoma cells and its underlying mechanisms. Treatments with 4-methoxy-TEMPO (0.5-5 mM for 2-6 hours) caused oxidative stress as demonstrated by increased intensity of the ROS indicator H2DCF-DA, decreased levels of glutathione. 4-Methoxy-TEMPO treatment also induced DNA damage as characterized by increased levels of DNA tail intensity is the Comet assay, increased phosphorylation of related proteins including γ-H2AX, p-Chk1, and p-Chk2, and activation of MAPK signaling pathways. In addition, 4-methoxy-TEMPO also induced autophagy as demonstrated by the conversion of LC3B-I to II, decreased level of p62, and the appearance of GFP-LC3B punctae. To investigate the crosstalk between different signaling pathways, pretreatment of HepG2 with N-acetylcystein (NAC), an ROS scavenger, attenuated 4-methoxy-TEMPO-induced DNA damage, suppressed JNK activation, and diminished autophagy induction. Furthermore, inhibiting JNK activation by a JNK-specific inhibitor, SP600125, decreased DNA damage levels induced by 4-methoxy-TEMPO. These results suggest that multiple mechanisms including ROS generation, DNA damage, and MAPK activation contribute to 4-methoxy-TEMPO-induced toxicity.
Genotoxicity assessment of pharmaceuticals or active ingredients used in cosmetology is mandatory for registration. In addition to the standard in vitro/in vivo test battery, other assays can be of interest because of their high throughput in the drug discovery stage. Among them, the γH2AX assay is suggested for detecting genotoxic properties of chemicals. The phosphorylation of γH2AX has been demonstrated to be a sensitive marker for DNA double strand breaks (DSB). Likewise, p53-binding protein 1 (53BP1) is an important regulator of the cellular response, promoting the end-joining of distal DNA ends. Keratinocytes are routinely used for skin toxicity studies. Large-scale cultures of primary keratinocytes demanded by high throughput approaches face obstacles such as a limited lifetime and in vitro differentiation, often compromising reproducibility. Here, we describe the expansion of primary keratinocytes after lentiviral transduction (upcyte® keratinocytes) for large-scale production and their subsequent use in γH2AX/53BP1 genotoxicity screenings. Resulting cells revealed typical keratinocyte morphology and were characterized by high expression of the basal marker CK5/14 while lacking expression of CK1/10. To further evaluate their suitability for genotoxicity studies, we challenged upcyte® keratinocytes and neonatal human epidermal keratinocytes (NHEK) with 16 model compounds including non-genotoxic controls (e.g. ampicillin trihydrate, D-threose, etoposide) and genotoxic compounds (e.g. paclitaxel, colchicine). Intranuclear γH2AX and 53BP1 spots were analyzed using an automated imaging approach. When looking at the γH2AX spot count, upcyte® keratinocytes were comparable to NHEK and allowed accurate identification of clastogenic compounds. In conclusion, we observed that γH2AX was not observed with non-genotoxic compounds. Interestingly, intranuclear 53BP1 spot counts revealed a base level 2 to 4.8 fold higher in upcyte® keratinocytes when compared to NHEK. This may explain that the fold induction of intranuclear 53BP1 spots after exposure to genotoxic compounds tended to be smaller in upcyte® keratinocytes when compared to NHEK. Direct sequencing of endogenous genes using error-corrected Next Generation Sequencing (NGS) has the potential to more accurately characterize mutagenesis, dose response, and ultimately cancer risk. In order to test the hypothesis that chemically-induced mutations in endogenous genes are comparable to the already validated cl t transgene, we directly examined the cl t gene and 4 endogenous genes in genomic DNA from the liver and bone marrow of BB mice treated with vehicle, N-ethyl-N-nitrosourea (ENU) and benzaldehyde (BpA) according to OECD guidelines. Using Duplex Sequencing (DS), the most accurate form of error-corrected NGS currently available (error rate <1/10^9), we compared the mutational frequencies (MF) and spectrum in the cl t transgene with a set of endogenous genes of varying expression level and chromosomal location: Polr1c, rhodopsin, haptoglobin and beta-cat- enin. As expected based on conventional BB plaque assay data, DS demonstrated that the cl t MF increased in response to chemical treatment to a degree that varied by tissue type. The endogenous genes also exhibited a clear increase in MF in response to mutagen treatment, similar to that seen in cl t, albeit with a reproducible 2-3 fold variation in magnitude between loci. The mutation spectrum in both the cl t gene and endogenous genes was also similar, and characteristic of ENU (T>A) and BpA (C>A) based on previous reports. The truncleotide spectral analysis demonstrated that adjacent nucleotide context strongly mod- ules mutagenic potential. For example, the number of base-pair substitutions was highly dependent on context, and additional active mutations in conventional BB, this could not be fully recognized with sequencing of individual plaques. These results support the con-
Measurement of DNA damage is an important factor in predicting human cancer risk of toxicants. In vitro mutagenic effects can be detected using reporter genes in bacteria and cells. However, in vivo mutagenesis was difficult to measure until development of transgenic rodent mutation assays. Assays, such as Big Blue® (BB), rely on recoverable bacteriophage shuttle vectors and loss of cII gene function measured using a bacterial-based plaque assay. Direct measurement of de novo mutations in DNA using standard next-generation sequencing is impossible due to an error rate (~10^{-3} mutants/base) well above the background mutation frequency of ~10^{-10} to 10^{-8}. Duplex Sequencing (DS) is a tag-based error correction method using both DNA strands that improves sequencing accuracy permitting detection of one mutant in ~10^{8} bases. The cII gene in BB animals is a qualified biomarker for in vivo mutation and the temperature selective plaque assay (PA) is a validated method to measure cII mutants (OECD TG 488). Here, we evaluated DS as an alternative method to measure cII mutant frequency (MF). We examined MF from liver (L5178Y) and BB mice treated with vehicle, N-ethyl-N-nitrosourea (ENU) or benzo[a]pyrene (BaP). The two methods gave similar fold increases in MF even though BB PA measures phenotypic mutants/gene and DS measures mutants/sequenced base. ENU gave increases in LV MF of 4.2-fold for PA and 4.3-fold for DS, and in BM MF of 10.6-fold (PA) and 7.1-fold (DS). BaP gave fold increases in LV MF of 4.9 (PA) and 3.6 (DS) and in BM MF of 17.7 (PA) and 9.5 (DS). Hierarchical clustering of cII mutants showed that ENU mutants from LV and BM and BaP in BM gave group specific signatures. Mutant spectra from vehicle animals overlapped somewhat with BaP spectra in LV. Spectra matched those classic for ENU (T→A) and signatures. Mutant spectra from vehicle animals overlapped somewhat with BaP spectra in LV. Spectra matched those classic for ENU (T→A) and BaP, consistent with literature. Mutant plaques were isolated and sequenced from duodenum and BM from similarly treated animals. Comparing cII mutant sequence from DS measurement of genomic DNA and cII mutant plaques showed the expected loss of function mutations in both while DS revealed additional synonymous mutations missed by PA. This work demonstrates that DS is capable of detecting background and induced cII mutants comparable to traditional plaque methods.

**1505 Comparison of In Vitro Toxicity of E-Vapor Condensates to Literature Data for Cigarette Smoke Condensates**

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The Food and Drug Administration Draft Guidance (2016) “Premarket Tobacco Product Applications for Electronic Nicotine Delivery Systems” recommends toxicological evaluations, including assessing in vitro genotoxicity and cytotoxicity, for new electronic nicotine delivery systems (ENDS) or e-vapor products. The Draft Guidance also recommends that the toxicity of ENDS be compared to the toxicity of traditional tobacco products such as combustible cigarettes. In the current analysis, we compared the genotoxic/cytotoxic potency (biological response per mass quantity) of condensates from selected e-vapor ENDS products marketed as MarkTen® with the condensates from combustible cigarettes. For the e-vapor products, the aerosol condensates were collected from MarkTen® products with a total of 7 formulations (a carrier (propylene glycol, glycerine, plus 5% nicotine) and 6 e-liquids (carrier plus flavor mixtures)). The e-vapor condensates were tested in two genotoxicity assays ( Ames Test and in vitro micronucleus assay (MN) using TK6 cells) and MN assays of cigarette condensates were genotoxic in the Ames and MN assays and cytotoxic in the NRU assay. The e-vapor condensates were not cytotoxic (NRU assay viability >80%) and not genotoxic in the Ames Test or the MN assay at any of the tested concentrations.

**1506 Absence of In Vitro Mutagenic and Genotoxic Properties of Two Supercritical Tobacco Extracts**


Super-critical carbon dioxide was used to extract tobacco constituents, especially aroma compounds from tobacco. The same method is used to extract natural substances for food, flavour, fragrance and pharmaceutical applications. The extracts were developed for use in a next generation tobacco product, where consumable ingredients are heated but not burned. Studies conducted on this next generation tobacco product indicated reduced exposure to toxicants in the aerosol generated, when compared to reference cigarette smoke. Part of the extracts’ stewardship was a series of in vitro mutagenicity and genotoxicity tests. Two liquid extracts were tested, derived from Virginia, Burley and Oriental tobaccos. The tests were for bacterial mutagenicity ( Ames test), mammalian mutagenicity (mouse lymphoma assay, MLA) and mammalian genotoxicity (the in vitro micronucleus test, IVMNT). All tests were conducted according to OECD guidelines, in an independent laboratory complying with GLP. The Ames test included bacterial strains TA98, TA100, TA102, TA1535 and TA1537. The MLA and IVMNT used LS178Y cells and V79 cells, respectively. All tests were with and without metabolic activation (rat liver S9). The vehicle control was dimethyl sulphoxide. Appropriate positive controls were used for each bacterial strain or cell line and level of metabolic activation. Test results were valid in terms of concurrent vehicle and positive control responses and historical ranges. All positive controls gave clear, statistically significant and reproducible responses, within their historical ranges. The extracts were tested up to 5mg/plate (Ames test) or 5mg/ml (MLA and IVMNT) which is the maximum recommended concentration for substances of unknown, variable or complex composition. Mutagenicity or genotoxicity were not observed in any of the tests. The conclusion was that these specific extracts were not mutagenic or genotoxic in vitro.
The exacerbatuse of animals in scientific research and industry has encouraged some researchers to create the 3Rs idea in 50th decade. These principles started the development and validation of alternative methods to the use of animals. In this context, we can highlight the use of three- dimensional cell culture for toxicological proposes, especially in the field of nutrition. Our model used in vitro hepatocyte culture itself as an integrated system. This combines metabolism and endpoint measurement in a hepatocyte culture, will increase confidence in results and could be used for chemical toxicity evaluation with results of concept, we determined genotoxic potential of octadiene (OCTA) and could be used for chemical toxicity evaluation with results more significant for human health when compared to monolayer cell culture.

Hepatocytes as a Metabolic-Activating System for Genotoxicity Testing: A Case Study with Octadiene


Use of the induced rat S9 as metabolic activating system in in vitro genotoxicity testing raises several issues including the high false positive rates seen with in vitro-based screening and therefore, questionable human relevance of in vitro results. The goal of this study was to demonstrate the importance of addressing both metabolic activation and detoxification to increase the predictivity of in vitro genotoxicity testing. As a proof of concept, we determined genotoxic potential of octadiene (OCTA) using a metabolically competent human primary hepatocyte suspension. OCTA requires cytochrome P450-mediated bioactivation to elicit genotoxicity. However, in vivo, OCTA’s active metabolites are detoxified by epoxide hydrolase and glutathione S-transferase, which cannot be fully captured by adding induced rat S9 to in vitro toxicity assays. As conventional genotoxic endpoints are not compatible with suspension culture, we measured DNA damage in HT1080 cells, a widely used in vitro genotoxicity testing model, by transferring media from primary hepatocytes to the suspension culture, we measured DNA damage in HT1080 cells, and the DNA adducts were identified in bronchial epithelial BEAS-2B cells. BEAS-2B cells were treated with 4-(methylhydroxynitrosamino)-(3-pyridyl)-1-butanone with Human Bronchial Epithelial Beas-2b Cells

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4-(methylhydroxynitrosamino)-(3-pyridyl)-1-butanone (NNK) is among the most important carcinogenic tobacco-specific nitrosamines (TSNAs) in tobacco products, which is powerful cancer-causing agents in animal models targeting mainly the lung in rats, mice, hamsters, and ferrets. Metabolic activation of the carcinogenic NNK results in the formation of 4-(3-pyridyl)-4-oxobutylation (POB)-DNA adducts, which have been previously identified in tissues of laboratory animals treated with NNK, but its carcinogenic potential in humans is not known. To obtain a better understanding of the genotoxicity of NNK in humans, we have investigated its metabolism and its ability to form DNA adducts in bronchial epithelial BEAS-2B cells. BEAS-2B cells were treated with NNK at doses ranging from 0.1–100 μM, and the DNA adducts were identified and characterized by UHPLC–Q-TOF/MS/MS and UHPLC–MS/MS. Six major methylation DNA adducts were identified, including three methylation DNA adducts, methyl-2’-deoxyguanosine, methyl-2’-deoxycytidine, methylyl-2’-deoxyadenosine and methyl-2’-deoxymethylguanine. In addition, four minor DNA adducts, including O6-[4-(3-pyridyl)-4-oxobutyl-1-yl]-2’-deoxyguanosine (O6-POB-dG), O6-[4-(3-pyridyl)-4-oxobutyl-1-yl]cytidine (O6-POB-C), O6-[4-(3-pyridyl)-4-oxobutyl-1-yl]thymidine (O6-POB-dT), 8-hydroxy-2’-deoxyguanosine (8-OH-dG), and 8-methoxy-2’-deoxyguanosine (8-MOP), were identified. Preincubation of BEAS-2B cells with 8-methoxypsoralen (8-MOP), a selective mechanism-based inhibitor of CYP2B6, resulted in a strong decrease in the formation of NNK metabolites and a concomitant decrease in DNA adduct formation. With the addition of S9 mixture solution, the concentration of NNK metabolites and DNA 8-OH-dG were identified, which were identified with 8-MOP. Therefore, under the tested conditions, these e-liquids were negative for genotoxicity, implying no biological relevance of very weak in vitro genotoxicity signals.
Among short-term tests developed to assess genotoxicity of chemical compounds, the micronucleus (MN) assay is largely used for detecting chromosome damage. MN test allows classifying clastogenic and aneugenic events, with easy measurement in both in vitro and in vivo experimental systems. The assay represents a tool in risk evaluation and developments for automation scoring. However, MN assay does not provide a dynamic assessment of the genotoxic event and also lacks a relevant human metabolically competent cell model. Our goal was to develop an in vitro MN test coupled with a metabolically well-equipped RGIHep 

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In April 2017, the China FDA (CFDA) released a draft guidance on Genotoxicity Testing and Data Interpretation for Pharmaceuticals Intended for Human Use for public comments. The guidance has yet to be finalized. To better understand these changes, the genotoxic guidelines from different agencies (e.g., CFDA, ICH Testing Guideline (TG), and OECD TGs) were compared. The current draft CFDA version was changed mainly based on ICH and OECD TGs. Compared to the current CFDA guidance, the main changes are listed as follows: 1) Genotoxicity battery option 2 is accepted; 2) in vitro micronucleus assay is considered equally suitable, besides in vitro chromosomal aberration assay (CAA) and mouse lymphoma assay (MLA); comet assay is recommended as a second in vivo assay; 3) genotoxicity endpoints can be incorporated into repeated dose toxicity studies; 4) Ames assay should still be carried out for compounds that are toxic to bacteria (e.g., some antibiotics); 5) the maximum top concentration recommended for in vitro cytogenetic assays and MLA is reduced to 1 mM or 0.5 mg/ml, whichever is lower; 6) relative population doubling (RPD) and relative increase in cell count (RCC) are the recommended cytotoxicity parameters for in vitro cytogenetic assays, and the cytotoxicity should not exceed a reduction of about 50% in cell growth; 7) when solubility is limiting, the maximum concentration, if not limited by cytotoxicity, should be the lowest concentration at which minimal precipitate is visible in cultures for in vitro cytogenetic assays and MLA; 8) automated analysis and rat blood are formally accepted for in vivo micronucleus assay if appropriately validated; 9) the scored number of cells to improve the statistical power is increased; and 10) more emphasis is placed on the evaluation of test results and follow-up test strategies. Although the draft version mainly refers to ICH TG, there are still several differences based on China’s actual condition. The main differences are listed as follows: 1) Ames experiment needs to be repeated even if the result is clearly positive or negative; 2) positive control should be included in every in vivo study; 3) genotoxicity battery should be fully investigated prior to clinical trials; and 4) special recommendations on traditional Chinese medicine should be included. Based on the above analysis, the draft version of CFDA TG is very similar to ICH TG, but there are still some special requests.
Acrylic bone cement is self-curing cement comprising of liquid and powder components of methyl metacrylate (MMA). It has been used extensively in orthopedics; however, adverse effects including exothermic reaction and bone cement implantation syndrome were associated with its use. In this case study, we investigated the genotoxic potential of two types of bone cement implant devices, bone cement loaded with and without antibiotic, which are both comprised of 97% MMA. The samples were fully cured and stored at -80°C prior to extraction. In Salmonella/E. coli Mutagenicity Test (Ames test), 0.316% and 3.16% of neat polar extract of antibiotic-loaded bone cement induced a statistically significant increase in the number of revertant colonies in TA100 strain in the presence of metabolic activation. However, the number of revertants was less than twofold of the number in the corresponding negative control. These data indicated that the extracts were not mutagenic.

We found that 25% of the neat polar extracts from bone cement with and without antibiotic induced 17% and 8% metaphases with aberrations, respectively, in the presence of metabolic activation. Interestingly, the increase of chromosome aberration was not observed in cured samples that were stored at room temperature. These data suggest that the clastogenic effect could be the result of a direct effect of MMA. In conclusion, our results provide initial evidence about the biocompatibility of the MMA bone cement.

The 3D Reconstructed Human Skin Comet and Micronucleus (MN) assays have been developed and validated to follow-up on positive results from the current in vitro genotoxicity test battery using cultured cells as they represent the exposure more realistically for dermally-applied chemicals such as cosmetic ingredients. Validation of these assays includes testing chemicals that are not genotoxic themselves, but can be converted into mutagenic metabolites. For example, 2-acetylaminofluorene (2-AAF) is a carcinogen when administered dermally, orally, or by intraperitoneal injection in rodents and shows positive effects in vitro primarily after metabolic activation. N-hydroxy-2-acetylaminofluorene (N-OH-2-AAF) and N-hydroxy-2-aminofluorene (N-OH-2-AF) are genotoxic metabolites of 2-AAF that form DNA adducts. In this study, 3D reconstructed human skin models were topically treated with 2-AAF and these metabolites. 2-AAF did not increase DNA damage in keratinocytes in the MN assay when administered in multiple (2-3) applications at 24 hr intervals but could be detected in the Comet assay in the presence of the DNA polymerase inhibitor aphidicolin. No increase in DNA damage was observed in N-OH-2-AAF in the MN assay after multiple treatments while a single 3h exposure to N-OH-2-AAF caused a large dose-related increase in the Comet assay. A significant increase in the MN assay was only obtained with the highly reactive N-OH-2-AF metabolite after multiple treatments over 72h, but N-OH-2-AAF caused a strong increase in DNA damage after a single 3h exposure in the Comet assay. In support of these results, DNA adduct formation, as measured by the 32P-postlabeling assay, was examined in parallel samples and adduct levels after 2-AAF treatment for 3h were minimal but increased >10-fold after multiple exposures over 48h. These results suggest that enzyme(s) involved in 2-AAF metabolism can be induced in the skin models. As expected, a single 3h exposure to N-OH-2-AAF and N-OH-2-AF resulted in DNA adduct levels that were at least 10-fold greater than that observed with 2-AAF after multiple exposures. Our results demonstrate that the type of DNA damage caused by the 2-AAF metabolites is more efficiently detected in the 3D skin Comet assay than the MN assay and that after enzyme induction, 2-AAF can be detected in the modified Comet assay.
Obesity is an increasing global concern with studies reporting a significantly elevated risk of and a poor prognosis for a variety of cancers in people with a high body mass index. Obesity stimulates oxidative stress that can cause DNA double-strand breaks (DSBs) and reduced DNA repair capacity. DSBs can result in gene translocations that can disrupt oncogenes in cancers like Burkitt lymphoma (BL) and Chronic Myeloid Leukemia. The c-MYC oncogene in BL harbors vulnerable breakpoint “hotspots” enriched in repetitive DNA sequences. Such sequences can adopt alternate DNA structures (i.e. non-B DNA; e.g. H-DNA) and differ from the canonical B-form DNA double helix. We have found that H-DNA structures are intrinsically mutagenic in human cells and mice. We speculate that the formation of non-B DNA structures can increase the accessibility of DNA double-strand breaks and damage and impact DNA repair efficiency and accuracy, leading to more cancer-associated genetic instability.

Thus, we hypothesize that obesity increases DNA structure-induced genetic instability in vivo, contributing to mutation “hotspots” in cancer. To determine the extent to which obesity stimulates the mutagenic potential of H-DNA, groups of transgenic mice harboring a mutation-reporter vector with H-DNA-forming sequence (mapping to a breakpoint hotspot in BL) were put on control, 30% calorie-restricted (CR) diet and diet-inducing obesity (DIO) for 13 weeks. Subsequently, the mutation-reporter vector was recovered from the spleen tissues (n=3 mice/group) and subjected to mutagenesis assay. The mutation frequencies in genomic (g) DNA from control, CR, or DIO mice were determined between 2.3, 2.4 and 9.6 respectively. The mutation spectra showed large deletions (300-900 bp), small deletions (50-200 bp), point mutations and transversion events. To determine the impact of DIO on DNA damage and expression of any of the “hotspots” in the same groups, we performed a limited inter-laboratory validation study. In total 27 compounds (24 test compounds and 3 assay controls) were tested at least three times by two independent laboratories. The within-laboratory (WLR) and between-laboratory reproducibility (BLR) of the assay as well as the concordance of classification (genotoxic versus non-genotoxic) for the tested compounds was determined. The WLR for the six different ToxTracker reporters was assessed per category of reporters (DNA damage, oxidative stress, protein damage) and for all reporters together. The overall WLR was on average 94% for both laboratories. For laboratory 1, the average WLR ranged from 92.2% (oxidative stress) to 95.1% (protein damage). For laboratory 2, the average WLR ranged from 90.6% (protein damage) to 94.9% (genotoxicity). Also the BLR was evaluated for the different reporters with the highest concordance for the genotoxicity reporters (96.3%), followed by the reporters for p53 activation (92.6%), oxidative stress (88.0%) and protein damage (85.2%). This resulted in an overall BLR of 90.5%. Predictive capacity for genotoxicity was calculated for each laboratory and for the cumulative results of the two laboratories. The sensitivity for identification of genotoxic compounds was 77.8% (7 out of 9 genotoxic compounds tested positive in ToxTracker). The specificity was 94.1% (16 out of 17 non-genotoxic compounds tested negative in ToxTracker for both laboratories. In this study, the overall concordance between ToxTracker and the standard battery of in vitro and in vivo genotoxicity tests was 85%. Overall, the interlaboratory validation indicates an excellent transferability and predictive capacity of the ToxTracker assay. Furthermore, the combination of the six GFP reporter cell lines can be applied to gain insight into the mechanisms of genotoxicity.
The replacement of animal testing for safety assessment of chemicals is the concern of many researchers. In this context, in vitro genotoxicity safety assessment tests are needed. Reconstructed skin models are particularly interesting test systems, especially for compounds with a dermal route of entry since they entail the barrier function of the skin. The FADU assay, (Fluorimetric Detection of Alkaline DNA Unwinding) has recently been introduced as a rapid and reliable method to detect DNA strand breaks in vitro. In an effort to set up genotoxicity testing of chemicals to be applied to the skin, the automated FADU assay and human reconstructed skin models were combined. In a first step, the principios suitability of skin models (e.g., cell systems) for the FADU assay was tested. Secondly we attempted to reduce the tissue processing time after treatment in order to minimize manual processing steps and to more rigidly control the DNA repair period. epoCS were treated for 3 h with two genotoxic agents (methyl methanesulfonate (MMS), 4-nitroquinoline 1-oxide (4NQO): 16 µM/cm²). Subsequently, cells were separated enzymatically and DNA strand breaks were measured.

In order to avoid time consuming enzymatic cell separation a second approach utilized biopsy punches (1 mm), to withdraw small samples of the treated skin models. The samples were analyzed on behalf of the automated FADU assay (AUREA qTOX-analyzer, 3T analytik). In short the tissue samples obtained were lyzed chemically and were analyzed for DNA strand break frequency. The DNA damage detection is based on progressive DNA unwinding under highly controlled conditions of alkaline pH, time and temperature. A fluorescent dye is used as a marker for double stranded DNA. Controls are run in parallel with the treated cells. Importantly, the MMS and 4 NQO resulted in a clear increase in DNA double strand breaks detected after 3h incubation time as expected from literature data. By combination of the reconstructed skin model epiCS and the FADU genotoxicity assay, it is demonstrated that that this double strand breaks in genotoxic chemicals are sensitively detected. Our results clearly show that the FADU assay with reconstructed skin models is a promising tool for safety assessment of compounds with a dermal route of entry while taking into account the influence of the skin barrier function.

There is still a lot of discussion on the genotoxicity of antimony compounds. Both trivalent and pentavalent compounds are generally negative in mammalian genotoxicity assays, but in mammalian cell lines, trivalent compounds have generated some positive results, while pentavalent compounds remained negative. Therefore, we investigated the genotoxic potential of a group of nine antimony compounds (5b-metal, four trivalent and four pentavalent) in the ToxTracker assay. ToxTracker is a panel of mammalian stem cells that contain different fluorescent reporters for induction of DNA damage, oxidative stress, and protein damage. The differential induction of the GFP reporters, as well as cytotoxicity of the tested compounds, is determined by flow cytometry. Selected compounds were analyzed in the absence and presence of an S9 rat liver extract-based metabolizing system. Quantitative data analysis is done using ToxPlot software. The validity of the ToxTracker assay results was confirmed by exposure to various reference compounds and assessing the specificity of the different reporter cell lines. None of the nine antimony compounds did induce the BscI2-GFP genotoxicity reporter that is associated with generation of pro-mutagenic DNA lesions or the Rtkn-GFP genotoxicity reporter for DNA strand breaks. Antimony, sodium antimonate, diantimony trioxide, antimony-3-sulfide, antimony glycolate, antimony-3-chloride, and antimony-5-chloride all induced the Srxn1-GFP and Blvrb-GFP oxidative stress markers significantly. None of the breaks induced were alanized by diantimony trioxide, antimony-3-sulfide, antimony glycolate, antimony-3-chloride, and antimony-5-chloride at cytotoxicity levels <75%. There was no indication in ToxTracker for metabolic activation of any of the antimony compounds. None of the tested antimony compounds showed genotoxicity in the ToxTracker assay even at the absence of or presence of a metabolizing system. Seven compounds induced significant levels of oxidative stress in the absence of a metabolizing system. Six of those compounds also showed a significant induction of oxidative stress in the presence of a metabolizing system.
DNA damage response proteins are stained and require vigilant analysis by the investigator, the proximity assay only positively stains foci at sites where DNA response proteins are physically binding during the DSB signaling cascade, thereby facilitating less biased and more time efficient analysis of DNA damage foci.

1527 The ToxTracker Reporter Assay Detects Indirect Genotoxicity by High-Lever of Oxidative Stress


ToxTracker is a mammalian stem cell-based reporter assay that detects activation of specific cellular signaling pathways upon exposure to unknown compounds (Hendriks et al, Tox Sci 2016). ToxTracker contains six different GFP-tagged reporters that allows discrimination between induction of DNA oxidative stress and protein damage in a single test. We first performed an extensive validation using 62 compounds from the recently updated ECVAM compound library. ToxTracker classified the genotoxic carcinogens as genotoxic with cytotoxicity of 94%. The non-genotoxic carcinogens and non-carcinogens were classified as non-genotoxic by ToxTracker with a specificity of 95%. Interestingly, various compounds that give misleading positive results in the conventional in vitro genotoxicity assays did not activate the DNA damage. However, it did induce high levels of oxidative stress or protein damage in ToxTracker. To further investigate indirect genotoxic effects of compounds that induce high levels of oxidative stress, a selection of 10 different metals, quinones, benzene compounds, and food additives were tested in ToxTracker in the presence of the ROS scavengers NAC (N-acetyl cysteine) or BHT (butylated hydroxytoluene). For each compound, three concentrations that induce 10-25/30% cytotoxicity in the reporter cell lines were tested in combination with increasing concentrations of NAC or BHT. In the absence of the ROS scavengers, all tested compounds activated the Snx1-GFP reporter for oxidative stress but often also activated the Rtkn-GFP genotoxicity reporter that is associated with induction of DNA strand breaks. Addition of NAC/BHT significantly reduced activation of the Snx1-GFP oxidative stress reporter. Interestingly, for many compounds also activation of the Rtkn-GFP DNA damage reporter was strongly reduced in the presence of the ROS scavengers, suggesting that the weak induction of DNA strand breaks by these compounds is likely a result of high levels of oxidative stress. The complete absence of induction of the Bsc12-GFP DNA damage marker, that is activation by DNA replication stress and induction of bulky, promutagenic DNA lesions, indicates that the tested compounds did not react directly with the DNA. Also cytotoxicity of the compounds was reduced by simultaneous incubation with a ROS scavenger, indicating that induction of oxidative stress is an important cytotoxic effect that can indirectly contribute to the genotoxic properties of compounds.

1528 Profiling the Budding Yeast Genome for Resistance to Heterocyclic Aromatic Amines

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The incidence of colon cancer is correlated with a diet containing red meat. Charred red meat contains heterocyclic aromatic amines (HAAs) that are carcinogenic. Since HAAs are also genotoxins, we hypothesize that there are genetic factors that confer a higher incidence of colon cancer in individuals exposed to HAAs, such as 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) and 2-amino-3, 8-dimethylimidazo[4,5-f]quinoline (MeIQx). These genetic factors may include polymorphic phase I and phase II genes that metabolically activate HAAs and genes that confer toxicity and DNA damage resistance. Because many Saccharomyces cerevisiae (budding yeast) genes are orthologous to human genes, budding yeast has been a useful model organism to identify basic housekeeping genes that confer resistance to toxicants, such as HAAs. We used the budding yeast diploid deletion library, which is a collection of ~5,000 strains each deleted for a single non-essential gene, to profile the yeast genome for HAA resistance. Budding yeast does not contain P450s that activate these compounds, so expression vectors that contain specific human P450s were introduced into the budding yeast genome for resistance to heterocyclic amines. The top five Genomic Ontology (GO) biological processes include transcription from RNA polymerase promoter, lipid metabolic process, responses to chemical, protein complex biogenesis, and regulation of organelle organization. Other GO biological processes include chromatin organization, response to chemical, and DNA repair. Among the DNA repair genes, genes involved in DNA damage tolerance and base excision repair (NTG1) were identified. NTG1 polymorphisms have previously been identified as genetic risk factors for colon cancer, and the yeast studies suggest that this risk may be aggravated when an individual’s diet contains HAAs. The yeast screens thus provide a novel methodology to identify genetic variants that confer resistance to HAA toxics. Support: National Institutes of Health, 1R15ES023685-01.

1529 Applicability of HepaRG Cell Line for Automated Assessment of DNA Strand Breaks

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In the context of testing genotoxic potential of chemicals the automated FADU (Fluorimetric Assay for Detection of Alkaline DNA Unwinding) assay provides a critical role in the assessment of the genotoxic potential of various noxes due to the requirement of bioactivation. The HepaRG cell line with the expression of a broad range of Phase I and II enzymes, transporters, and nuclear factors is one of the best candidates mapping the response expected from human hepatocytes. The aim of the presented study was the assessment of the principle applicability of the HepaRG cells in the FADU assay. Two common genotoxic agents without the need of bioactivation (methyl methanesulfonate (MMS) and etoposide) as well as D-mannitol as negative control were investigated. Furthermore, applicability of HepaRG was tested for aflatoxin, a genotoxic agent with the need of bioactivation. HepaRG cells were supplemented with well-known genotoxic substances with different modes of action etoposide, MMS, and aflatoxin. The maximum concentration of aflatoxin was not cytotoxic tested with a common cytotoxicity assay. The DNA damage detection is based on progressive DNA unwinding under highly controlled conditions of alkaline pH, time and temperature. A fluorescent dye is used as marker for double stranded DNA. Controls are run in parallel with the treated cells in the conventional in vitro genotoxicity assay. The DNA damage detection was reproducibly and reliably for well-known genotoxic noxes with and without need of bioactivation. Our data indicate that this combination is a promising rapid method for safety assessment of chemicals.

1530 Withdrawn by Author

1531 Integration of Liver Micronuclei, Blood Micronuclei, and Blood Pig-a Assays into a 28-Day Rat Study as a 3Rs Friendly Approach for Evaluating Chemicals’ In Vivo Genotoxic Potential—Proof-of-Concept with Diethylnitrosamine


Regulatory guidance documents note the value of assessing multiple tissues and the most appropriate endpoints when evaluating chemicals for in vivo genotoxic potential. However, conducting several studies to consider multiple endpoints and tissue compartments is time-consuming and resource-intensive. Furthermore, conventional approaches for scoring genotoxicity endpoints are slow, tedious, and less objective than the ideal. We are striving to address these deficiencies with the current state-of-the-art by i) employing flow cytometry-based methods for scoring liver micronuclei, blood micronuclei, and blood Pig-a mutation, and ii) integrating the assessments into a common general toxicology study design, the rat 28-day repeat dose study. As proof-of-concept, an experiment was performed with 6-week-old male Sprague Dawley rats exposed to diethylnitrosamine (DEN) for 28 consecutive days at 0, 5, 10 or 15 mg/kg/day. Blood was collected for the micronucleus reticulocyte (MN-RET) assay on Days 4 and 29. A aliquot of each Day 29 blood sample was also evaluated for the frequency of Pig-a mutant cells. Day 29 left lateral lobe liver sections were collected from exanguinated rats and processed for liver micronucleus scoring (MNHEP). Whereas MN-RET frequencies were not affected by DEN exposure, MNHEP exhibited dose-related increases (9.1-fold for the high dose group). Furthermore, the MNHEP values were highly cor-
related with parallel assessments accomplished via microscopy (R2 = 0.89). Concurrent with MNHEP analyses, assessments of Ki-67-positive events and the proportion of 2n, 4n, and 8n nuclei provided evidence for treatment-related changes to hepatocyte proliferation. Pig-a mutant cell frequencies were slightly elevated, suggesting DEN can induce gene mutation to hematopoietic cells in the bone marrow, despite the low level of genotoxic metabolites that reach this compartment. Collectively, these results suggest augmented genotoxicity assays can be successfully integrated into repeat-dose studies for higher efficiencies, and better utilization of animals.

**1532 Double Strand Break Repair by Capture of Unintentional Sequences, an Emerging New Possible Risk for the Leading-Edge Technology**

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Among DNA damages, double strand breaks (DSBs) are one of the most toxic to a cell. Failure in DSB repair could lead to genomic instability and cancer. DSBs can be caused by various assaults by both exogenous environmental factors (e.g. chemical agents, ionizing radiation, ultraviolet light) and endogenous cellular events (e.g. transposition, meiotic double strand break formation). The CRISPR/Cas system allows the introduction of DSBs at particular loci in the genome, therefore, it is possible to investigate the DSBs and DSB repair events accurately by using the CRISPR/Cas system. As a result, DSBs introduced into the mouse zygote by the CRISPR/Cas system are occasionally repaired by the capture of retrotransposons and/or mRNA sequences. This DSB repair mechanism with reverse transcription (RT)-mediated DNA repair of retrotransposon-mediated DSB repair (RMDR), was observed at least 2% of the DSB-induced yzogotes irrespective genome loci., because among the captured sequences, some were apparently derived from RT-mediated spliced mRNAs (Ono et al. 2015). In this study, lengths of the indels introduced by the CRISPR/Cas system in vivo and in vitro were determined by deep sequencing of PCR products amplified with two primers across the target DSB site. One of the novel findings of our high-throughput sequencing analyses is that they generate novel long-range fusion proteins between DSB-introduced gene and truncated retrotransposons and/or mRNA sequences. Both of which can contribute both to genome instability and cancer. A DSB repair by capture of unintentional sequences have a potential to lead to genomic instability. The findings of this study highlight an emerging new possible risk for this leading-edge technology. Reference: Ono R., Ishii M., Fujihara Y., Kitagawa M., Usui T., Kondo-Eshino T., Kanno J., Ikawa M., Ishino F. 2015. Double strand break repair by capture of retrotransposon sequences and reverse-transcribed spliced mRNA sequences in mouse zygotes. Scientific reports 5: 12281.

**1533 Evaluation of Germline Mutations Induced by Ethyl Methanesulfonate in Different Reproductive Development Stages of Caenorhabditis elegans Using Whole Genome Sequencing**

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Germline mutation is a heritable change in the DNA that occurred in a germ cell and can be passed on to their descendants. Germline mutation is a test result for more than 30 known inherited disorders. A practical assay for detecting germline mutations is desired for regulatory purpose. Caenorhabditis elegans (C.elegans) has a short hermaphroditic life cycle, a relatively small genome, and a large number of offspring, making it an ideal model for assaying germline mutagenicity of chemicals. In this study, a panel of transgenic strains of different germline development stages of the worm to mutagenic insults to assess whether the model can appropriately detect chemical-induced mutations in male and female germs. Parent worms were treated with 25 mM ethyl methanesulfonate (EMS), a model germline mutagen, for 4 hours in L3 larval stage. After the germl line undergo robust proliferation, L4 larval stage when the hermaphrodite develops spermatogenesis, and young adult stage when the hermaphrodite ceases spermatogenesis and switches to oogenesis. The whole genome of F1 offspring from each individual worm treated with EMS or the vehicle was sequenced and the sequencing data were analyzed with a bioinformatics pipeline including VarScan, a variant calling program. The mutation frequencies (MFs) were 185-fold, 130-fold and 175-fold higher in the L3, L4 and young adult treatment groups, respectively, than the control group (MF is 2 ×10–6). More than 90% of the induced mutations are G:C >A:T transitions, a signature mutation type induced by EMS. The MFs induced in sperm germs were slightly lower than those in early germ cell proliferation stage and in egg germs. The results suggest that C. elegans can sensitively detect exposure of germline mutagens in both male and female germ cells, as well as in early germline proliferation stage.

**1534 Validation of the ToxTracker Reporter Assay for the Genetic Toxicology Assessment of Petroleum Products**


Certain poorly refined petroleum streams that contain relatively high levels of 3-7 ring polycyclic aromatic hydrocarbons (PAHs) are known to have mutagenic properties and cause skin tumors in mice. Since the guideline carcinogenicity test has obvious time, cost and animal-welfare constraints, screening assays were developed such as the IP346 assay and Modified Ames test. To further underpin these assays and increase their predictivity by including a mechanistic component, 8 petroleum substances (PS; highly refined, with various levels of 3-7 ring PAHs) were tested in the ToxTracker reporter assay. ToxTracker is a panel of six validated mammalian stem cell lines each containing a GFP reporter to detect induction of DNA damage, oxidative stress and protein damage in a single test. DMSO extracts, containing the “biologically active” fraction (i.e. mostly 3-7 ring PAHs) of the petroleum substances were prepared following standard procedures. These DMSO extracts were tested in the ToxTracker assay at 3 different levels of cytotoxicity (10, 25 and 50%), along with 4 positive control samples. The DMSO abstracts were analyzed in the absence and presence of 59 liver extracts to include a mutagenic mixture. PAHs are known to be autofluorescent, which could interfere with analysis of the GFP reporters. Therefore, activation of the six ToxTracker biomaker genes was also confirmed by quantitative RT-PCR. In parallel, aliquots of these DMSO extracts were tested in the standard Modified Ames test for validation purposes and PAH levels were analyzed by ring class. The ToxTracker protocol was modified to allow analysis of autofluorescent test substances. ToxTracker showed that poorly refined petroleum streams containing high levels of 3-7 ring PAH are genotoxic as observed by induction of the Bsc12-GFP and Rtkn-GFP genotoxicity reporters. Activation of the Bsc12-GFP reporter is associated with induction of bulky, promutagenic DNA lesions and the Rtkn-GFP reporter with induction of DNA strand breaks. The poorly refined petroleum extracts also induced high levels of oxidative stress and protein damage, in line with the toxicity profile of these extracts. Highly refined products, containing virtually no (3-7 ring) PAHs, did not induce any cytotoxicity or activation of the ToxTracker reporters. These observations were confirmed by the results of the Modified Ames assay.

**1534a Mycoplasma Infection Induces Oxidative Stress and Affects Cellular Base Excision Repair**

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Mycoplasma contamination is a major concern for in vitro cell culture models. Mycoplasma is resistant to antibiotics, so the most difficult challenges are the prevention and treatment of mycoplasma contamination. Numerous studies show that mycoplasma infection generates a variety of cellular responses, in a variety of cell lines. However, there is a lack of information about the effects of mycoplasma infection on genomic instability. In this study, a dopaminergic neuronal cell line (BE-M17), which is popular in vitro model of Parkinson’s disease, was used to evaluate genomic instability and base excision repair (BER), during mycoplasma infection using the hOGG1-modified comet assay. The results showed that mycoplasma infection resulted in significantly elevated levels of DNA damage, in the form of strand breaks, alkaline-sensitive sites (SB/ALS) and oxidized purines, compared to uninfected cells (P < 0.0001). Uninfected cells repaired SB/ALS more rapidly than infected cells over first 1 h (P < 0.001). However, SB/ALS were fully repaired in both uninfected and infected cells 2 h after H2O2 challenge. Uninfected cells showed complete repair of oxidized purines after 24 h, but in infected cells, these were not fully repaired until 30 h (P < 0.0001). In conclusion, the study showed that not only does mycoplasma infection induce oxidative stress and DNA damage, but it also affects the main pathway responsible for the repair of oxidatively damaged DNA (BER). In this in vitro model, there is no mechanism for infection-induced inflammation, a source of reactive oxygen species. Therefore, further studies are needed to evaluate how mycoplasma infection causes oxidatively damaged DNA, and how it modulates the cellular DNA repair ability.
Chemosensitivity is an area of concern that has arisen due to the implementation of cancer chemotherapy. Several platinum compounds, including cisplatin, are currently used as anticancer drugs; however, some cancer types, such as ovarian cancer, tend to become resistant to cisplatin chemotherapy and this limits its usefulness, and results in increased mortality. Therefore, there is a significant need to discover an effective treatment for cancer, which reactivates chemosensitive tumors. This study aims to understand how ovarian cancer cell lines become resistant to cisplatin chemotherapy, which will help in identifying factors which may confer sensitivity in resistant tumors. Modified comet assays were used to measure interstrand cross-link (ICL) formation and repair, together with base excision repair (BER) in chemosensitive (SKOV-3) and chemosensitive (OCI-P5x and A2780) ovarian cancer cell lines. This method analyzes DNA damage formation and repair at the level of a single cell. The data revealed considerable differences among the ovarian cancer cell lines in response to cisplatin treatment. Although we discovered that the peak of crosslink formation for all three cell lines was at 12 h; we noted a significant attenuation of DNA crosslink formation in the chemoresistant cell line SKOV-3, compared to the chemosensitive lines, which was attributed to increased nucleotide excision repair over the first 12 h. In contrast, no differences in BER were noted between the cell lines. The ICL comet assay was also used to study the role of nuclear organization on DNA damage formation, through comparison of response in nuclear bodies and intact cells. The results demonstrated the same rank order of dose-response in nuclear bodies as in intact cells, but with chemoresistant cells being considerably more resistant to damage formation, at all doses cisplatin. These data provide interesting insights into differences in the DNA damage response between chemoresistant and chemosensitive cells, and future experiments will explore the molecular basis of these differences.

Toxicity of Cigarillos Versus Cigarettes: Smoke Chemistry, Cytotoxicity, and Genotoxicity

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There has been limited toxicity testing of cigarillos, including comparison to cigarettes. The present study compared smoke chemistry and cytotoxic/genotoxic potential of ten conventional cigarettes and cigarillos selected based on largest market share. Aerosolized whole smoke and total particulate matter (TPM) were generated using the Health Canada Intense (CI) and the International Organization for Standardization (ISO) puffing protocols. Smoke chemistry was examined for select tobacco-specific nitrosamines, polycyclic aromatic hydrocarbons (PAH) and formaldehyde using liquid/gas chromatographic-mass spectrometric methods. Cytotoxicity was assessed using the neutral red uptake assay. The genotoxic potential of the products was assessed using the Ames test with TA98 and TA100 strains and the thymidine kinase (TK) gene mutation assay. TPM from cigarillos were more potent than cigarettes in inducing cell death. Micronuclei formation at the highest dose of TPM showed significantly greater genotoxicity for cigarillos compared to cigarettes in the presence and absence of rat liver S9 fraction. In Ames test, both tobacco products exhibited significant dose-dependent increases in mutation frequency with TA98 and TA100, in the presence of S9. Enhanced mutation frequency was also observed for both tobacco products in the TK assay; however, effects were significantly greater with cigarillos in the presence of S9. The levels of all measured PAHs and carbonyls (except acrolein), N-nitrosonornicotine and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butane were significantly greater in cigarillos than cigarettes. Benzo[a]anthracene and chrysene were the most abundant PAHs in all products. The CI puffing protocol led to increased aerosol constituent levels compared to the ISO protocol. As the gas/vapor phase was not studied, the results represent only a subset of the potential toxicity related to whole smoke of tested products. Under conditions of this study, these data showed that cigarillos have significantly more toxic potential than cigarettes.
Mitochondrial Toxicity has been shown to contribute to a variety of organ toxicities, such as liver, cardiac and kidney. In the past decade, two high throughput applicable screening platforms (isolated rat liver mitochondria: glucose-galactose grown HepG2 cells) to assess mitochondrial toxicity have been deployed in many pharmaceutical companies. However, only two publications have demonstrated the utility of these screens as a predictor of human drug induced liver injury. In the present study, we screened 73 hepatotoxicants, 49 cardiotoxicants, 46 nephrotoxicants and 60 compounds not known to cause human organ toxicity for their effects on mitochondrial functions in the assays mentioned above. Predictive performance was evaluated using specificity and sensitivity of the assays for predicting particular organ toxicity. Our results show that the highest sensitivity for all the drug classes was when compounds were tested at 100 µM (drug concentration in human plasma). The sensitivity was 63%, 33% and 28% for hepatotoxicants, cardiotoxicants and nephrotoxicants, respectively, and the overall specificity was 93% and above. We were not able to detect compounds that induced toxicity via mechanism such as induction of mitochondrial membrane permeability transition pore, or mitochondrial membrane swelling and depolarization, or inhibition of fatty acid oxidation, or exhibit toxicity at higher doses (outside our testing range). The sensitivity was increased to 78%, 39% and 38% for hepatotoxicants, cardiotoxicants and nephrotoxicants, respectively when additional mechanisms were included in the predictive analysis. Furthermore, we examined the physicochemical space associated with such liability and found that compounds which tested positive in the mitochondrial toxicity assays had higher median clogP (Calculated Log Partition Coefficient) values and lower median TPSA (Topological Polar Surface Area) values than compounds that were negative in the mitochondrial assay. There were no differences between the different organ toxicity test sets, which prevents the assays to be used to predict particular organ toxicity.

In Vivo Toxicological Outcomes Assessment by Physicochemical Properties and In Vitro Screening Data


Limiting toxicity-driven attrition of drug candidates is a key challenge for the pharmaceutical industry. To address this issue, guiding medicinal chemists to safer, more productive chemical space would clearly be an effective strategy. An analysis of exploratory toxicity studies of 245 Pfizer internal compounds, suggests that drug candidates in the chemical space low-clogP and high-TPSA, are considerably less likely to be cause toxicological effects at total plasma concentrations below 10 µM. Following this report, an internal Eli Lilly analysis showed partial support to the rule, whereas AstraZeneca’s assessment concluded the opposite. This difference is likely due to the different chemical space within each company, but the influence of the toxicological endpoint measured (e.g., preclinical vs. Phase 1 survival) cannot be excluded. According to a patent analysis, Takeda’s compound library has a different chemical space, as defined by clogP, MW, HBD, HBA, RotB, chiral C, sp3, Ar and ArHet, when compared to those of Pfizer, Eli Lilly and AstraZeneca. This situation motivated us to analyze our internal compounds to help identify our own safer chemical space. Interestingly, assessment of 193 internal Takeda compounds suggested that both low-clogP and high-TPSA are well-correlated with toxicity occurrence at 10 µM total concentrations, similarly to Pfizer’s analysis. If ATP-competitive kinase inhibitor project compounds were removed from the analysis, the odds are dramatically enhanced. Despite the difference in chemical space between Pfizer and Takeda, our results provide support to the 2750 nM useful guideline for medicinal chemists. In addition to physicochemical properties, high-throughput in vitro ADME screening data were also investigated to understand if any of these contributed towards the prediction of toxicity at 10 µM. Among them, decreased microsomal metabolism and high-Toxscore were correlated with toxicity occurrence yet interestingly, there was no correlation with lipophilicity. A combination of these orthogonal features (clogP, TPSA, and microsomal metabolic stability) significantly improves the prediction of toxicity occurrence.

Detection of Pre- and Post-Metabolism Compound Toxicity Using Co-Cultured Autobioluminescent Human Cells

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In vitro cell-based assays are critical components of the drug discovery workflow to serve as early-stage screening tools to identify potential therapeutic compounds as well as eliminate compounds with undesired toxicity. It is critical that new in vitro assay system be developed that can perform these screens at lower costs, with higher throughput, and with improved prediction of in vivo outcomes. However, this need is currently limited by the lack of cost-effective in vitro assays capable of replicating the complex interactions of multiple tissues types. In this study, we evaluated the use of autobioluminescent human cells to support higher-throughput and more data intensive co-culture assay formats as a promising solution to this problem by co-culturing in 3D of one type Ewing’s sarcoma (EFT) cell line (A-673, TC-32, and TTC-446) in two primary EFT cell lines (TC-32 and CHLA-10) as well as an unrelated HEK293 renal epithelial cell line. The average PMO efficacy (EC50) in TC-32 cells was 670 ± 130 nM with a range from 420 to 890 nM; in CHLA-10 cells the average EC50 was 250 ± 130 nM with range from 110 to 560 nM. In contrast, the average EC50 in HEK293 cells was 3,070 nM with range from 460 to 16,700 nM. Synergy was observed for a cocktail of all PMOs at low doses, but the cocktail was less effective than single agents at oligomer doses above 1 µM. Synergy was also observed for both EFT cell lines when single agent targeting PHGDH was combined with XAGE1 or CYP4F22. Antagonism was observed for both EFT cell lines when CCND1 was combined with XAGE1 or CYP4F22, and when IGFBP2 was combined with CCND1 or RBM11. The k-mer based transcriptomic approach to antisense drug target identification is effective, as it consistently identified gene targets with dose dependent effects on EC50 and FC. The in vivo assay setup resulted in two synergies and antagonism, which are as yet not predictable. Synergistic combinations of effective targets hold significant potential to identify highly effective anticancer therapy (HEAT), however, care must be exercised in selecting optimal combinations.
Genetically engineered rodent models (i.e. gene knockout (KO)) have been critical tools in early safety de-risking of drug development. Temporally controlled conditional KOs (cKOs), where the loxP-flanked gene of interest is deleted at a given time point by use of a tamoxifen (TAM)- or tetracycline-driven Cre recombinaise (Cre), can bypass developmental phenotypic influence and be beneficial to understand target safety in the intended patient population. However, there are often not available cKOs or phenotypic analysis for novel targets, and the appropriate model needs to be developed. For target safety assessment of gene X, we recently generated a cKO mouse using a CAG-Cre strain (loxP/loxP; CAG-Cre). Cre negative mice with the loxP-flanked gene X (loxP/loxP; CAG-Cre) served as control animals. Both cKO and control mice (12-14 week-old) were dosed with TAM at 400 mg/kg food for 3 weeks. Following a 2-week wash out period, there were no differences in clinical observations, hematology, and chemical between cKO and controls. However, the cKO had minimal to marked pancreatic acinar atrophy. To understand whether pancreatic findings in the cKO were due to the deletion of gene X or Cre activity, we repeated the study by having additional control animals, which are Cre positive mice without loxP-flanked gene X (wt/wt; CAG-Cre). Pancratic lesions were found in TAM-treated Cre positive mice with or without loxP-flanked gene X. However, control Cre positive mice without loxP-flanked gene X or TAM-treated Cre negative mice with loxP-flanked gene X had no pancreatic findings. These results suggested that pancreatic lesions were related to Cre activity but not the deletion of gene X. Additionally, our historical data revealed that pancreatic acinar atrophy is likely to be a Cre strain specific since these findings were not noted in TAM-treated Rosa26-Cre mice without knocking out the target gene. Our results highlight the importance of including proper control groups for phenotypic analysis when using cKO models to differentiate effects of non-specific background lesions (i.e. Cre activity) from the effects of knocking out the target gene.

Thrombocytopenia in Cynomolgus Monkeys Induced by a Therapeutic Human Monoclonal Antibody


In pre-clinical cynomolgus monkey studies, decreases in circulating platelets were observed within 24 h following a single intravenous administration of mAb X, mAb Y, or mAb Z (monoclonal IgG1 antibodies with a modified IgG1 tail lacking effector function) at several doses tested (0.5, 1.5 and 10 mg/kg). In some animals the decrease in circulating platelets continued over several days/weeks and some animals developed thrombocytopenia. The effect was not dose-dependent, but platelet numbers recovered immediately once the antibody was cleared. All three antibodies bind to the same target, with mAb Y binding to a different overlapping epitope compared to mAb X and mAb Z. The target of these antibodies was known to be expressed on both human and cynomolgus monkey platelets. Treatment with the IgG1 wildtype isotype of these antibodies resulted in activation of platelets in vitro, as determined by upregulation of CD62P and activation of GPIb/IIa receptors. It was demonstrated that addition of Fc block or engineering the isotype of these antibodies resulted in activation of platelets. It was demonstrated that addition of Fc block or engineering the isotype of these antibodies resulted in activation of platelets. It was demonstrated that addition of Fc block or engineering the isotype of these antibodies resulted in activation of platelets.

Drug-induced mitochondrial toxicity (DIMT) has gained growing interest during the early stage of drug discovery and development. Accumulating evidence implicates drug-induced mitochondrial dysfunctions as a leading cause of undesired side effects and/or off-target toxicities. Hence early-stage identification of compounds that cause mitochondrial dysfunction is informative during lead optimization and ultimately candidate selection. Glycolysis/Galactose Assay (GGA) has been traditionally employed to assess DIMT, but suffers from technical feasibility issues: low sensitivity and specificity to identify clinically relevant toxicants. Pharmaceutical companies recently employ assay systems to provide more feasible information of DIMT using a cell-based Seahorse XF96 Analyzer (Seahorse assay) and an isolated mitochondrial assay system for mitochondrial toxicity (iMitox). In this study we compared performance across assay systems using a selected set of 30 commercially available compounds with suspected mitochondrial toxicity and clinical adverse events, with the objectives to increase sensitivity and specificity in detecting DIMT and thus to facilitate risk categorization of each compound. A quantitative analysis with defined thresholds was applied for each assay and the results were compared across all Seahorse assay, iMitox and the existing GGA. While 11 out of 30 compounds showed the same risk category (6 low, 5 mito-tox) in all 3 assays, 4 out of 30 that displayed cytotoxic effects in GGA were categorized in low risk in both Seahorse assay and iMitox. Fifteen out of 30 were identified as responders based on oxygen consumption rate (OCR) compared to DMSO vehicle control by both (5), or either Seahorse assay (7)/ iMitox (3), respectively. These results demonstrate that iMitox and Seahorse assay replicate each other, as each screening system provides higher sensitivity, specificity and confidence in identifying potential
Drug-induced irregular heart beat (pro arrhythmia) and/or changes in contractility (inotropic liability) can limit the utility of potential novel therapeutics. Since abnormal ventricular repolarization can cause not only electrical disorders, but also affect the heart’s contractile function, the main motivation of this study was to develop a new model based on adult human primary cardiomyocytes to provide a preclinical tool for the simultaneous assessment of drug-induced pro-arrhythmogenic and pro-inotropic effects. To facilitate the scalability of the model to high throughput methodologies, we recorded fractional sarcosome shortening (SS) using a digital, cell geometry measurement system (IonOptix®) and then record changes in the contractility transients to infer both isotropic (SS) as well as pro-arhythmogenic risk (aftercontraction (AC), contractility escape and time to 90% relaxation). To address the clinical relevance of this approach, we performed a validation study in which we assessed the effects of a set of reference drugs with known clinical outcomes. Both positive and negative controls were selected, including 23 torsadogenic and 10 non-torsadogenic drugs. The approach provided very reliable prediction of pro-arhythmogenic and isotropic risks. For example, we found that AC incidence with doxetilide, a torsadogenic drug, was seen starting at 10x the free Effective Therapeutic Plasma Concentration (fETPC), while verapamil, a non-torsadogenic drug, did not induce ACs up to the highest possible of fETPCs tested in our study (222x). When the assessment of drug risk was based on effects observed at 10x the fETPC, human cardiomyocyte-based model differentiates between torsadogenic and non-torsadogenic drugs with excellent assay sensitivity and specificity values: 96% and 100%, respectively. Our data also demonstrate that human cardiomyocytes also identify drugs associated with inotropic effects. hERG channel blockers, like dofetilide, have no effects on SS, while multi-channel blockers, like verapamil, inhibited SS. Thus, adult human primary cardiomyocytes can simultaneously predict risks associated with pro-arrhythmogenic and inotropic activity. This approach enables the generation of reliable and predictive data for human cardiac safety assessment in early drug discovery, and appears to be more predictive than the stem cell-derived cardiomyocyte models.
gate the link between off-targets and toxicological effects. With the off-target module, potential off-targets can be predicted for any of the 20,000 reviewed, human proteins in UniProt. The predicted off-targets combined with a TargetTissueSA are an useful approach to elucidate toxicities that might not be target-related.

1549 An Unanticipated Toxicity Profile of Anti-Fucosyl-GM1 Antibody Conjugated to a Tubulysin Payload after a Single-Dose Intravenous Administration to Cynomolgus Monkeys

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Fucosyl-GM1, a sphingolipid monosialoganglioside and tumor-associated antigen (TAA), has been detected in about 72% of small cell lung cancer (SCLC), 24% of adenocarcinomas, and 14% of squamous cell carcinomas, while its expression in normal tissues is almost non-existent. Potential new treatments for SCLC include the use of conjugated monoclonal antibodies. Here we describe the toxicity of a novel antibody-drug conjugate ADC consisting of a fully human IgG1 antibody linked to a small molecule microtubule polymerization inhibitor via a site-specific conjugation (bacterial transglutaminase) with drug-antibody ratio of 4. The objective of this study was to evaluate the toxicity of BMT-125961 when given as a single dose to cynomolgus monkeys and to determine changes in peripheral nerve conduction velocity. This ADC was administered as a single intravenous injection at doses of 1, 4, and 8 mg/kg to 3 groups (mixed females and males) of monkeys. Parameters evaluated included clinical observations, body weight, food consumption, peripheral nerve conduction, clinical pathology, gross and microscopic pathology, and serum toxicokinetics. Serum exposure (AUC (0-infinity)) was approximately dose proportional with a profile consistent with ADC experience. Observed clinical effects included redness at injection site progressing to other areas with posterior black blotching. Persistent cough was observed at 8 mg/kg beginning on Day 38. Reduced food consumption and sustained body weight loss, particularly at 8 mg/kg (~10%). There was a progressive increase in serum AST (max @8.7X) and ALT (max @2x) on Days 4 and 15 with partial recovery on Day 21, at 4 and 8 mg/kg. Hematologic indicated an inflammatory process beginning Day 9 or 21 at the same doses, with vacuolated monocytes evident in peripheral blood smear. Unexpected pulmonary effects presented grossly as consolidation and reddening of ventral (gravity-dependent) lung lobes with a very clear delineation between normal and abnormal lungs. Histologically, the consolidated areas of lung were characterized by fluid expansion of alveoli (exudative alveolitis) and granulomatous foci with vacuolated macrophages and fibrosis. There were no effects on peripheral nerve conduction velocity.

1550 New iPSC-Based Neural In Vitro Approach for Seizure Liability Testing


Standard animal models have shown insufficiencies in predicting adverse effects of therapeutic compounds particularly on the CNS, mostly because of limited concordance with human neurotoxicity. The high attrition rate of new drugs in clinical studies is largely attributed to CNS-related safety failures unidentified in pre-clinical testing. One of the most common issues encountered during safety assessment over the past 5 years is the induction of seizures at relevant therapeutic concentrations. Moreover, GABA	extsubscript{A} receptors as primary mediators of inhibitory neurotransmission, represent a vulnerable target for neurotoxic environmental chemicals, many of which lead to seizures in humans. Therefore, new human in vitro assays are urgently needed for de-risking of new drugs and higher throughput testing of multitude of chemicals with daily human exposure. Based on direct reprogramming of induced pluripotent stem cells (iPSCs) into highly functional neurons of defined subtypes, we developed a pure human neuronal/glial co-culture platform for comprehensive electrophysiological measurements using multi-electrode arrays (MEAs). When combining defined ratios of glutamatergic to GABAergic neurons together with astroglial cells, robust neuronal activity, including synaptically driven spontaneous synchronized network bursting can be recorded at 3-4 weeks post seeding. Due to parallel acquisition of multiple parameters, our platform allows detailed characterization of neurotoxicity effects of test compounds. Here, we optimized our platform to specifically and quantitatively assess chemically-induced seizure-like activity in a semi high-throughput setting. As part of the HESI NeuTox Group, we tested a set of 8 compounds with clinically reported seizurogenic effects in patients, which parameters remained detected in iPS-based testing.

Importantly, our human system was able to identify specific alterations in neuronal activity of all test compounds in a dose-dependent manner, and determine seizure-like firing patterns in the most potent subset. We also used our platform to test a set of environmental neurotoxicants with well-established effects on human GABA	extsubscript{A}Rs, and successfully identified changes in activity indicative of seizurogenic effects over a large range of concentrations. Hence, we report the development and proof of concept of a novel iPSC-based neuronal/glial in vitro approach for the assessment of seizure liabilities of chemical compounds in a human-relevant cell context.

1551 Improved Prediction of Arrhythmia Potential in Drug Discovery and Development


Predicting the potential to be associated with the arrhythmia Torsades de Pointes (TdP) is a key activity in early drug discovery. Classically, a 30-fold margin between therapeutic concentrations and the inhibitory potency at the hERG potassium channel is used to predict arrhythmic potential. Kramer et al (2013) showed a mixed ion channel inhibition approach improved the prediction. The best model described was the ratio between the IC50 values at the hERG channel and the L-type calcium channel. This was “Model 5” and had a sensitivity of 97%, specificity of 83%, and an overall accuracy of 91%. “Model 5” does not take to account therapeutic concentrations, target potency, or the non-linear relationship between concentration and effect. We illustrate two techniques that perform as well or better than “Model 5” but take in to account relative potency and channel balance. Using the “Model 5” data set, we calculate the changing balance across concentrations, as a net inhibition. Net inhibition at 25-fold, the therapeutic concentrations as a predictor of TdP had an accuracy of 93%, a sensitivity of 94%, and specificity of 91%. The two false positive and false negative calls had very close net degrees and would be susceptible to errors in the estimates of ion channel potency or the contribution of metabolites. Net inhibition is more predictive than “Model 5” and other recent predictors and is analogous to the net current metric recently suggested as a measure by others using an in silico cardiac model. “Model 5” could have an advantage early in drug discovery, as it does not require knowledge of the therapeutic concentrations. We applied another intuitive triaging process, Fast Frugal Trees. This requires information on hERG and calcium channel potency along with an in vitro measure of target potency. All such data are available early in discovery. It has an accuracy of 91%, with sensitivity and specificity of 91%. This is better than previously published predictors of TdP, as good as “Model 5,” and is compatible with the discovery process. In summary, the net inhibition metric takes in to account the therapeutic levels and nonlinear relationship between response and concentration, and is an effective predictor of TdP potential and does not require the use of an in silico model. Early in discovery, Fast Frugal Trees can also be used to make quick decisions on compound progression.

1552 Transcriptomic Biomarkers to Assess the Liver and Metabolic Responses Associated with Bioactivation Mechanisms of Drug-Induced Liver Injury


Drug induced liver injury (DILI) is a major reason for attrition, denied commercialization, marketplace withdrawal and restricted prescribing in the marketing of new pharmaceuticals. Metabolic bioactivation is thought to contribute to a significant number of liver associated adverse drug reactions (ADR) in humans that fail to be detected in standard preclinical animal studies. A challenge for pharmaceutical drug safety is to identify which drug candidates form chemically reactive metabolites (CRM) that pose a high risk in the clinic and projected therapeutic doses. We developed a rat liver transcriptional biomarker and an in vitro cellular model, to discern doses of drugs with bioactivation mediated hepatotoxic potential, by measuring transcriptional pathways activated by electrophilic CRMs. Here we describe establishment of this system using benchmarked CRM forming test compounds and systems using a curated list of commercial drugs and internal Merck compounds anchored in clinical experience with respect to ADRs and hepatotoxicity. Based on 130 compounds run in short-term rodent studies and consideration of the clinical dose administered, the approach yielded 33% sensitivity and 95% specificity for discriminating safe from hepatotoxic
Bioengineered let-7c is Effective at Reducing Orthotopic Hepatocellular Carcinoma Tumor Burden and is Well Tolerated in Mouse Models


Given pleiotropic molecular targeting mechanisms, restoration or inhibition of endogenous microRNAs (miRNA) is a promising pharmacological strategy. Recently, our group has bioengineered a collection of miRNA agents in live cells using a non-coding carrier RNA (nCAR) based technology. In vitro screening identified nCAR/let-7c as the most potent at inhibiting hepatocellular carcinoma (HCC) viability; this was found to be processed to the mature let-7c molecule and suppressed target gene expression. As such, in vivo efficacy and safety of this agent was characterized in the present study using polyethyleneimine (PEI) and liposomal-polyplex (LPP) RNA formulations. Huh7 cells stably expressing luciferase and green fluorescent protein were orthotopically engrafted into immunocompromised mice. To evaluate efficacy, 40 μg of nCAR/let-7c or a truncated control agent was administered systemically every other day for two weeks, followed immediately by necropsy. Live animal bioluminescent and ex vivo fluorescent imaging suggested that LPP-formulated nCAR/let-7c reduced tumor burden to a greater degree than all controls and PEI-formulated nCAR/let-7c. Additionally, both nCAR/let-7c and truncated control agents were well-tolerated compared to untreated mice, as evidenced by gross evaluation and similar body-weights throughout the study; however, further studies are warranted in immunocompetent animal models to elucidate the complete toxicological profile and immunogenic potential of nCAR/let-7c. These data are the first to demonstrate that let-7c restoration using a biologically-derived agent can serve as a potent antineoplastic agent, presenting a new, clinically-relevant strategy for HCC therapy.

Seizure Liability Assessment in Human IPS-Neurons Using Microelectrode Array and High-Throughput Screening via Calcium-Flux

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New, higher throughput predictive models are always required to address the evolving needs of preclinical R&D and safety assessment. Human neurons derived from induced pluripotent stem cells present a predictive, flexible and potent tool due to their unlimited availability, physiological relevance and lack of interspecies translatability issues. iP5-derived neurons express a plethora of neurotransmitters at physiologically-relevant levels. This can help to accelerate the assessment of clinically relevant issues such as psychological disorders, drug-induced seizure liability, peripheral neuropathy, and other CNS-related issues. Here we present two models that take advantage of instrumentation featuring 96 (or 384) well plates with automated compound handling/robotics and measurements for a cost-effective and animal-free neurological phenotypic assessment. 1. 96/384 well optical readout: calcium transient flux induced by neurotransmitters using the Hamamatsu μCell. Here we show that multiple monoamine neurotransmitters, glutamate, NMDA, acetylcholine, etc. induce increases a fluorescent calcium reporter in a concentration-dependent manner. 2. 96 well electrical (extracellular field potential) recordings: three separate neuronal populations were co-cultured with isogenic IPS astrocytes until mature, synchronous burst firing was achieved. The effects of 9 seizure-related compounds (as part of the HESI Neutox MEA consortium) on the neuronal activity using the Axion Maestro MEA technology were assessed and results were correlated to established primary rodent models.

Qualification and Deployment of an In Vitro Liver Model (HepatoPac) Used Early in Pharmaceutical Development to Help De-Risk for Drug-Induced Liver Injury


Drug-induced liver injury (DILI) remains a major safety concern for pharmaceutical development despite significant efforts across academia and industry to detect DILI early in development. Merck has deployed a set of rat liver transcriptional biomarkers in a short-term in vivo rat study to inform on the potential of drug candidates to generate high liver burden of chemically reactive metabolites (CRM) and to help predict risk for clinical hepatotoxicity. Here we describe a 9-day in vitro assay using micropatterned human and rat hepatocyte co-culture systems (HepatoPac®) as a compound, animal and time-sparing approach to de-risk compound series shown to have high CRM burden in vivo. We established a transcriptional biomarker signature in vitro, and qualified the use of the signature in the HepatoPac models using 90+ commercial drugs and Merck internal compounds, including liver safe and unsafe analogs. The assay showed a high concordance (80%) with results obtained in vivo with the same compounds. Taking into the consideration calculated human liver in vitro clinical exposures, the in vitro rat model provides ~80% sensitivity and ~90% specificity for distinguishing liver safe from hepatotoxic drugs. While overall performance between the in vitro human and rat models was similar, appreciable differences were observed for several compounds, suggesting species differences in drug-metabolic pathways, turnover rates, and/or xenobiotic responses that could drive discrepant biomarker responses. In conclusion, we show that the HepatoPac micropatterned co-culture liver model is a resource sparing approach to improve DILI risk assessment from CRM early in preclinical development.

In Vivo-In Vitro (IVIV) Correlation of the Response to Prototypical Rat Liver CYP-Inducers—Assessment of Between-Strain, Gender, and Species Variability

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Since the potential risk of liver cytochrome P450 (CYPs) induction to cause drug-drug interactions and adverse effects needs to be considered during the development of any new medication, regulatory studies using primary human hepatocytes are included as part of the regulatory package requested during drug development. Toxicology studies performed in preclinical species often suggest a species-dependent response of liver CYPs, it is thus appropriate to assess which test species is closest to humans (if any) and should be chosen for pivotal toxicological studies. The between-species variability, including human, can be assessed in vitro using primary hepatocyte cultures, as long as IVIV correlation in preclinical species has been confirmed. In the present investigation the induction potential of prototypical CYP1A (β-naphthoflavone), 2B (phenobarbital), 2C (3a-demethasone) and 4A (bezafibrate) inducers was revealed by the increases in mRNA expression and isozyme-specific activities, both after in vivo treatment of male Sprague Dawley (SD) rats and after in vitro treatment of primary male SD rat hepatocytes cultures. Strain, gender and species differences (mRNA induction) were effectively evaluated in vitro primary cell cultures: for example the response of SD rat hepatocytes was higher to both phenobarbital (10-fold higher CYP2B2 induction) and bezafibrate (2-fold higher CYP4A induction) compared to Wistar rat hepatocytes; the response of males was higher than females (Wistar) to phenobarbital and demethasone (3-fold higher induction of respectively, CYP2B and CYP3A); the response of rodents was higher than non-rodent species to bezafibrate (4- to 100-fold higher CYP4A induction). CYP3A response to rifampicin compared to demethasone was higher only in human hepatocytes, with large inter-donor variability in the response. In conclusion, this approach allows not only in vitro-in-vivo extrapolation (IVIVE), but it also offers a potential significant reduction in the number of laboratory animals needed for drug research, in accordance with the 3Rs (Refinement, Reduction, Replacement).
The disposition and clearance of drugs by the kidney relies largely on a well-characterized subset of membrane transport pumps collectively known as solute carrier or SCL proteins. Among the SLC family proteins, OAT1, OCT2 and OAT3 are the most important transporters in kidney tissue, and are recommended by the FDA, ITC and the EMA as targets for drug-drug interaction studies. Therefore, there is a large demand for kidney toxicity models, especially in-vitro models, that have a normal human kidney origin, functioning transporters, accurate clinical predictability, and consistent data output for initial drug interaction studies. Unfortunately, primary renal epithelial cells lose OAT1 and OCT2 transporter expression over time in culture. Transiently expressing these transporters in primary renal epithelial cells yields large variations between experimental models making data hard to interpret. Current cell line-based models are available using MDCK, CHO, U2OS, or other lines which either do not have human kidney tissue origin or are themselves cancer lines, significantly compromising clinical predictability. In our study, we have generated kidney transporter cell models using well-characterized hTERT-immortalized primary Renal Proximal Tubule Epithelial Cells that stably overexpress either the OAT1, OCT2 or OAT3 gene. After confirming the SLC mRNA expression for each gene by RT-PCR, we performed immunostaining that showed OAT1, OCT2 and OAT3 were only trafficked to the plasma membrane. Notably, these clones show typical epithelial morphology, functionality, and expression of the appropriate epithelial and kidney tissue specific markers. Most importantly, we verified that the overexpressed transporters have normal transport activities using 5-CF, 6-CF, ASP+, and EAM5+ assays. Furthermore, uptake of these compounds are blocked in a dose-dependent manner by well-known SLC inhibitors, indicating that the overexpressed kidney transporters are functioning as expected. Overall, our data demonstrates that these modified renal epithelial cell lines maintain kidney transporter expression over time, are useful tools, and can provide physiological and human kidney function, and are more consistent and reliable than comparable in-vitro models currently used to determine the effect of exogenous compounds on renal membrane function. 

The availability of human primary cell-based 3D small intestinal (SMI) microtissues that recapitulate the structure and function of the in vivo counterpart is critical to predict the safety and bioavailability of drugs intended for oral administration. Here we present drug permeability of a newly developed in vitro 3D-human small intestinal (SMI) microtissue model. The SMI microtissues are cultured using primary human intestinal fibroblasts and enterocytes and their 3-dimensional polarity and morphology mimics that of native in vivo tissues. Characterization of the microtissues included evaluation of structural features, barrier properties, and expression of drug transporters and drug metabolizing enzymes. To evaluate the suitability of the microtissues for drug absorption, the apparent permeability coefficients (Papp) for a panel of 18 benchmark drugs with known human absorption values were determined. Drug-drug interactions were examined using compounds known to be substrates or inhibitors of efflux transporters. Results showed that the microtissues are highly reproducible with physiological TEER values averaging 146±20.8 Ω*cm² (% CV: 14.2%) and 142±13.8 Ω*cm² (CV:9.7%) in the US (N=128 lots) and Slovakia (N=9 lots) facilities. The microtissues expressed drug transporters and metabolizing enzymes known to be present in vivo. Drug permeation results showed that the microtissues could discriminate between low and high permeability drugs with 94% accuracy. The in vitro Papp values correlated well with human absorption data (r² = 0.85). SMI microtissues exposed to drug efflux transporter substrates and inhibitors reduced the drug efflux ratio while increasing the bioavailability of the test drug, providing evidence of drug efflux transporter activity. In conclusion, the SMI microtissues appear to be a useful pre-clinical tool for predicting drug activity and bioavailability of orally administered drugs. 

Lineozid (LZD) is an oxazolidinone with potent activity against drug-resistant M.tuberculosis; however, prolonged courses and high doses of LZD therapy are associated with adverse effects resulting from LZD-induced mitochondrial toxicity (MT). We have previously developed a panel of in vitro assays that encompass three major adverse events resulting from MT namely (1) energy metabolism disruption, (2) increased oxidative stress, and (3) altered apoptosis. We utilized the same panel to test LZD-associated MT in K562 (chronic myelogenous leukemia) cells treated with 0, 2.5, 5, 10 and 20 mg/L of LZD for two weeks in culture. Cells were harvested on Days 2, 4, 7,9,11 and 14 following LZD exposure, lyed and evaluated using assays detecting oxidative phosphorylation (OXPHOS) complex 1-5 levels, ATP, total glutathione, ROS/RNS and caspase 3 content. LZD treatment resulted in a panel of 1 in vitro assays that encompass three major adverse events resulting from MT namely (1) energy metabolism disruption, (2) increased oxidative stress, and (3) altered apoptosis. We utilized the same panel to test LZD-associated MT in K562 (chronic myelogenous leukemia) cells treated with 0, 2.5, 5, 10 and 20 mg/L of LZD for two weeks in culture. Cells were harvested on Days 2, 4, 7,9,11 and 14 following LZD exposure, lysed and evaluated using assays detecting oxidative phosphorylation (OXPHOS) complex 1-5 levels, ATP, total glutathione, ROS/RNS and caspase 3 content. LZD treatment resulted in a 40-90% decrease in OXPHOS Complex 1, 3, and 4 and levels in K562 cells over controls. While ATP and caspase 3 levels were marginally elevated post LZD treatment, the oxidative stress biomarkers were similar to those reported in controls. We also characterized the LZD induced MT in K562 cells cultured in either glucose- or galactose-based medium for two weeks. This was done to ensure that the MT biomarkers determined were not misinterpreted, as cancer cells have the ability to bypass OXPHOS cycle to generate ATP through glycolysis in glucose rich conditions. Similar results were obtained for toxicity evaluations conducted in both media regardless of the energy source. In addition, we evaluated whether secondary substrates of MT such as OAT1 and OAT3 were affected. Both substrates were conserved in K562 cells over the duration of the treatment. K562 cells were cultured for 14 days with 20 mg/L of LZD. On Day 14, treatment drug was removed in one subset of K562 cells and these cells were allowed to propagate in LZD-free culture medium for an additional two weeks. LZD treatment was continued for a second subset of cells until the end of study. All MT effects were resolved in K562 after discontinuation of LZD treatment, two weeks after therapy cessation. Overall, our panel of in vitro assays
characterized the LZD-induced MT profile in K562 cells and this information can be used as a benchmark for MT screening for future generation oxazolidinones. Work supported by NIAID Contract HHSN272201400006I and Lifespan/Tufts/Brown Center for AIDS Research (CFAR), an NIH/NIAID/ Miriam Hospital funded program P30-AI-042853.

1561 H/R Stress and Circumventing the Crabtree Effect Enhance Susceptibility of HepG2 to MPT-Inducer (troglitazone)


Troglitazone (TRO), developed as an antidiabetic agent that improves insulin resistance, withdrew from the market because of its rare but severe liver injury. In our line of study, it was found that 1) TRO induced mitochondrial permeability transition (MPT), a trigger of apoptosis, in isolated rat liver mitochondria (1), 2) activation of Ca2+-independent phospholipase A2 was involved in TRO-induced MPT (2), 3) Hypoxic reperfusion (H/R) stress sensitized rats to diclofenac (MPT inducer)-induced liver injury. However, it has yet been elucidated whether TRO-induced MPT leads to apoptotic cell death and that is exacerbated under H/R condition. In this study, we tried to verify these hypotheses using HepG2 cells. To increase detection of drug-induced mitochondrial effects, HepG2 cells were forced to rely on mitochondrial oxidative phosphorylation rather than glycolysis by substituting galactose for glucose in the growth media. To reproduce H/R stress in vitro, HepG2 cells were exposed with 1% O2 condition (hypoxia for 4 h) and then substituted with 20% O2 condition (re-oxygenation). Generation of reactive oxygen species (ROS) was assessed by dichlorodihydrofluorescein diacetate (DCFDA). Cellular MPT induction was detected by mitochondrial Ca2+ retention assay. Cell death was assessed by release of LDH and by co-staining of Annexin V/Zombie dye. In galactose-substituted medium, generation of ROS was promoted by H/R condition. Because oxygen concentration is raised drastically under H/R condition, mitochondria might accordingly induce ROS through its activated respiratory chain. Moreover, under H/R condition, TRO-induced-MPT was further enhanced compared with that under normoxic condition. Furthermore, TRO-induced increase of Annexin V positive cells was enhanced by H/R condition. These observations are suggested that H/R condition-induced ROS production enhances susceptibility to MPT induction by TRO and therefore exacerbates apoptosis cell death in HepG2 cells. Substitution of sugar source with galactose and simulating H/R condition increase sensitivity of hepatocytes to TRO-induced MPT and apoptosis in vitro likely as a result of increased mitochondrial oxidative stress. References: (1) Masubuchi Y. et al. Toxicology 222, 233-239. (2) Okuda T. et al. Toxicol. Appl. Pharmacol. 248, 242-248.

1562 Exploration of Biomarkers for Predicting Drug-Induced Liver Injury in Co-Culture Systems of Human Peripheral Blood Mononuclear Cells with Hepatocellular Carcinoma-Derived Cells

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Prediction of drug-induced liver injury (DILI) risk remains a major challenge because of its multifactorial mechanisms. Immune and inflammatory reactions are considered one of the mechanisms for DILI; however, biomarkers in vitro systems using immune cells have not been comprehensively studied yet. In this study, we aimed to find potential biomarkers for predicting DILI in in vitro co-culture systems of peripheral blood mononuclear cells (PBMCs) with human liver cell lines. HepG2 or differentiated HepaRG cells were seeded onto the lower compartment of the Transwell® insert system and, after 24 h, freshly isolated human PBMCs were added onto the upper compartment. Immediately after the PBMC seeding, the co-cultured cells were treated with DILI-positive drugs (amiodarone, ketoconazole, tiotBABIC acid, diclofenac, and trovafloxacin) or DILI-negative drug (chloroquine, mebendazole, ethacrinic acid, lactulose) for 24 h. Then, total RNA was isolated from PBMCs and analyzed using the Agilent SurePrint G3 human gene expression microarray. Overall, 160 and 50 genes were upregulated more than 2-fold in PBMCs/HepG2 cells and PBMCs/HepaRG cells, respectively, by the treatment with 5 DILI-positive drugs. By excluding genes that were upregulated by the treatment with 5 DILI-negative drugs, 113 and 42 genes were extracted. Among them, five genes were commonly upregulated between PBMCs/HepG2 cells and PBMCs/HepaRG cells, however, quantitative PCR could not validate the microarray data probably due to their low expression in PBMCs. Next, we extracted upregulated 476 genes from PBMCs/HepaRG cells by the treatment with 3 or more out of 5 DILI-positive drugs. By considering expression levels in intact PBMCs with reference to a database, 9 genes were selected and 6 genes were correctly validated. These included IL-1α, IL-1β, and IL-8. Taken together, we establish co-culture systems of PBMCs with liver cell lines and suggested that selected biomarkers might correctly predict DILI risk in the in vitro assay. Ongoing investigations to understand differences in responses between fresh and frozen PBMCs also will be described.

1563 Profiling of Liver Toxicity Protein Biomarkers in Rat Plasma by Mass Spectrometry

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Pre-clinical assessment of pharmaceutical compounds for regulatory submission is performed using classical toxicological analyses. However, ‘omics technologies may offer a quick readout of a compound’s potential for liver toxicity. We have previously demonstrated a proteomic signature for prediction of DILI biomarker assays from rat plasma in which >90% of 83 biomarkers measured such as apolipoproteins and fumarylacetoacetase predicted Acetaminophen (APAP)-induced hepatotoxicity. The objective of the current study was to examine the performance of this assay using compounds with varying hepatotoxicity mechanisms while considering other contextual factors such as the fed-state of the rats. Rats were treated orally with dose vehicles or a single high dose of different hepatotoxic or nephrotoxic compounds. Groups were included to compare fed-state of the animals prior to and during treatment. Protein biomarker data from rat plasma as measured by LC-MS/MS was assessed using multivariate statistical analysis to categorize the treatments. We found the changes in major secretory proteins were highly-associated with APAP toxicity. We also found that changes in biomarkers for APAP toxicity did not overlap substantially with biomarker profiles for other drugs used in the study - including Buspirone, Finasteride, Fluamamide, and Nefazodone. While biomarker profiles differed from the treatment group, we noted overlap between compounds with varying hepatotoxicity mechanisms. We found that changes in biomarkers for APAP toxicity did not overlap substantially with biomarker profiles for other drugs used in the study - including Buspirone, Finasteride, Fluamamide, and Nefazodone. While biomarker profiles differed from the treatment group, we noted overlap between compounds with varying hepatotoxicity mechanisms. We found that changes in biomarkers for APAP toxicity did not overlap substantially with biomarker profiles for other drugs used in the study - including Buspirone, Finasteride, Fluamamide, and Nefazodone.
recruitments in our case were predominately comprised of M1 macrophages. Pro-inflammatory condition of monocytes was likely to be associated with thrombus formations. Although there remain additional investigations to be done, characterization of whether pro-inflammatory- or tissue repair-types of macrophages provided clue for further elucidation of mechanisms of action.

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Bacterial mutagenicity, absorption, thereby increasing efficacy and reducing systemic toxicity. Breast cancer drugs may increase local delivery and reduce systemic dose levels with the aim to identify NOAELs and/or LOAELs. Knowledge of the pharmacokinetic profile in the selected animal species is key to decide suitable in vitro photo-safety testing.

41 systemic photo-LLNA studies (7 reference compounds, 34 drug candidates) demonstrated the utility of this approach. Phototoxicity in vivo is clearly a dose-dependent effect. The applied level of simulated sunlight (normalized to 10 J/cm² UVA) is sufficient to elicit phototoxic responses using reference compounds and corresponds well to typical sunlight exposure (Bau50). Therefore, NOAEL-derived safety margins versus therapeutically relevant drug levels based on Cmax are an appropriate method to support human risk assessment and are regulatory accepted (ICH S10, 2013). In some cases, e.g. for drugs with very limited systemic exposure, even the IC50 value obtained under irradiation in the in vitro 3T3 NRU Phototoxicity Test (OECD TG 432) can be used to estimate the margin versus therapeutically relevant drug levels and to support risk assessment in a weight-of-evidence approach.

In comparison to oral or iv administration, direct topical application of breast cancer drugs may increase local delivery and reduce systemic absorption, thereby increasing efficacy and reducing toxicity. Bacterial mutagenicity, in vitro clastogenicity, in vitro phototoxicity, and in vivo dermal sensitization studies were performed to evaluate the safety of a gel formulation of endoxifen being developed for breast cancer prevention. E2/EE2-endoxifen was not mutagenic in four strains of S. typhimurium or in E. coli WP2 uvrA, either with or without metabolic activation; positive control articles were mutagenic in all tested strains. E2/EE2-endoxifen did not induce structural chromosomal aberrations in Chinese Hamster Ovary cells, either with or without metabolic activation; two positive control articles did demonstrate significant clastogenicity. E2/EE2-endoxifen was not phototoxic in the neutral red uptake assay in BALB/c 3T3 fibroblasts; the positive control article did induce significant phototoxicity. Dermal sensitization was evaluated in female Hartley guinea pigs (modified Buehler method) using four study groups (vehicle control gel: 0.5% (E/Z)-endoxifen gel; 1.0% (E/Z)-endoxifen gel; and 5% (E/Z)-4-dinitrochlorobenzene; DNCB); DNCB caused erythema well-defined to severe erythema in all animals. Possible evidence of dermal sensitization in endoxifen-treated groups was seen at 24 h after the first challenge very slight to well-defined erythema in 5/10 and 4/10 animals in low and high dose groups versus very slight edema in 1/10 vehicle controls). After rechallenge, very slight edema was seen at 24 h in 4/10 vehicle control animals and in 4/10 animals in groups receiving low or high doses of (E/Z)-endoxifen gel. These data demonstrate that (1) (E/Z)-endoxifen is not mutagenic in a bacterial (Ames test) battery that is used widely for mutagenicity evaluations; (2) (E/Z)-endoxifen does not induce chromosome aberrations in a mammalian cell system that is commonly used to identify clastogenic agents; and (3) (E/Z)-endoxifen is not phototoxic in a standard in vitro assay. The possibility of weak sensitizing activity of (E/Z)-endoxifen gel appears to be caused by the component of the gel vehicle rather than by (E/Z)-endoxifen itself. [HHSN2612015000241]

The recent change by European authorities in legislation regarding the minimum housing requirements for large animals on safety assessment studies has highlighted the need to refine certain practices. Historically, for infusion studies using a surgically implanted catheter, single housing was accepted since few alternative products were available. In order to better comply with European requirements as well as to ensure the highest standard in terms of animal welfare, our laboratory developed an ambulatory model for infusion studies in large animals. Four cynomolgus monkeys were surgically implanted in the femoral vein as per standard procedures, with the exception that the catheter length was shortened as the animals were not tethered. The catheter was exteriorized into a jacket backpack, which also held the pump and the infusion bag.

Animals were group housed and recovered from surgery for 12-14 days with a saline intravenous infusion maintenance rate of 2 mL/hr. The rate was then increased to 2.5 mL/kg/hour, a rate commonly used for continuous infusion toxicology studies, and animals were administered saline continuously (24 hrs/day) for 28 days. The infusion was then stopped and animals underwent 4 weeks of once weekly intermittent infusion at 20 mL/kg/hr for 1 hr, and then 4 weeks of once weekly intermittent infusion at 0.2 mL/kg/hr for 4 hrs. Clinical condition was monitored daily and body weight once weekly. To evaluate any effect of the additional weight carried by the animals, heart rate, blood pressure, body temperature and activity level were monitored by telemetry for 24 hrs with incremental weights (corresponding to different sizes of infusion bags) placed into the jacket backpack. Accuracy of the monitoring equipment was tested at each occasion of dosing. There was no abnormal clinical sign noted, and no significant changes in body weights. Weights between 330 and 730 g in the backpack had no impact on any of the physiological parameters measured. Group housing of the animals was successful for the duration of the project, and no damage to the jackets or pumps was observed. Less repair surgeries were required than with the tethered approach, which suggests this model is more stable. Overall, these results show that the ambulatory system can be successfully used for both continuous or intermittent intravenous infusion studies for a duration of up to 4 weeks in the monkey while improving the welfare of the animals.

Phototoxic properties of systemically applied pharmaceuticals may be the cause of serious adverse drug reactions. Despite being clinically manageable in principle, they can limit clinical use of a drug depending on the indication. Protective measures against sunlight can be applied very reasonably during a few days but may not be practicable for chronic treatments. Thus, both patients and health authorities are unlikely to accept a relevant photosensitization risk, including skin tumors, in such situations. Although definitive clinical testing is an option it is rarely considered as it can only be done late during clinical development and it is significantly more expensive than any preclinical testing (Bauer, Photochem Photobiol Sci, 2016). Thus, a reliable preclinical photosafety assessment strategy combining in vitro and in vivo approaches is usually applied early on. For most drug candidates, photosafety evaluation can be based purely on spectroscopic measurements (Bauer, Regul Toxicol Pharmacol, 2014) and in vitro results (Schümann, Toxicol Sci, 2014). However, a few compounds will need confirmation in vivo, for instance those with oral or nasal presentation. Therefore, NOAEL-derived safety margins versus therapeutically relevant drug levels based on Cmax are an appropriate method to support human risk assessment and are regulatory accepted (ICH S10, 2013). In some cases, e.g. for drugs with very limited systemic exposure, even the IC50 value obtained under irradiation in the in vitro 3T3 NRU Phototoxicity Test (OECD TG 432) can be used to estimate the margin versus therapeutically relevant drug levels and to support risk assessment in a weight-of-evidence approach.
lack of off-target interactions. TK revealed dose proportional increases in AUC and C_{max} over the dose range of 50 to 500 mg/kg and accumulation of mAb114 from the first to last dose interval. The mean terminal half-life for mAb114 ranged from approximately 7 to 15 days. Ex vivo binding to recombinant Ebola glycoprotein was demonstrated in serum and cerebrospinal fluid (CSF), suggesting that mAb114 was pharmacologically active and distributed through the central nervous system via CSF. Based on these data the NOAEL was considered to be 500 mg/kg/week and associated with a mean C_{max} of 24,206 μg/mL and mean AUC of 237,090 day·μg/mL. Simulation of human PK indicated that for the proposed clinical high dose of 50 mg/kg, the safety margins based on the cerebrospinal and AUC were 19-fold and 20-fold, respectively. In summary these data indicate that mAb114 is safe and has a broad therapeutic window.

**1569 Repeat-Dose Toxicity and Metabolism of Topical (E/Z)-Endoxifen Gel in Minipigs**

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When compared to oral or iv administration, direct topical application of breast cancer drugs to the breast may increase local delivery and reduce systemic absorption, thereby increasing efficacy and reducing toxicity. A 28-day repeat-dose study was performed in female minipigs to characterize the toxicity and metabolism of an (E/Z)-endoxifen gel being developed for breast cancer prevention. Groups of 8 Göttingen minipigs received topical (dermal) exposure to E/Z-endoxifen gel (E/Z-endoxifen doses of 2 or 4 mg/day) or vehicle gel only for 28 consecutive days. Gels were applied daily to the same nipple of each minipig; the high dose was applied daily over a dermally doseable to the nipple. In addition, a pharmacology comparator group of 4 minipigs received daily gavage exposure to Z-endoxifen for 28 days. Endpoints included mortality; body weights; clinical observations; local tolerability; clinical pathology; drug and metabolite levels in plasma and liver; and histopathology. Topical application of E/Z-endoxifen gel was well-tolerated by female minipigs: no mortality, clinical evidence of toxicity, local toxicity, body weight effects, changes in clinical pathology parameters, or gross or microscopic findings were observed. Dose-related decreases in absolute and relative ovary weights were seen in both groups receiving topical (E/Z)-endoxifen gel, reflecting the pharmacologic action of this drug. Endoxifen is rapidly glucuronidated in minipigs; with few exceptions, plasma levels of endoxifen were below the limit of quantitation, regardless of route of exposure. Endoxifen glucuronide was measurable in the mammary gland (but not plasma or liver) of minipigs receiving topical (E/Z)-endoxifen gel. By contrast, high levels of endoxifen glucuronide were present in the plasma of minipigs receiving oral exposure to Z-endoxifen. Both endoxifen and endoxifen glucuronide were measurable in the liver of minipigs receiving oral exposure; endoxifen glucuronide was also present in the mammary glands from the oral exposure group. Repeat-dose topical administration of E/Z-endoxifen gel for 28 days was well-tolerated in female minipigs, and resulted in mammary gland levels of endoxifen glucuronide that were comparable to those seen following administration of a much higher oral dose of Z-endoxifen (HHSN2612015000241).

**1570 Preclinical Safety Evaluation of KBP-5074, a Novel Non-Steroidal Mineralocorticoid Receptor Antagonist for the Treatment of Cardiorenal Diseases**


Mineralocorticoid receptor antagonists have been demonstrated to reduce the risk of mortality in patients with heart failure and to decrease proteinuria in patients with cardiorenal diseases. KBP-5075 binds selectively to mineralocorticoid receptors with excellent in vitro antagonistic activity (IC50 2.7 nM). The primary objective of this study was to evaluate the preclinical safety of KBP-5074 in CD-1 mice. Safety pharmacology studies included in vitro and in vivo proarrhythmia assays. The NOAEL was 15 mg/kg. Genotoxicity studies of KBP-5074 included in vitro mutagenesis and chromosomal aberration studies as well as in vivo micronucleus test in CD-1 mice. KBP-5074 demonstrated no genotoxicity potential. A pilot study of KBP-5074 in SD rats was performed to select the dose levels for the IND-enabling one-month oral toxicity study. Once daily dosing of KBP-5074 at 50 mg/kg/day or higher for 14 days was not tolerated. Based on these results, the one-month oral rat toxicity study was performed using 0 (Vehicle control) 2, 6 and 20 mg/kg/day. No apparent drug-related toxicity was observed rats treated with 2, 6, or 20 mg/kg/day. The NOAEL was determined to be 6 mg/kg/day for both male and female rats. A pilot study of KBP-5074 was also performed to select the dose levels for the one-month oral toxicity in Beagle dogs. Once daily dosing of KBP-5074 at 100 mg/kg for 7 days showed significant toxicity findings. Based on these data, the GLP oral dog toxicity study was performed using 0, 2, 6 and 20 mg/kg/day. The NOAEL of KBP-5074 was determined to be 6 and 2 mg/kg/day in male and female dogs, respectively. Based on the above studies, it was concluded that the preclinical safety profile of KBP-5074 would support the initiation of clinical trials in man.

**1571 Calculation of a Permitted Daily Exposure Value for the Solvent 2-Methyltetrahydrofuran**

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In response to increasing concerns around the potential environmental impact of industrial chemicals, the pharmaceutical industry is seeking alternatives for traditional solvents used during the manufacturing process. Taking into consideration the principles of green chemistry, 2-methyltetrahydrofuran (2-MeTHF) is proposed as a suitable replacement for the structurally similar solvent tetrahydrofuran (THF). 2-MeTHF is derived from renewable sources and is more easily recovered thereby facilitating its reuse. However, 2-MeTHF is currently not included in the International Conference on Harmonisation (ICH) Q3C residual solvent guidelines and their Pre-Registered Daily Exposure (PDE) limit was proposed below which there would be negligible safety concerns for patients exposed to it as a residual impurity in a drug product. To enable the calculation of a PDE, a GLP compliant 3-month repeat-dose oral toxicity study in rats with a 1-month recovery period was conducted with weights of 80, 250, 500, and 1000 mg/kg of 2-MeTHF. Administration of doses of up to 1000 mg/kg/day was tolerated. Based on minimal observed effects on the liver at ≥500 mg/kg/day, the NOAEL in this study was considered to be 250 mg/kg/day. Following the ICH methodology including this NOAEL and a safety factor of 250, a PDE of 50 mg/day was derived to support the safe use of 2-MeTHF in the pharmaceutical industry.

**1572 Optogenetic Pacing for Assessment of Proarrhythmic Potential of Drugs in Induced Pluripotent Stem Cell-Derived Cardiomyocytes**


Induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) have been proposed to be used as a part of the Comprehensive in vitro Proarrhythmia Assay (CIPA). Currently available iPSC-CMs contract spontaneously with various frequencies. Dependence of CMs action potential duration on the beating rate is most often corrected using empirical formulas derived for clinical QT interval dependence on heart rate that may not be appropriate for iPSC-CMs, especially with wide beating rate ranges. Using a viral vector (rAAV9-CAG-CHR2-GFP, University of North Carolina) we expressed channelrhodopsin2 (Chr2) in iPSC-CMs (iCell2, ProPrep study) and used a 48-well light delivery device (Lumos, AstraZeneca) to simultaneously pace cell cultures at various frequencies (0.1, 1, 10, 50, and 150 Hz) while recording drug-induced field potential duration (FPD) prolongation and arrhythmias using a microelectrode-array system (Maestro, Axion). After optimizing the intensity and duration of the stimulating light pulses, we recorded iPSC-CMs responses to three drugs: dofetilide (0.3-10 nM, a sodium channel blocker); ranolazine (0.1-100 μM, a calcium blocker) and ranolazine (0.1-100 μM, a balanced hERG and late sodium current blocker) with and without pacing. Dofetilide-induced dose-dependent FPD prolongation in all experiments with clinical observations; local tolerability; clinical pathology; drug and metabolite levels in plasma and liver; and histopathology. Topical application of E/Z-endoxifen gel was well-tolerated by female minipigs: no mortality, clinical evidence of toxicity, local toxicity, body weight effects, changes in clinical pathology parameters, or gross or microscopic findings were observed. Dose-related decreases in absolute and relative ovary weights were seen in both groups receiving topical (E/Z)-endoxifen gel, reflecting the pharmacologic action of this drug. Endoxifen is rapidly glucuronidated in minipigs; with few exceptions, plasma levels of endoxifen were below the limit of quantitation, regardless of route of exposure. Endoxifen glucuronide was measurable in the mammary gland (but not plasma or liver) of minipigs receiving topical (E/Z)-endoxifen gel. By contrast, high levels of endoxifen glucuronide were present in the plasma of minipigs receiving oral exposure to Z-endoxifen. Both endoxifen and endoxifen glucuronide were measurable in the liver of minipigs receiving oral exposure; endoxifen glucuronide was also present in the mammary glands from the oral exposure group. Repeat-dose topical administration of E/Z-endoxifen gel for 28 days was well-tolerated in female minipigs, and resulted in mammary gland levels of endoxifen glucuronide that were comparable to those seen following administration of a much higher oral dose of Z-endoxifen (HHSN2612015000241).
The daily oral administration to rats of an investigational drug in neurodegenerative disease at a dose of 300 mg/kg/day resulted in body weight loss leading to early termination. Diffuse villous atrophy, epithelial erosion and variable hyperplasia were observed in the duodenum. At 100 mg/kg/day, the test item was well tolerated for 14 days and histopathology of the duodenum was limited to multinucleated giant cells with/without clefts in the mucosa. Examination of snap-frozen unstained sections of the duodenum by polarized microscopy revealed the presence of minute birefringent material in the duodenal mucosa and triggered further investigations by Mass Spectrometry Imaging. The slides of snap-frozen unstained duodenum were analyzed by MALDI-FTICR-MSI in positive polarity mode and identified the test item as the polarizing material in multinucleated giant cells of the duodenum. We interpreted that the absorption of the test item led to pH-dependent precipitation out of a supersaturated solution of the test item in the duodenum mucosa and formation of multinucleate giant cells scavenging this poorly soluble material. At higher doses, the scavenging phenomenon was overwhelmed by the amount of precipitate, leading to mucosal epithelial damage (villous atrophy, epithelial erosion) and physical consequences on animal health status (decreased nutrient absorption and body weight loss). When unaccompanied by structural damage, the mucosal APP precipitation was considered a local dose-dependent reversible effect. Advancements in Mass Spectrometry Imaging allowed fast resolution of the issue with minimal additional experiments.

**Plethysmography Versus Blood-Gas Parameters: Overlap and Considerations in Nonclinical Safety Assessment Studies**


Evaluation of blood gas parameters is a suggested method for assessing test article effects on respiratory function in nonclinical safety pharmacology and toxicology studies. The sensitivity of the ABL80 FLEX CO-OX instrument to measure arterial blood gas measurements has not been thoroughly investigated in rats. This study evaluated intra-precision, inter-precision, stability, intra-individual variability, and type of collection apparatus for blood gas collections. Additionally, this study compared blood gas values to head-out plethysmography data in Sprague Dawley rats administered a respiratory stimulant; 20 mg/kg theophylline-line via oral gavage. Blood was collected from the femoral arterial catheter and analyzed with the ABL80 FLEX CO-OX blood gas analyzer using Radiometer assays. Intra-precision and inter-precision variability was acceptable for all endpoints, with the exception of standard base excess, actual base excess, and anion gap (K⁺). The following endpoints exhibited acceptable nook parameter stability through 30 minutes post-collection: bicarbonate, concentrations of hemoglobin, sodium ions and chloride ions, fractions of methemoglobin and carboxyhemoglobin. Although results from Pico syringes, Westmed (Pico 3100-25) syringes, and capillary samples were generally similar, it is recommended that one type of collection device be used within a study, rather than using these devices interchangeably. In head-out plethysmography evaluation, theophylline significantly increased tidal volume, respiration rate, and minute volume within 2 hours postdose. However, fractions of carboxyhemoglobin and methemoglobin and concentrations of bicarbonate, calcium ions and hemoglobin were minimally decreased by the theophylline; other blood gas parameters were not significantly altered during this same postdose time span. These data suggest that blood gas measurements are less sensitive than plethysmography for nonclinical safety evaluation of respiratory safety pharmacology, but blood gas parameters can be valuable supplemental data.

**Drug-Induced Toxicity in Rats Due to API Precipitation in the Intestinal Mucosa**


Histone deacetylases (HDACs) are crucial players of epigenetic regulation, largely through their influence on gene transcription, and therefore have important roles in numerous biological processes. Levels of these proteins are implicated in different disease states such as cancer, cardiovascular, neuronal and musculoskeletal diseases, which makes them promising therapeutic targets. The goal of this study was to generate a comprehensive knowledge base to account for epigenetic targets and their safety assessment. We made computable all publically available data about molecular functions, biological processes as well as the data supporting involvement in pathologies/diseases. We explored mechanisms that underlie target effect of candidate drug or perturbed biological pathway. In order to test our system, we focused on the histone deacetylases HDAC1 and HDAC3 and their characterization as valid and safe therapeutic targets in colon cancer. Here we confirmed that developed HDAC1 and HDAC3 functional and several HDAC1/3 associated pathologies and diseases that are related to cancer. In addition the system associated HDAC1 and HDAC3 to some other diseases on cellular, organ and organ system level related to cardiovascular system (congestive heart failure, cardiac hypertrophy), smoking, obesity, diabetes, musculoskeletal system and formation and Brain function. We identify proteins related to colon carcinoma and HDAC1/3, including those involved in cell cycle regulation, proliferation, angiogenesis, apoptosis and growth factor receptor signaling. In order to confirm the therapeutic potential of HDAC1/3 inhibitors, we used a gene expression data from human colorectal carcinoma cells treated with Largazole, an HDAC1/3 inhibitor. Bioinformatics analysis provides the molecular mechanism of how Largazole induces downstream transcriptional changes in cell cycle inhibitors p21, p15 and CDK6, proapoptotic BCL2 protein and growth factor receptors EGFR, HER-2 and RS-1, all triggered by HDAC1/3 inhibition and other partnering proteins within the colon carcinoma model. Here we confirmed that developed database and software can contribute to a better understanding of therapeutic potential of HDAC1/3 inhibition in colon cancer and other disease indications and can increase the coverage of traditional safety reports.

**Intravenous Toxicity of Fentanyl, Remifentanil, and Carfentanil in the New Zealand White Rabbit**

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Fentanyl is a narcotic estimated to be 100x more potent than morphine. It acts primarily through interaction with mu-opioid receptors located in brain, spinal cord and smooth muscle. Fentanyl is prescribed to patients who have developed tolerance to less potent analgesic drugs. In extreme potential of overdose and death, Fentanyl elicits respiratory depression by slowing signal transduction in the respiratory centers of the brain and causing rigidity in skeletal and thoracic respiratory muscles. Fentanyl also been shown to depress the respiratory brainstem center’s ventilator response to rising levels of CO2. Remifentanil is an opioid, agonist and antagonist with an extremely short duration of action. It was approved for use in heptically compromised patients, as it is metabolized by circulating esterases within the liver. Carfentanil is estimated to be 100x more potent than fentanyl in regards to its analgesic potency, which may or may not correlate to toxicity. While not used in human medicine, carfentanil was...
used as a large animal sedative. It is hepatically metabolized and has been shown to exert its effects longer than that of both fentanyl and remifentanil. This study was conducted to investigate the biological effects of commercially available opioid analgesics, specifically fentanyl, remifentanil and carfentanil, administered by the intravenous route to New Zealand White rabbits. The effects of interest were the median lethal dose and the median effective dose. As the endpoint of incapacitation. Additionally, the progression of signs related to drug response and toxicity were monitored to characterize the symptomology of these compounds; this includes cardiac damage biomarkers, saturated oxygen, as well as opioid-targeted toxic signs. Carfentanil was observed to be approximately 10x more lethal than fentanyl and 500x more lethal than remifentanil. Generally, symptomology was the same among these compounds with the greatest difference being time to recovery. Rabbits exposed to remifentanil recovered more quickly than those exposed to fentanyl or carfentanil, which demonstrates the ability of remifentanil to be rapidly cleared, limiting its propensity to accumulate in tissues and prolong the exposure.

**1578 Examining the Impact of Eliminating the Concomitant Satellite Cohort in Toxicology Studies**

M. Hackett. Battelle Memorial Institute, West Jefferson, OH. Sponsor: S. Anand

The ICH is considering a revision to guidance S3A to allow blood microsampling of the main study cohorts of a general toxicology study for toxicokinetic (TK) analysis. Published data shows only minor hematological effects when 32-200 μL of blood is collected up to 6 times/day in healthy animals (Kerris-Glover, 2014; Caruso, 2015). The purpose of this study was to show the bleed effects from microsampling would also be negligible in animals treated with a toxic dose of an approved drug and confirm the bleeds would not impact the interpretation of study results. Female and male Sprague Dawley rats were dosed with either 500 mg/kg ascorbic acid or normal saline via IP injection daily for 7 days. Animals in both groups were 1) not bled, 2) serial bled 6 times/day via the tail vein at 100 μL/bled on Days 1 and 7 or 3) bled twice/day via the retro-orbital venous plexus at 600 μL/bled on Days 1 and 7 (composte). Endpoints included clinical signs of toxicity, body weight measurements, and clinical and anatomic pathology. VPA induced ataxia, lethargy and temporary coma consistent with previously published results (Zieve, 1989). VPA-treated animals also exhibited a body weight loss of 3-11% over the week of treatment, testicular atrophy, immuno-suppression, and minor liver impairment. Minor effects of the blood collection were observed in the hematology of the VPA-treated, microsampled animals consistent with the corresponding saline controls; however, no parameter reached statistical significance when compared to their respective unbled controls. Further, the changes measured in the microsampled groups at the end of the dosing period all successfully reverted to baseline following a 14-day recovery period, though the microsampled group at the end of the dosing period all success-

**1579 Assessment of the Efficacy and Safety of Synergistic Florfenicol and Thiamphenicol Combination as a New Drug against Actinobacillus pleuropneumoniae and Pasteurella multocida**

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Florfenicol (FF) and thiamphenicol (TAP) have been reported to exhibit both in vitro and in vivo synergism against clinical isolates of Staphylococcus aureus from pigs and Pasteurella multocida from chickens. Synergistic combination of existing antibiotics is a desirable practice because it could provide a broader antibacterial spectrum and minimize toxicity as well as the emergence of resistant bacteria. The current study aims to investigate if FF and TAP synergism also occurred for Actinobacillus pleuropneumoniae and Pasteurella multocida, the two important upper respiratory pathogens in pigs. Clinical toxicological assessment of FF-TAP including acute oral toxicity, Ames mutagenicity as well as resistance induction by P. multocida under sub-inhibitory concentrations were evaluated. Potential synergistic effect was observed on 58% of A. pleuropneumoniae (n=50) and 45% of P. multocida (n=79) isolates. Fractional inhibitory concentration index indicated that 38% of A. pleuropneumoniae and 50% of P. multocida had values ≤ 0.5. Overall, the MIC of FF could be reduced to less than ½ and ¼ of its original strength (1 MIC) against A. pleuropneumoniae and P. multocida respectively, when combined with 1/2 MIC of TAP. The in vitro and in vivo toxicity results suggested no significant toxic effects of FF-TAP combination. In addition, the MIC of FF/TAP combination on P. multocida was not changed after 12 passages. Therefore, this combinational antibiotic synergism may offer an attractive alternative for effective therapy against infections of these two pathogens in susceptible pigs. Furthermore, since the dosages are significantly reduced compared to the labeled recommendation dose, it is reasonable to also expect reduced residue time and improved safety as indicated in this study.

**1580 An Integrated Strategy for Leveraging Novel Tissue Transcriptional Biomarkers, Translational Safety Biomarkers, and Advanced In Vitro Models to Reduce Drug Attrition and Improve Pharmaceutical Safety in Drug Discovery and Development**


Historically ~13% of drug candidates fail due to findings seen in first GLP animal toxicology studies, and ~40% of all candidate attritions are due to safety concerns seen in animal toxicology studies (Waring, M.J. et al. (2015) Nat Rev Drug Disc). To combat this, Merck investigators introduced several changes that have brought attrition rates down to less than 5% through first GLP studies, and the contribution of animal toxicities to overall attrition to less than 10%. Here we describe some of the components contributing to this improvement. Transcriptional biomarker signatures focused primarily on adverse drug-induced alterations in liver, heart, kidney and skeletal muscle were designed into a 5-day rat study conducted at 2 dose levels to identify sources of high dose intolerability, and safety issues that could be expected near the desired safety margin. Drug safety has been assessed in this candidate selection (CS) safety lead optimization “stage gate” study before making decisions to enter regulated stages of drug development. Complex in vitro 3-D liver models have also been deployed in parallel, to accelerate timelines and reduce resources for identifying improved molecules when flagged by certain findings in such CS studies. Many but not all of the endpoints, either in vivo or in vitro, are focused on sensitive detection of predominant mechanisms and key events associated with organ toxicities, including, for example, excessive chemically reactive metabolite burden, activation of certain nuclear-receptor transcriptional pathway signatures, severe systemic inflammatory responses, mitochondrial injury, and perturbation of bile acid homeostasis. For certain drugs that enter regulated stages of drug development and still present with significant animal toxicities, attrition reductions have been achieved on a strategic case-by-case basis, using in some cases new translational safety biomarkers, including imaging, to establish safe clinical monitorability or customized investigative approaches to inform mechanism and human relevance. Experience supporting the value of the approach and examples describing integration of these approaches are presented.

**1581 Intra-Arterial Administration of Alprostadil in Peripheral Arterial Obstructive Disease: A Toxicological Comparison of Alcoholic Solutions with Lyophilised Powder Containing α-Cyclodextrin**

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This was a comparison of local tolerance of intra-arterial (IA) infusions of prostaglandin E1 (PGEl, Alprostadil) prepared from ethanolic (ETHO) concentrates of PGE1 (Pridorx) with PGE1 lyophilized with cyclodextrin (Prostavasin). PGE1 is used in PAOD in single doses up to 60 μg, up to 80 μg/day IV, and up to 20 μg/day IA. IA infusion is not yet approved for ETQH concentrates. Aqueous solutions of PGE1 have poor stability, unless prepared from lyophilized formulations with α-cyclodextrin.
no macroscopic findings at day 8. Animals dosed with Pridax® and the treatment site on day 3; pale kidneys were noted in one; there were no macroscopic findings at day 8. On day 3, rabbits treated with Pridax® had a marked red area (2-5 mm) at the injection site that were interpreted as typical effects of IM or ID dosing as changes were no longer present after the 28-day recovery period. No increased spleen weights in several groups; these effects were reversible, regardless of route of exposure, as all rabbits developed neutralizing antibodies against the target virus. Both vaccines were well tolerated by rabbits curious whether clinical IA use of an ETOH formulation of Pridax® would be safe, compared with formulations lypolysiphied with α-cyclodextrin.

### Bone Marrow Immunophenotyping Reference Data—Integrated Biopsies in a Study with Cynomolgus Monkeys


In the non-clinical safety assessment of biological medicinal products, often cynomolgus monkeys (Macaca fascicularis) are the only relevant species. We have established a method for immunophenotyping (IPT) of bone marrow cells from cynomolgus monkeys that can be used to study pharmacology and immunotoxicity in this animal model. Bone marrow cells were harvested from cynomolgus monkeys in the scope of an intravenous infusion study. Bone marrow cells were harvested proximal from the left humerus of 7 male (up to 6.5 kg) and 14 female monkeys (up to 4.8 kg) that were anaesthetized for implantation of the port catheter system. After repeated dosing with a test article, animals were anaesthetized before necropsy, and bone marrow cells were then harvested proximal from the right humerus. The bone marrow was sampled using a veterinary bone marrow biopsy needle and a syringe filled with EDTA fluid. Following the pre-dose sampling, skin wounds were sutured. All animals were treated with the analgesic meloxicam (0.1 mg/kg, subcutaneously) and the antibiotic sulfadiazine/trimetprim (24 mg/kg intramuscularly). Following lysis of erythrocytes, cells were stained with specific antibodies against CD8, CD14, CD20, CD25, CD38, CD69, and CD138. Following the reference formulation showed arterial thrombosis on Day 3 (marked in all 3 animals treated with the reference and mild in 1/3 animals treated with Pridax) and Day 8 (1/ group). IA injection of Pridax® or Prostavasin® was irritant to the arterial wall resulting in thrombosis. The effect was less marked after Pridax® showing that Pridax® was better tolerated. These results show that IA injection of Pridax® was well tolerated and that its effects were less than those with Prostavasin® with no renal change. It is considered that these studies show that clinical IA use of an ETOH formulation of Pridax® would be safe, compared with formulations lypolysiphied with α-cyclodextrin.

### Altered Bile Acid Homeostasis and Mitochondrial Function: Potential Mechanisms for BMS-986020-Induced Human Hepatobiliary Toxicity

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In a Phase 2 clinical trial, BMS-986020, a lysophosphatidic acid 1 (LPA1) receptor antagonist under development for idiopathic pulmonary fibrosis, produced hepatobiliary (HB) toxicity (1 ALT, AST, and ALP; cholecytisitis). In the absence of liver toxicity in rat and dog, additional non-clinical assessments were undertaken retrospectively to identify a potential mechanism(s) of toxicity. BMS-986020 inhibited the canicular bile acid (BA) efflux transporter BSEP (4.8 µM IC50) and MRP2 (37 µM), but did not increase oxidative stress. HB toxicity was not predicted in rats (6 months; 100x human AUC) or dogs (9 months, 7x), but, in a retrospective rat study (7 days, 100x), a prototypical response to rat Bsep inhibition was observed (↑ plasma BA [≤ 14x], ↑ expression of MRP3 [9x], MRP4 [2.4x], and Bsep [1.9x], and L expression of the BA uptake transporter, NTCP [0.8x]). In a retrospective monkey study (8 days, 2x human AUC), BMS-986020 increased ALT (2x) and GLDH (4.9x) and bile ductule hyperplasia, cholangitis, and cholestasis, demonstrating that the monkey was the only nonclinical species sensitive to BMS-986020-induced HB toxicity. DILysm simulations predicted 17.5% compared with 20.8% incidence of patients with ALT > 3x ULN in the clinic when a combination of BA transporter and mitochondrial ETC inhibition was represented. These results are consistent with reports that combinations of altered BSEP, MRP, MDR3, and mitochondrial function in the clinic when a combination of BA transporter and mitochondrial ETC inhibition was represented. These results are consistent with reports that combinations of altered BSEP, MRP, MDR3, and mitochondrial function studies show that clinical IA use of an ETOH formulation of Pridax® would be safe, compared with formulations lypolysiphied with α-cyclodextrin.

### Human Hepatobiliary Toxicity

Funding provided by The Geneva Foundation through NIAID contract HFN52201200019C.
β-catenin, a core component of Wnt/β-catenin signaling is a key molecule in the pathogenesis of Wnt-dependent familial and sporadic colorectal cancer (CRC). CEQ508 is an oligonucleotide therapeutic using live-attenuated *Escherichia coli* genetically engineered to deliver β-catenin short-hairpin RNA to mediate RNA interference (RNAi) in the gastrointestinal (GI) epithelium of patients with CRC. The long-term safety of daily oral administration of CEQ508 was evaluated in Cynomolgus monkeys. CEQ508 contained the plasmid pMBV43-H3 encoding the sequence for a shRNA against the mRNA of β-catenin, as well as the ability to enter the host cells and release the expressed shRNA through the use of two unrelated proteins, invasin and listeriolysin. CEQ626 was used as nonspecific shRNA control. Groups of three Cynomolgus monkeys/sex/group (n = 18) received the highest dose anticipated in the dose-escalating clinical trial of CEQ508 (10^11 colony forming units [CFU]/day) or CEQ626 and control vehicle via nasogastric gavage once daily for 280 days with a 7-day recovery period. Serum collected pretreatment and monthly throughout the study was analyzed for Th1 cytokine induction. Colon tissue was collected pretreatment and after 1, 3, 4, 5 and 6 months and local cytokine response was monitored using gene expression arrays of 84 different cytokines, chemokines and related receptors, and compared to serum collected from LPS-treated monkeys and corresponding controls. CEQ508 did not induce serum and local cytokine response in the panel of proinflammatory markers analyzed. In 280 days of daily oral dosing, no test article-related adverse effects were observed. In 280 days of daily oral dosing, no test article-related adverse responses were identified in a variety of parameters, including clinical observations, body weights and temperatures, serum chemistry, coagulation, hematology, and anatomical pathology assessments. Evaluation of fecal material confirmed the presence of CEQ508 or CEQ626 in the GI tract. Monkeys were evaluated up to Bup doses of 80 mg/kg in a full-thickness skin incision. Pigs were euthanized on Days 3, 14, or 28. LIQ865A was well-tolerated, at all doses with no evidence of systemic toxicity. Local incision site responses were the same as those described in the rat. Particles were noted in the injection sites of all LIQ865A groups on Day 3. By Day 14, the inflammatory response had resolved, all incisions appeared healed and particles, noted in only a few LIQ865A animals, showed significant degradation. On Day 28, no microparticles were observed in incisional tissues. LIQ865A was well-tolerated, both locally and systemically, up to Bup doses of 80 mg/kg in the rat and 36 mg/kg in the minipig. The local changes associated with LIQ865A were consistent with a degradable foreign body and what is reported for Bup. No novel findings or safety concerns were identified. These studies provided adequate safety data/multiples to support Phase 1 LIQ865A clinical trials.

ANX005 is a humanized IgG4 recombinant antibody against C1q that inhibits its function as the initiating molecule of the classical complement cascade. The safety and tolerability of ANX005 is currently being evaluated in a phase I trial in healthy volunteers (www.ClinicalTrials.gov Identifier:NCT03010046). Inhibition of C1q can be applied therapeutically in a broad spectrum of diseases, including acute antibody-mediated autoimmune disease, such as Guillain-Barré syndrome (GBS), and in chronic diseases of the central nervous system involving complement-mediated neurodegeneration (CMND), such as Alzheimer’s disease (AD). To support the clinical development of ANX005, several nonclinical studies were conducted to assess the pharmacology, pharmacokinetics, and potential toxicity of ANX005. The rat and the Cynomolgus monkey were determined to be pharmacologically relevant and the most appropriate nonclinical species for toxicity studies with ANX005. Results from the toxicology studies showed that intravenous administration of ANX005 once weekly for 4 weeks was well tolerated in rats and monkeys, with no ANX005-related adverse findings. Serum levels of ANX005 in monkeys correlated with a reduction of free C1q levels, both in the serum and in the cerebrospinal fluid. In summary, ANX005 showed proof-of-concept in the *in vitro* and *in vivo* nonclinical pharmacology models, with no toxicity in 4-week repeat-dose toxicology studies in rats and monkeys. The non-observed-adverse-effect level (NOAEL) was 200 mg/kg/dose, which is 200-fold higher than the first-in-human (FIH) starting dose of 1 mg/kg in healthy volunteers.

ANX005 is a mixture of Curcumin (C)and Doxorubicin (D). This mixture is designed to have a more targeted effect on cancer cells. IMX-110 administered by single IV slow bolus at 12.0 C/3.0 D mg/kg to Crl:CD (SD) rats induced changes in hematology on Day 6 after dosing (all dose groups). The single dose of IMX-110 at the level of 21.1 C/5.3 D mg/kg was considered the maximum tolerated dose (MTD). IMX-110 administered by IV slow bolus for 5 consecutive days to rats at 6.0 C/1.5 D and 12.0 C/3.0 D mg/kg/day resulted in mortality in the high dose group, clinical signs of toxicity and induced decreases in body weights, changes in hematology (both dose groups but much more pronounced in the high dose group), clinical chemistry and coagulation. The dose of IMX-110 at the level of 6.0 C/1.5 D mg/kg/day was considered the MTD after dosing for 5 consecutive days. The dose of IMX-110 at the level of 12.0 C/3.0 D mg/kg/day was considered the severely toxic dose (STD) after dosing for 5 consecutive days. The dose of IMX-110 at the level of 6.0 C/1.5 D mg/kg/day was considered non-toxic. IMX-110 administered by IV slow bolus for 5 consecutive days to dogs at 0.9 C/0.225 D and 1.8 C/0.45 D mg/kg/day resulted in moribund sacrifice of both animals in the high dose group, clinical signs of toxicity, decreases in body weights, decreases in food consumption, changes in hematology and clinical chemistry (both dose groups but much more evident in the high dose group), and coagulation. The dose of IMX-110 at the level of 0.9 C/0.225 D mg/kg/day was considered the STD after dosing for 5 consecutive days. Based on these results, pivotal murine and canine studies are being performed. The study was sponsored by Immix Biopharma Australia Pty Ltd.
of proarrhythmia risk assessment but experimental methods and factors that influence variability of these ECG parameters in non-clinical species need to be further evaluated. In addition, evaluation of a broader range of positive and negative control drugs may help establish their translational and discriminative value.

**1591 A Flow-Cytometry Assay to Assess B-Cell Maturation Antigen Expression in Peripheral Whole Blood of Cynomolgus Monkeys**

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Multiple myeloma (MM) is the second most common hematologic malignancy. MM is characterized by abnormal proliferation of monoclonal plasma cells (PC) resulting in monoclonal immunoglobulin, osteolytic bone disease and immunosuppression. Among new immunotherapeutic targets for MM, the cell surface transmembrane protein B-cell maturation antigen (BCMA/TNFRSF17/CD269) is most promising target for immunotherapy due to its key role in PC survival, high expression on PC, malignant PCs but not on naive and memory B cells, CD34+ hematopoietic cells, or any other normal tissue cells. BCMA modulates optimal PC survival by inducing signal cascades upon binding to proliferation-inducing ligand (APRIL) and/or B-cell activating factor (BAFF). Several high affinity anti-BCMA therapeutics such as BCMA-T cell bispecific antibody (EM801), bispecific T cell (BiTE) targeting BCMA and CD3e (Bi 836909) and anti-BCMA antibody drug conjugate (GSK2857916) are currently in various stages of preclinical/clinical assessment. Preclinical toxicology testing of anti-BCMA therapeutics requires a suitable non-human primate model. Cynomolgus monkeys are pharmacologically relevant species for non-clinical safety assessment, pharmacokinetics and pharmacodynamics studies of anti-BCMA therapeutics due to BCMA expression on their peripheral blood B cells and PCs. We report here, development of a flow cytomtery assay to assess BCMA expression in peripheral blood of various species of non-human primates and B cell subset immunophenotyping and BCMA detection was performed using commercial CD269, CD20, CD27, CD40, CD38 and CD138 antibodies. Natural killer (NK) and T cells were excluded based on CD159a and CD2 expression and B cells were assessed in the CD45+/SSCINT CD159a+CD159a+CD2+leukocyte population. BCMA expression on different B subsets (CD27+/CD10-, CD27-/CD10+) and CD138+ PCs in peripheral blood as well as species and sex-specific variations in BCMA expression were studied. In summary, this is the first report of detection of BCMA expression on B cells in peripheral blood of cynomolgus monkeys and its species-specific variations. This study also highlights the use of flow cytomtery to detect/screen BCMA expression for toxicity and PK/PD assessment of anti-BCMA therapeutics.
Impairment of mitochondria by drugs and chemicals could lead to organ toxicity, or even mortality. A number of in vitro platforms and assays have been developed in recent years to identify and de-risk this liability during early drug development. Currently established in vitro models rely largely on the use of cancer/immortalized cell lines with limited metabolic activity, or isolated mitochondria, with mitochondrial dysfunction measured by cellular respiration or ATP production and cell viability under selected nutrient conditions. It has been suggested that some compounds known to cause drug-induced liver injury (DILI) by damaging mitochondria may require drug metabolism capability and/or a longer-term treatment for the toxicity to manifest. Complex micropatterned co-culture model (HepatoPac™) may therefore offer an advantage in detecting certain drug-induced mitochondrial liabilities. In the present work, we first evaluated metabolic responses of rat and human HepatoPac to a set of paradigm mitochondrial toxicants with various known modes of action. In addition, selected DILI drugs and Merck internal compounds demonstrated a high degree of concordance between results obtained in this model and those with other detection methods. Media metabolite changes were profiled and we found that baseline biochemical metabolite profiles and the responses to mitochondrial insults in HepatoPac differed significantly from those of hepato- toma cell lines, and also sometimes between rat and human HepatoPac. In HepatoPac, concentration-dependent media urea decreases follow-owing drug exposures in the absence of cytotoxicity provide a sensi-tive indicator of mitochondrial dysfunction. In addition, metabolic "fingerprints" indicate disturbances in metabolic pathways associated with established mechanisms of mitochondrial toxicity. For example, increases in glucose consumption and lactate production track well with chemicals known to disrupt the electron transport chain, while distinct metabolomics patterns are seen with other mechanisms of mitochondrion toxicity. Overall, our data suggest that drug-induced mitochondrial toxicity can be monitored using changes of certain culture media metabolites of HepatoPac, and this approach could provide an additional risk assessment tool to mechanism-based in vitro toxicity testing.

We conducted a 6-month GLP safety study in rats with sutezolid (STZ; PNU- 100480), an oxazolidinone for the treatment of tuberculosis. STZ was given twice daily (BID; ~6-hr interval) by gavage for 180 days to adult male, female and ovariecto-mized (OVX) female rats followed by a 30-day recovery. Male and female rats were given STZ at 7.5, 15, 30, 60 or 120 mg/kg/day BID. Starting Week 4, all STZ-treated animals showed decreased gain and/or loss of body weight relative to controls. We decreased the high-dose from 120 to 100 mg/kg/day, the mid-dose from 60 to 50 mg/kg/day, and from BID to single daily dose. Intermittent convulsions were observed during handling in the non- OVX females starting on Day 80 and continuing through Day 210. A total of 31 convulsions were observed in 5 rats, in the 15, 30 and 100 mg/kg non-OVX female groups; no convulsions were seen in the control or low-dose, or in any males or OVX females. Other STZ-related clinical observations were considered related to the body weight decreases. STZ and its active metabolites (M1: PNU-101603; M2: PNU-101244) had a Tmax of ~1 hr after the second dose. Major dose dependent sex differences were noted in the plasma exposure of STZ, with females, including OVX females, having significantly higher (up to 12- or 16-fold) plasma levels of STZ than males, which may be due to differences in the metabolism of STZ between males and females. Males had significantly higher plasma levels of the metabolites M1 (30- to 40-fold) and M2 (2- to 3-fold) than STZ. Non-OVX females and OVX females also had a higher (17- to 48-fold) plasma exposure of M2 than that of STZ. Third microscopic findings in all STZ-treated groups included oval cell hyperplasia and numbers of portal macrophages containing gold pigment were present in the livers of non-OVX females at ≥7.5 mg/kg (a finding reported in a previous 13-week study). Oval cell hyperplasia reversed after recovery, while increased pigment was still observed in portal macrophages in all STZ-treated groups. STZ was well tolerated by males and ovariectomized females, but sporadic convul-sions were seen in several hormonally normal females. The maximum tolerated dose (MTD) is considered to be ~100 mg/kg/day. A no observed adverse effect level (NOAEL) could not be determined, but is considered to be ≥7.5 mg/kg/day. Work supported by NIAID Contract HHSN272201400006E.

Extracellular Field Potential (FP) and impedance recordings of SC-CMs are currently used to detect drug induced cardiac arrhythmia liabilities. Major endpoints include the field potential duration (FPD), the duration of the impedance twitch, and the presence of proarrhythmic markers including early and delayed afterdepolarizations and fibrillations. We used the cardioECR instrument (ACEA Biosciences) and cellCM™ cardiomyo-cyte (cardiac Dynamics) assay to 1) identify the FP parameter (that best captures the intrinsic frequency-dependence of repolarization and 2) assess the extent the system can detect the mitigating effect of drugs with multiple ion channels effects (MICE) on proarrhythmic activity. Cardiac repolarization in FP recordings is encapsulated by one positive (T1) and two negative (T2) peaks. We plotted a linear relation with comparable slopes of 0.133 and 0.129 respectively (n=2) between T2 and T3 durations and beat period in the spontaneous frequency range. T1 shows a shallower slope (0.031) suggesting that the parameter does not follow closely the repolarization process and consistent with its limited response to drugs that affect the FPD. MICE effects were investigated by assessing the effect of different combinations of pure hERG (E-4031) and hCav1.2 (nifedipine) channel blockers designed to simulate distinct IC50 hERG/hCav1.2 ratios (HCR). The functional relationship between the number of wells showing proar-rhythmic markers and log HCR was fitted to a logistic function with an inflection point at -0.148 and a HCR of 0.71. Thus, compounds with HCR values ≤0.71 are predicted to induce proarhythmic activity in SC-CMs as we here show for dofetilide (0.001), sotalol (0.58), cisapride (0.002), quinidine (0.11), moflaxacin (0.50) and ibutilide (0.003). As expected for hERG (3667) showed a proarhythmic activity. Notably, the 0.71 ratio observed in SC-CMs is comparable to the 0.89 cutoff for drug-in-duced torsadogenic activity observed in the clinic (Kramer, Obejero-Paz et al., 2013). The evidence indicates that 1) T2 and T3 durations are reli-able endpoints to assess the effect of drugs on cardiac repolarization and 2) SC-CMs are useful tools to detect MICE with HCR cutoff values for proarrhythmic activity comparable to the clinic.
The infectious bursal disease virus (IBDV) is an immunosuppressive virus of chickens causing the infectious bursal disease. One promising strategy for preventing this highly contagious disease is using recombinant subunit vaccine, employing viral protein 2 (VP2) as epitopic antigen. Traditionally, the quality of vaccine was mostly evaluated by the host response or herd immunity against the pathogen that was immunized with, which normally happened after the vaccine was given. In the present study, an affinity capillary electrophoresis (ACE) method for VP2 detection and quantification was successfully developed in an attempt to evaluate the vaccine quality before and after its use, including the degree of adjutant-VP2 complex and the existence of specific antibody against VP2. Fixed concentration (100 μg/ml) of anti-IBDV VP2 IgG monoclonal antibody (mAb) was mixed with varying concentrations (10-100 μg/ml) of VP2 subunit viral particle (SVP) and analyzed by ACE using in a 60-cm fused-silica capillary (75 μm-inner diameter) with 20 kV voltage and 50 mM boric acid buffer (pH9) containing 1 mM sodium dodecyl sulfate as running buffer. Samples were diluted with boric acid buffer and injected by pressure of 0.5 psi for 3 sec. The detection wavelength (UV) was 214 nm. The migration times of mAb and SVP were 4.69 and 5.65 min, respectively, while those of mAb-SVP complex was in the range of 4.80-5.34 min which prolonged with increasing SVP level.

Linear relationship between the remaining mAb and SVP concentration was established with binding saturation at the ratio of 0.42. Potential applications of this method include quantifying VP2 concentration in the vaccine product as quality control measure of vaccine product and its stability. Specifically, the degree of association/dissociation between VP2 and adjutant could be evaluated by this technique. In addition, the ACE-UV analysis can also be applied for measuring anti-IBDV antibody in chicken serum for evaluating host immunity response against the virus.
Colorectal cancer (CRC) is the second leading cause of cancer-related death in the US and risk is on the rise in both middle aged and young adults. Despite 70-90% of all CRC incidence being attributed directly to diet, Americans routinely consume highly processed foods that are energetically-poor. The overall objective of this study was to determine the impact of trans-generational or multi-generational consumption of the total Western diet (TWD), a Western-style diet formulated for rodents using human US nutrient intake data, in a murine model of inflammation-associated colorectal carcinogenesis. The hypotheses tested were that 1) ancillary feeding TWD would promote colitis-associated caecal colorectal cancer (CAC) in F1 offspring, and 2) inter-generational feeding TWD would further exacerbate disease development. C57BL/6J mice were bred for three generations, during which they were fed either a standard diet (AIN93G) or TWD during the F0 generation only, for the duration of F1 or F2 generations, or the F3 generation only. The azoxymethane and dextran sodium sulfate model of CAC was employed in F3 offspring, subsequently necropsied at 24 weeks of age. Notably, tumor incidence was increased by indirect exposure to TWD (92%) when compared to consecutive AIN93G exposure only (56%). Moreover, successive exposure to TWD markedly increased tumor burden (3-fold increase) when compared to direct TWD exposure. Neither ancestral nor cumulative TWD exposure increased body weight or fat mass. In summary, trans-generational TWD exposure increased CAC incidence and disease severity in third generation offspring that were not directly fed this diet, and continuously feeding TWD for three generations distinctly exacerbated disease outcome in third generation offspring. Ongoing analysis will include gene expression profiling of colonic mucosal cells, followed by promoter methylation analysis that is expected to reveal an epigenetic mechanism of action.

Development of a Consensus Approach for Botanical Safety Evaluations

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Interest in botanicals (in consumer care products; most notably in foods, over-the-counter medicines and supplements) coupled with a desire to minimize animal testing, presents unique challenges for this category of ingredient. For both food botanical ingredients there are various safety assessment approaches from heavy reliance on post market surveillance of adverse events, to tiered strategies built on existing data and new data generated to meet specific safety needs (e.g., including but not limited to chemical characterization, genotoxicity/carcinogenicity testing, developmental/ reproductive testing, ADME considerations). A multi-stakeholder roundtable was held at the 2017 EUROTOX meeting in Bratislava, Slovakia to review a decision tree methodology for botanical safety evaluation. The presentations covered an overview of the European Food Safety Agency’s 2009 report on data needs for food supplements and a proposed holistic approach; evaluation of the safety of botanical dietary supplements; natural product characterization methods; and challenges in assessing disposition of these complex mixtures. The participants developed a series of statements in order to: discuss and provide their perspective on the proposed decision tree methodology; and identify key elements needed to build a robust botanical safety evaluation; highlight and debate vulnerabilities in these tactics; and share additional perspective to ensure this end-to-end safety approach is sufficient, actionable and timely. Critical areas and data gaps were identified as opportunities for future focus, and elements include better context on systematic assessment of weight of evidence, use of in silico approaches, inclusion of threshold of toxicological concern considerations, assessing natural product-drug interactions, and adoption of in vitro and in vivo physiologically-based pharmacokinetic modeling.

1602 Development of a Consensus Approach for Botanical Safety Evaluations

Safety Evaluation of Daily Oral Administration of Egg Shell Membrane Via Soft Chew to Male and Female Beagles

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Eggshell membrane has been evaluated in several clinical trials demonstrating beneficial effects for joint and skin health in companion animals in recent years. However, no data demonstrating the safe use of eggshell membrane in targets species are available in the public domain. The protocol for the present study was based on the EPA Health Effects Test Guideline for Companion Animal Safety. Eight beagle dogs (4 per sex; mean 8.7 kg) received one soft chew orally per day on Days 0–83, followed by 5 soft chews/day on Days 84–110. Each 2 g soft chew contained eggshell membrane (66 mg), hyaluronic acid (8 mg), Boswellia serrata extract (5 mg; 65% boswellic acids), astaxanthin (250 mg; algae), and vitamin D3 (50 IU). Body weights were recorded on Day 0 and weekly thereafter. Blood was collected for hematology and clinical chemistry evaluations on Days 0, 83, and 111. On average, the majority of animals consumed the test article wholly with a dose acceptance score of 1. Physical examinations on Day 1 revealed abnormalities in bilateral otitis externa in two male dogs and one female dog and right ear otitis externa in one female dog. On Days 83 and 111, all dogs were observed to have dental tartar ranging from light to moderate, which was determined not to be treatment related. There were no observed abnormalities during the clinical observations for the duration of the study. Body weights were statistically higher after treatment with the soft chew on Days 84 and 111, relative to pre-treatment body weights; the average weight gain was 1.2 kg. Significant increases in creatine kinase values were observed on Day 111 (combined mean 157.9 U/L), relative to Days 0 and 83 (99.0 and 103.6 U/L, respectively). In addition, aspartate aminotransferase levels were significantly increased on Day 111 (32.1 U/L) compared to Day 20 (23.8 U/L) and Day 83 (25.1 U/L). While some significant differences in pre- and post-treatment serum chemistry results were observed, they remained within the normal reference ranges, and therefore were determined not to be clinically significant. Only bicarbonate on Day 83 was outside the normal range, with a combined mean value of 26.4 mmol/L slightly above the reference range of 15-25 mmol/L. In conclusion, the eggshell membrane product was found to be safe when administered to dogs orally for 111 days up to the targeted margin of safety of 5x the recommended dose.

Safety Evaluation of Daily Oral Administration of Egg Shell Membrane Via Soft Chew to Male and Female Beagles

1604 Dietary Early Glycation Products Protect Type 1 Diabetic Mice against Hyperglycemia through Altering Immune Homeostasis

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Early glycation products (EGPs) are proteins modified with reducing sugar moieties (e.g., glucose). They are generated in the first step of Maillard reaction/glycation, and are present in pre-processed/processed food and food-based products. These compounds have been shown to affect the bioactivity of food-delivered end-products (AGEs). This modification enhances the physiochemical properties of proteins (e.g., solubility), and has been proposed as a strategy to improve food protein qualities. However, the health effects of EGPs are rarely known. Our previous studies suggested that EGPs are anti-inflammatory. In contrast, AGEs, which are pro-inflammatory, are tightly associated with aging and hyperglycemia and are proved to be detrimental to almost all the organs and systems. We hypothesized that EGPs was protective for type 1 diabetes (T1D) through modulating the Th1/Th2 balance. Food-borne EGPs were produced from a whey protein isolate-glucose model system, with non-reacted samples (NR) and AGEs as controls. Two T1D models were used: streptozotocin (STZ)-induced T1D in female C57BL/6J mice, and non-obese diabetic (NOD) mice (non-diabetic females and males, and diabetic females). The C57BL/6J mice were dosed with water, glucose solution, NR, EGPs and AGES (600 mg/kg body weight, 8 wk) and then intraperitoneally develop T1D with 4 consecutive i.p. injection of STZ (50 mg/kg Bw), and they were continuously dosed for another 4 wks. NOD mice were dosed with NR and EGPs for 8 wks. Bw and blood glucose levels were measured weekly; diabetic incidence (blood glucose > 200 mg/dL) was recorded; glucose tolerance test (GTT); and insulin challenge test. Sera were separated, and spleen and thymus were harvested for flow cytometry analysis of the immune cell populations. The results showed that EGP-treated NOD mice showed significant lower blood glucose levels, delayed diabetes onset, less responsive to glucose challenge, and reduced insulin challenge test. EGP-treated T1D mice showed normal fasting glucose levels, normal glucose tolerance test (GTT), and reduced insulin challenge test. In addition, the diabetic females displayed lower or even normal blood glucose levels after EGP-treatment. The flow cytometric data showed that EGP-treated mice had a significantly higher CD4/CD8 ratio (an

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for a Langmuir type equation to be compared. Sample C-5 showed a pattern compatible with Langmuir (I-2) equation (R² = 0.99), while C-12 showed a linear trend more compatible with the Freundlich model (R² = 0.96). Currently we are working with CEC determinations among other complementary analysis in order to find the best characteristics for aflatoxin binders. Funded by Industry Award by Minerals Technologies.

**1607 Low/No-Calorie Sweeteners and Effects on the Gut-Microbiota: Assessing the Nonclinical and Clinical Data and Implications for Food Safety**

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Low/no-calorie sweeteners (LNCS) are repeatedly under the spotlight with regards to their benefits and safety in foods. A study published in 2014 concluded that LNCS as a group of compounds induce glucose intolerance, which is mediated by alterations in the composition of the gut microbiota. This study captured widespread media attention; however, several other studies published prior to this did not report such alterations in the gut microbiota and LNCS. In response, we set out to undertake an in-depth analysis of the nonclinical and clinical data existing in the public domain relevant to LNCS and gut microbiota function and health. To investigate whether an association exists between LNCS exposure and modulation of the gut microbiota, a comprehensive review of the scientific literature was conducted. The search generated 115 hits, of which 13 were identified as relevant primary research articles investigating LNCS (including aspartame, saccharin, sucralose, and rebaudioside A) in vivo and measuring changes in microbial populations in the gut. The design of each study was critically reviewed and reported outcomes were evaluated. Several common critical issues were identified in study design including the lack of proper control groups, particularly isocaloric controls, as dietary factors are key determinants in gut microbial composition. Furthermore, LNCS doses administered in the majority of nonclinical studies were greater than the currently established acceptable daily intakes (ADI), greatly limiting the relevance to humans in a dietary context. In all, 3 nonclinical studies that employed LNCS doses at or below the ADI reported a change in gut microbiota, all of which were conducted in mice, a species with a gut microbiome that is significantly different than humans. No clinical studies reported changes in gut microbiota that could be definitively linked to LNCS exposure. The sum of the data establishes no clear evidence of any adverse effect of LNCS on the gut microbiota at doses relevant to human use. The safety databases that have been developed over decades for several structurally unrelated LNCS indicate that these compounds as a group, or individually, pose no safety concerns at their currently approved levels, a viewpoint that is supported by all the major international food safety and health regulatory authorities.

**1608 A Safety Analysis of an Energy Drink**


Concern has centered around possible health effects from drinking energy drinks. Yet Americans consume hundreds of millions of bottles of energy drinks every year. Is it safe? We addressed this question first by evaluating the safety of caffeine in an energy drink in a manner consistent with Generally Recognized as Safe (GRAS). We also researched current caffeine consumption, quantified added consumption from energy drinks, reviewed key toxicity and epidemiology studies, and conducted a quantitative analysis. Next, we evaluated whether caffeine consumption in an energy drink, combined with caffeine intake from other sources, would fall within safe limits of total caffeine intake. Finally, we convened a panel similar to a GRAS panel, which conducted an in-depth evaluation of caffeine safety under the conditions of its intended use in an energy drink. The GRAS panel panel found little evidence of an association between cardiovascular disease (CVD) or other adverse health effects and caffeine (coffee) intake. Major studies found either an inverse association or no significant association between chronic coffee consumption and CVD, heart failure, stroke, myocardial infarction, and atrial fibrillation. Some individuals, especially individuals who are not regular coffee drinkers, and thus not habituated to caffeine, may be at increased risk of a coronary event or stroke, but it is unclear if caffeine or other risk factors are responsible for these outcomes. We also evaluated the correlation between caffeine consumption and other health endpoints including bone mineral density (BMD), intraocular pressure (IOP) and glaucoma, diabetes and blood glucose, reproductive health (male and female), and cancer. In addition, we evaluated other constituents in energy drinks such as vitamins, amino acids, and nutrients. For the product evaluated, we concluded that these ingredients are safe if the energy drink is consumed in accordance with the product-label direc-
tions. Based on our safety assessment, the calculated margins of safety (MOSs) for all vitamins, amino acids, and nutrients for a 70 kg adult are slightly greater than or less than 1, indicating that no adverse effects would be expected. We concluded that consumption of the energy drink in a manner consistent with the product-label is safe.

### 1609 Microbiological Quality and Aflatoxin Contamination of Roasted Cashew Nuts Sold in Lagos, Nigeria

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This study aimed at assessing the microbiological quality of roasted cashew nuts sold in Lagos State, Nigeria. Moisture content, Total Viable Count, Aflatoxigenic potential of aspergillus isolates and Total aflatoxins concentration of the cashew nuts were determined using standard methods and ELISA method respectively. The Moisture content, total plate count and fungi count ranged from 5.00 - 86.0% (Mean; 9.3%), 5.5 - 64.5 x 10^5 cfu/g (Mean; 35 x 10^5 cfu/g) and 1.0 - 4.5 x 10^5 cfu/g (mean ; 27.5 x 10^5 cfu/g) respectively. Nine different fungi species belonging to 5 major genera were isolated with Aspergillus flavus, Rhizopus oryzae and Fusarium oxysporum having the highest percentage occurrence (50% each) across the state. In addition, E. coli was present in all the samples but one (1-12x10^5 cfu/g), while Staphylococcus aureus was detected in 25% of the samples. Furthermore, aflatoxin concentration ranged from 0.1 - 6.8 µg/kg (80% above EU limit of 4µg/kg). Cashew nut consumers in Lagos State are at risk of exposure to aflatoxins and food borne diseases. This calls for mitigation measures from appropriate governmental organizations.

### 1610 Beyond the History of Safe Use of Botanicals and Their Extracts in Foods: A Proposed Standardized Framework for Their Safety Assessment

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In recent years, an industry wide trend, driven by consumer preference for more ‘natural’ foods and food ingredients, is occurring. This preference is often fed by the consumer's belief that everything coming from nature is safe or at least, safer than manufactured or processed food ingredients. This has led to a prominent trend in which more botanically derived functional and technological useful ingredients are added to food, often replacing their traditional and historically proven safe synthetic counterparts. For product developers and risk assessors, this trend presents many challenges. Although botanicals (and their extracts) often make the claim that they have been safely consumed in some form for many years, there is most often a general lack of robust safety and toxicological data to support such claims. As the first tier in a newly proposed decision tree approach for the risk analysis for botanicals, the literature (preferably published by a regulatory body (ESFA, FDA, EMA) is searched for the available safety data on the botanical and its primary constituents. Although additional data are becoming available on many botanicals, there is still a large data gap for most, in particular dealing with repeated exposure and vulnerable subpopulations (e.g., children, pregnant women, elderly). This lack of data drives on to a second tier in which the plant’s compounds are assessed individually. Important to consider in the second tier is the various extracts of the botanical. The essential oils containing flavors, scents and other functional chemicals are often concentrated in ethanolic extracts, which may contain up to 90 chemicals. These extracts are extremely complex and the chemicals in the extracts need to be identified and evaluated for their toxicity. Tools are constantly being developed that identify what often are called ‘compounds of concern’ or compounds with known biological activity. Additionally, harvesting methods and seasons, plant parts, and extraction methods all lead to different extracts which in turn may lead to different chemicals of concern. These tools together with better chemical characterization of the extract by suppliers should allow for more precise identification of these compounds. In the third step, these identified compounds undergo individual and specific risk assessment approaches (e.g. TTC).

### 1611 Occurrence of Selected Mycotoxins in Maize Destined for Human Consumption in South Africa

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Contamination of foods by mycotoxins has been linked to various health and economic implications to both man and animals. This study was carried out to evaluate the occurrence of mycotoxins contaminating commercial and small scale maize grains and to evaluate potential health risks for consumers based on South African and international regulations. A total of 100 maize samples were randomly collected from commercial farmers and small-scale farmers across the North-west province of South Africa. Mycotoxin analysis was done using Immuno-affinity column (IAC), Thin Layer Chromatography (TLC), High Performance Liquid Chromatography (HPLC) and Enzyme Linked Immunosorbent Assay (ELISA). Results obtained revealed that Fumonisin B1 (FB1) was the most contaminant mycotoxin in the small-scale and commercial samples with incident rate of 100% and 98.6% respectively. Aflatoxins contamination in samples occurred at incidences of 26.7% in small-scale samples and 25.0% in commercial samples. The levels of AFs were varied between 0.080-9.34 µg/kg and 0.32-8.60 µg/kg in small scale and commercial samples respectively, though still within the EU acceptable limits of 10 µg/kg (Total aflatoxin). Furthermore, ochratoxin A (OTA) has a high incident rate of 97.8% and 93.0% and ranged from 3.60-19.44 µg/kg and 1.60-9.89 µg/kg respectively in small-scale and commercial maize samples while zearealenone (ZON) occurred in 50% and 55% of small-scale and commercial samples respectively. These results demonstrate that maize especially those from small-scale farmers may contribute to dietary exposure to mycotoxins. Farmers and consumers should be alerted to the dangers of mycotoxins contamination in maize with resultant health risks.

### 1612 Assessment of Aflatoxin B1-Induced Adverse Impacts on Nutritional Compositions of Gut-Microbial Metabolome in F344 Male Rats

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Gut-microbial metabolome, a collection of thousands of micronutrients and functional metabolites, was previously found to be severely affected by oral exposure to Aflatoxin B1 (AFB1) in F344 male rats. In this study, we assessed the changes of nutritional composition in this metabolome through HRLC-LTQ-Orbitrap Elite MS and GC-EI-Q MS based metabolomics analysis. The rats were orally exposed with AFB1 at doses of 0, 5, 25 and 75 µg AFB1/kg body weight (B.W.) for 5 weeks, and a total of 120 samplings were conducted for GC/MS based metabolomics analysis in the pooled feces samples collected in each dose group. There were 1490 features aligned from the dataset. Among the top 100 significant features, 61 metabolites were identified by searching NIST database, OPLS-DA and Random Forests together extracted 10 distinct metabolites, including D-lactic acid, 3-hydroxybutyric acid, erucic acid, cholesterol, 20α, 22R-dihydroxycholesterol, N-acetyl-L-alanine, α-trehalose, L-arabinose, galactitol, and turanose. The MS based metabolomics analysis was further conducted. Data was collected via the CID-MS/MS mode. Raw files were processed through both on-line XCMS and off-line MZmine modules. The two modules provided 2498 and 3925 MS1 features, respectively. A total of 86 metabolites were identified from the 140 remarkable features (adjusted-p < 0.05) by searching HMDB and METLIN databases with precursor ions or MS/MS spectra. In order to search for the correlated biochemical pathways and diseases, the integrated dataset was undergone enrichment analysis using KEGG and HMDB. We found that oral exposure to AFB1 in rats reduced total content of carbohydrates and amino acids, gut lactic acid bacteria, production of short chain fatty acids and related secondary aliphatic acids. AFB1-exposure also disrupted the production of amino acids and neurotransmitters e.g. melatonin and 5-HT, the absorption of food-contained long chain fatty acids, as well as bile acid secretion and metabolism. The correlated diseases included seizures, liver damage, diabetes, autism and multiple sclerosis, etc. The affected pathways mainly included glucose metabolism, glycolysis, metabolism of amino acids and neurotransmitters. These results provide us an insight into the impact of oral exposure to AFB1 on the nutritional composition of the gut-microbial metabolome, as well as the affected biochemical pathways and diseases.
### 1613 Evaluating the Applicability of Read-Across Tools and High-Throughput Screening Data for Food Relevant Chemicals

J. Wynder, J. Ovesen, A. Maier, R. Judson, N. Kleinstreuer, and M. Krishan.

Alternative toxicity methods to characterize the hazards of chemical substances have been proposed to reduce animal testing and efficiently screen thousands of chemicals. Relevant resources include large in vitro datasets from efforts such as the high-throughput screening (HTS) ToxCast/Tox21 programs and read-across tools such as the Organization for Economic and Cooperation Development (OECD) QSAR toolbox.

The goal of this work is to compare the results from traditional toxicity studies with predictions from these alternative testing methods for food relevant chemicals in ToxCast. Computational models developed using ToxCast HTS data were used to predict the activity of food relevant chemicals against the estrogen receptor (ER) and androgen receptor (AR) pathways. We identified 94 putatively estrogen- or androgen-active food relevant chemicals in ToxCast. To reduce possible confounding from cytotoxicity and cell stress, the ER and AR model results were filtered based on observed in vitro cytotoxicity, which resulted in 89 putatively active, non-cytotoxic food chemicals. As a proof of concept, we further shortlisted these 89 chemicals based on the availability of in vivo data related to developmental and reproductive toxicity (DART).

This resulted in 10 putatively active, non-cytotoxic, endocrine disrupting chemicals. Structural similarity and similar mode of action were used to identify potential analogues for the 10 chemicals. We used read-across approaches to compare the pattern and potency of DART for the analogues to that of the target chemical. These methods identified 3 target chemicals for which the analogue approach and computational models successfully predicted the DART potential of the food relevant chemicals. This study demonstrates that ToxCast/Tox21 HTS assay data can be used for prioritization along with weight of evidence from read-across tools to evaluate food relevant chemicals, although the limitations in the approaches are evident. This abstract does not necessarily reflect US EPA policy.

### 1614 Evidence on the Intake of Intense Sweeteners, Appetite Regulation, and Body Weight Development: A Scoping Review of Reviews


Observed associations between consumption of diet foods and prevalence of overweight have sparked controversy over whether intense sweeteners, despite contributing negligibly to energy intake, may promote weight gain. In this scoping review, we assessed reviews published from 2006 to May 2017 to obtain an overview of hypotheses, research approaches and features of the evidence on intense sweeteners’ potential relationships to appetite regulation and weight development. The 40 included reviews present hypotheses both on how intense sweeteners can reduce or maintain body weight and on how these can promote weight gain. We classified only five publications as systematic reviews; another nine presented some systematic procedures, while 26 reviews did not describe criteria for selecting or assessing the primary studies. Evidence was often presented for intense sweeteners as a group or unspecified, and against several comparators (i.e. sugar, water, placebo, intake levels) with limited discussion on the interpretation of different combinations. Apart from the observational studies, the presented primary evidence in humans is dominated by small studies with short follow up - considered insufficient to assess weight development. Systematic reviews of animal studies are lacking in this topic area. The systematic evidence only partly explore forward hypotheses. Primary studies seem to be available for systematic exploration of some hypotheses, but longer-term experimental studies in humans are sparse.

### 1615 Polyphenol Oxidase/Caffeic Acid Reduces the Allergenicity of Ovalbumin in a Balb/c Mouse Model

P. Tong, S. Chen, J. Wang, J. Gao, and H. Chen.

Food allergy has been recognized as a common public health problem and affects an estimated 6-8% of children and 2-5% of adults. Egg is one of the “top 8” most allergenic foods in children and infants, with an estimated annual incidence of 1.6-3.2%. To mitigate the allergic adverse effects and develop hypoallergenic food products are still a challenging task for the research field and food industry. In this study, we tried to modify the food allergen, ovalbumin (OVA), with caffeic acid-assisted cross-linking technique catalyzed by polyphenol oxidase (PPO). OVA is the most abundant egg white protein and is also a well-known food allergen. The modulation effects were evaluated in a Balb/c mouse model. 8 female mice per group were orally sensitized with OVA or cross-linked OVA using cholera toxin as adjuvant. Clinical signs of allergy, specific antibody levels, serum histamine levels, mast cell protease-1 (mMCP-1) concentrations, morphological structure of duodenum, and cytokines were further determined. Both OVA and cross-linked OVA induced allergic diarrhea in Balb/c mice, however, the histological symptoms of the small intestines were much milder in mice fed with cross-linked OVA than in those fed with OVA alone. A tendency toward decreased allergen-specific IgE, IgG, IgG1 and IgG2a levels, as well as serum histamine and mMCP-1 concentration were observed in cross-linked OVA group (p<0.05), accompanied by an inhibition of IL-4, IL-5, IL-13, and IFN-γ production in the stimulated splenocyte cell (p<0.001).

Results of this study demonstrate that caffeic acid-assisted PPO-catalyzed cross-linking technique was an effective method to significantly reduce the potential allergenicity of OVA, but may not completely eliminate it.

### 1616 Withdrawn by Author

### 1617 An Occurrence of Aflatoxigenic Fungi and Aflatoxin Contamination in Raw Groundnut from Selected Markets in Obafemi Owode Local Government, Ogun State, Nigeria

T. A. Dada, M. Adetunji, N. Awa, O. Atanda, and M. Mwanza.

Groundnut is one of the most nutritious oil-seed used in Africa, and it is valued for its protein and oil content. This study aimed at investigating the microbiological quality and level of aflatoxin contamination in raw groundnut sold in Obafemi Owode local government. A total of 15 different composite groundnut samples were collected from five major markets in the zone; and the Moisture content, total microbial count, frequency ratio of isolates and aflatoxins level in the samples were determined using standard methods and ELISA method. The mean moisture content of the groundnut ranged from 5.10 - 7.21%, while Total Viable bacteria count ranged from 58-89 x10^3 cfu/g and Total Fungi Count ranged from 4 - 24 x10^3 cfu/g. The most frequent organisms isolated from the samples included Penicillium spp (87.5%), Aspergillus flavus (83.3%), Aspergillus niger (42.85%), Fusarium spp (12.5%) among others. The total aflatoxin concentration ranged from 29 ppb - 33.8 ppb. The aflatoxins level in all the samples were far higher than the recommended EU permissible limit of 4 ppb and the US FDA recommendation of 20 ppb. Groundnuts consumers at Obafemi Owode are at risk of exposure to aflatoxins especially the young and infant. Appropriate intervention by concerned agencies is recommended.
California Proposition 65 requires businesses to provide a warning if a product sold in California contains a carcinogen at levels that would result in exposures above its Safe Harbor Level (SHL) or No Significant Risk Level (NSRL). Furfuryl alcohol (FFA), a flavoring ingredient and by-product of thermal processing of foods, was added to the Proposition 65 list via the authoritative bodies mechanism after US EPA’s conclusion that FFA is likely to be carcinogenic to humans based on results from 2-year NTP inhalation bioassays in rats and mice. Like acrylamide, also on the list, FFA is formed in baked and roasted foods, including coffee. As OECDHA has not derived an SHL/NSRL for FFA, the current work was undertaken to (i) derive an NSRL and to (ii) determine if FFA in different types of coffee is likely to exceed the NSRL. Derivation of the NSRL was based on OECDHA methodology. Modelling of the incidence data from the rat for nasal tumors, using a linear dose-response model, yielded the most conservative (lowest) NSRL for FFA of 16 micrograms/day. From data from NHANES 2013-14 were used to identify the mean daily consumption of coffee products (brewed/fILTERED, specialty, and instant), which may contain up to 408 ppm (mg FFA/kg dry coffee). Based on this concentration estimate, FFA exposures from consumption of brewed/fILTERED coffees were up to 1,100,000 micrograms/day. FFA levels in specialty coffees and instant coffees, estimated at 2,300 and 1,600 micrograms/day, respectively. For exposures to be below the NSRL, the concentrations of FFA on a dry weight basis would need to be below approximately 0.57, 2.8, and 4.1 ppm for brewed, specialty, and instant coffees, respectively. While coffee products may require a warning for FFA, such products might already have the warning due to acrylamide content in which case one label could address both substances. As coffee is consumed in significant amounts and is known to contain amongst the highest amounts of FFA, other foods would need to be evaluated independently to determine whether a warning label is warranted under California Proposition 65 legislation. If such foods are consumed in lower amounts and have lower FFA concentrations than coffee, a warning might not be required.
Cyclodextrin is a unique compound produced by cyanobacteria, and it is increasingly perceived as a global water-quality issue growing in scope and persistence. It has hepatotoxic, general cytotoxicity, and neurotoxic effects, affecting plants, several aquatic organisms, and mammals with different degrees of damage. The present work studies the genotoxicity of pure CYN by the in vitro micronucleus (MN) assay (OCDE 487) in LS178T TE-21 cell line and the comet assay in Caco-2 cells, including in this case also the study of the indirect oxidative damage of DNA using restriction enzymes. The results obtained showed that the potential genotoxicity of the cyanotoxin could be related with CYN metabolites. Acknowledgements: Spanish Ministry of Economy and Competitiveness (AGL2015-64558-R, MINECO/FEDER, UE) for the financial support and the Microscopy Service of CITIUS from the University of Sevilla for the technical support.
were the main effects of E+ grazing. The genera and Ruminococcaceae Max-Q and E+ steer MB. Significant early decreases of Erysipelotrichaceae of toxic fescue on grazing beef microbiota (MB) and the role it might in the southeastern United States forage grass, can become infected with a tions of various environmental stressors. Tall fescue, the predominant food-producing animals, such as beef cattle, especially under condi-
tions that either associate with, or, contribute to, FT pathophysiology.

Our data characterize the grazing Angus steer fecal MB and indicate that E+ grazing results in MB shifts that either associate with, or, contribute to, FT pathophysiology. Leticia Díez-Quijada also acknowledges for the grant BES-2015-64558-R, MINECO/FEDER, UE for financial support. Acknowledgements: Ministerio de Economía y Competitividad of Spain (AGL2015-64558-R, MINECO/FEDER, UE) for financial support. Leticia Díez-Quijada also acknowledges for the grant BES-2016-07873 associated with this project (MINECO/FEDER).

The cyanobacterial toxin cylindrospermopsin (CYN) is recognized as one of the most globally important of the freshwater algal toxins due to the expanding distribution of CYN producers into different regions, representing serious human and environmental health risks across many countries. Its ability for bioaccumulation in tissues of different organisms intended for human consumption may have implications in food safety. In this sense, edible tissues of plants and vegetables could represent a possible route of human intoxication if they are irrigated or in direct contact with CYN-contaminated waters. A method for determination of CYN, ergoalkaloid (EA)-producing endophyte that results in numerous the first time. The procedure is based on solid phase extraction with graphitized carbon cartridges, and quantification by Ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS). The method exhibits good linearity (r² = 0.9999) with linear range from 5-500 ng CYN/g fresh weight (f.w.), and sensitivity, with limits of detection and quantification of 0.22 and 0.42 ng CYN/g f.w., respectively. Mean recoveries ranged 85-104% and intermediate precision values from 12.7 to 14.7 %, acceptable according to the tabulated values. The validated method demonstrated to be robust for three different variables tested. Moreover, it has been successfully applied for the extraction and quan-
tification of CYN in real vegetables samples such as lettuce and spinach, previously exposed to 10 and 50 µg CYN/L in the laboratory. Results reveal that CYN uptake in both lettuce and spinach leaves was greater when the exposure concentration was lower. This work emphasizes the need to develop validated methods to monitor the occurrence of CYN in crop products irrigated with waters affected by cyanobacterial blooms. This method will improve the real human exposure evaluation required in risk assessment. Acknowledgments: Ministerio de Economía y Competitividad of Spain (AGL2015-64558-R, MINECO/FEDER, UE) for financial support. Leticia Díez-Quijada also acknowledges for the grant BES-2016-07873 associated with this project (MINECO/FEDER).

Toxic Endophyte-infected Tall-Fescue Dysregulates the Fecal Microbiota of Grazing Beef

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Microbiome homeostasis and dyshomeostasis are understudied in food-producing animals, such as beef cattle, especially under condi-
tions of various environmental stressors. Tall fescue, the predominant southeastern United States forage grass, can become infected with a toxic, ergoalkaloid (EA)-producing endophyte that results in numerous physiological deterrents, ultimately decreasing weight gains. This eco-
nomically costly condition is known as fescue toxicosis (FT). The impact of toxic fescue on grazing beef microbiota (MB) and the role it might play in FT etiology, has yet to be investigated. So, we used 16S rRNA sequencing and a metatranscriptome from Angus steer grazing toxic (E+; n = 6) or non-toxic (Max-Q; n = 6) tall fescue over a 28 day grazing trial. Firmicutes and Bacteroidetes phyla comprised 90% of both Max-Q and E+ steer MB. Significant early decreases of Erysipelotrichaceae and delayed increases of Ruminococcaceae and Lachnospiraceae families were the main effects of E+ grazing. The genera Oscillibacter increased after 14 days of E+ grazing. Many operational taxonomic units within the Ruminococcaceae and Lachnospiraceae families negatively correlated with weight gains (decreased by E+) and positively correlated with respiration rates (increased by E+). Thus, E+ grazing induces MB dysbiosis in a way that could contribute to the altered lipid metabolism and enteric nervous and immune system dysfunction associated with FT. Moreover, E+ grazing-induced MB shifts could influence MB metabolism, potentially modulating FT-related feeding behaviors through enteroendocrine activities. Detection of decreased Prevotellaceae and increased Clostridiaceae likely reflect MB selection pressure from degra-
diation of EA and other plant/endophyte-derived metabolites. Overall, our data characterize the grazing Angus steer fecal MB and indicate that E+ grazing results in MB shifts that either associate with, or, contribute to, FT pathophysiology. Support: USDA, NIFA, Grant Number 67030-25004.

Cherry Intake Potential to Improve Intestinal Health

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Quercetin and its glycosides have been shown to possess potential ben-
efits to human health. Several flavonoids are available to consumers as dietary supplements, promoted as anti-oxidants; however, incorporation of natural quercetin glycosides into food and beverage products has been limited by poor miscibility in water. Enzymatic conjugation of multiple glucose moieties to isoorientin to produce alpha-gly-
cosyl isoorientin (AGIQ) has been shown to enhance solubility and bioavailability. AGIQ is used in Japan as a food additive and has been granted generally recognized as safe (GRAS) status. However, although substantial genotoxicity data exist for quercetin, there is very little avail-
able data for AGIQ and isoorientin. To support expanded global mar-
eting of food products containing AGIQ, comprehensive good labora-
tory practice-compliant testing of the genotoxic potential of AGIQ and isoorientin was conducted in accordance with current regulatory test guidelines. Both chemicals tested positive in reverse mutation assays in several bacterial strains with and without metabolic activation, and 4-hour, but not 24-hour, exposure to isoorientin resulted in chromo-
osomal aberrations in CHO-WBL cells in the presence and absence of metabolic activation. All other in vitro mammalian micronucleus and chromosomal aberration assays, micronucleus and comet assays in male and female B6C3F1 mice and female Sprague Dawley rats, and mutation assays evaluating multiple potential target tissues in transgenic mice (Muta™ mouse) were negative for both chemical. These results supplement existing toxicity data to further support the safe use of AGIQ in food and beverage products.
An OECD 408 dietary 90-day toxicity study was conducted on test material (10-undecenal, a widely used flavor and fragrance ingredient). Sprague Dawley rats (10 rats/sex/dose) were fed diets containing 0, 200, 2000, 6000 or 20000 ppm (equivalent to a mean achieved dosage of 0.143, 138.6, 382.3 and 1135.9 mg/kg/day, respectively) of 10-undecenal for 90 days. Estrous cycling, sperm analyses and reproductive organs were also evaluated. A dose-related reduction in body weights was observed among males of the 2000, 6000 and 20000 ppm dose groups and females of the 6000 and 20000 ppm dose groups. Body weight gains were significantly decreased among high dose males throughout the study and among 6000 ppm males and 20000 ppm females only during the first few weeks of the study. Overall food consumption was reduced in animals of both sex treated at 2000, 6000 and 20000 ppm. Food efficiency was also reduced among the high dose group animals during the first week of the study. Centrilobular hepatocellular hyper trophy was reported in males of the 2000, 6000 or 20000 ppm dose groups during microscopic examination, which did not exceed minimal severity limits. There were no corresponding degenerative or inflammatory changes observed, and was therefore considered to be an adaptive response. Microscopic examinations revealed epithelial acanthosis of the limiting ridge of the stomach among males and females of the 2000 and 20000 ppm dose groups and also in females of the 6000 ppm dose group. This finding was considered to be indicative of a local irritation potential associated with the route of administration, and not related to the systemic toxicity of 10-undecenal. Thus, the NOAEL for systemic toxicity was considered to be 2000 ppm or 138.6 mg/kg/day, based on reductions in food consumption and body weights among the higher dose groups. There were no treatment-related effects on the reproductive organs up to the highest dose tested. Thus, the NOAEL for reproductive toxicity was considered to be 20000 ppm or 1135.9 mg/kg/day, the highest dose tested.

The introduction of GM plants modified for nutritional purposes into the food supply requires a comprehensive evaluation of the potential influence of GM food on nutrient intake. Codex guidelines have been established which state that this assessment should be undertaken by simulating the replacement of consumption of the unmodified food (“conventional counterpart”) with the modified food. If the nutrient profile is modified such that it is more comparable to another food, Codex indicates this is also appropriate to conduct an assessment for that food (“appropriate comparator”). However, the guidelines are general and accommodating to different methodological approaches. We therefore set out to develop a decision matrix that incorporates key methodological considerations related to such a nutrient intake assessment. The dietary assessment at intake is baseline and after substitution of foods from the original plant, and requires two basic inputs: food consumption data and the concentration of the nutrient(s) of interest in the original and GM food. In terms of the former, a food consumption database which allows a comprehensive assessment, and manipulation of intake is essential. Intakes should be examined in the context of the total diet for the entire population, while also taking consideration of potentially vulnerable population groups, and individuals with extremes of intake. The selection of the nutrient(s) to be examined may consider factors such as compositional differences between the original and modified food(s), nutrient intake recommendations, and the availability of reliable nutrient data. Sources of concentration data include standard recipes, market and supply data, and technological considerations. These data are also used to determine whether the assessment should be conducted only for the conventional counterpart, or also for the appropriate comparator. Finally, the extent of the replacement must be decided; in the first instance, it may be appropriate to assume a 100% replacement, however, this may be refined to consider more realistic scenarios considering production volume and market share data. This decision matrix serves to assist in identifying the specific information to be incorporated into a GM nutrient assessment using considerations that are unique to each situation and dependent on the nature of the modification.

Ready to eat manufactured baby foods, are natural puree that contain more than one food, usually a mixed fruits, veggies and grains. Due to their physico-chemical characteristics, these components are able to be contaminated by fungi such as Alternaria, Fusarium, Penicillium, and Aspergillus in turn lead to the occurrence in these foods of mycotoxins that are secondary metabolites produced by fungi, presenting varied chemical structures that have the common characteristic of toxicity against humans and animals. The Rapid Alert System for Food and Feed of the European Union reports mycotoxins on the third positions according to the total number of hazard notifications (RASFF, 2015). The objective of the study was to evaluate the presence of 15 mycotoxins (DON, 2AcDON, 15-AcDON, NEO, DAS, NIV, ZON, α-ZOL, β-ZOL, α-ZAL, β-ZAL, FUS X, T2, HT2 and PAT) in ready-to-eat baby foods samples (n=80) by QuEChERS extraction and determination gas chromatography-tandem mass spectrometry determination (GC-MS/MS).

The methods accuracy was evaluated by recovery assays at four concentration levels, and precision, expressed as the intra- and inter-day relative standard deviations (RSD%) were calculated. Good validation results in terms of recoveries (63-117%), reproducibility (RSDs <15%) and repeatability (RSDs<20%) were reached. Moreover, limits of detection (0.5-2.5µg/kg) and quantitation (1-10µg/kg) achieved were lower than the legislated limits. Matrix effect was evaluated and matrix-matched calibrations were used for quantitation. Results showed presence of at least one mycotoxin in 21% of analyzed samples. The mean contamination level of NIV, DON, HT2 and PAT were 2.5, 3.75, 6.2 and 15 µg/kg respectively. The obtained results indicated that mycotoxins are present in different baby food samples which point out the need to perform continuous surveys to insure babies’ health. The dietary exposure of the child population was estimated using the deterministic approach, through the evaluation of the consumption and data of ready-to-eat baby foods mycotoxin contamination to assess the estimated daily intake (EDI) and values obtained resulted below the tolerable daily intake for the selected mycotoxins. Acknowledgements: The Spanish Ministry of Economy and Competitiveness (AGL2016-77610-R) and Government Scholarship program Carlos Antonio López, Paraguay.

**1634a** A Survey of Mycotoxins in Infant/Toddler Foods and Breakfast Cereals in the US Retail Market

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Mycotoxins are ubiquitous secondary metabolites produced by fungi, and they are commonly found in foods. Mycotoxin consumption is associated with adverse human and animal health outcomes making it important to understand their concentration and distribution among food. The goal of this study was to conduct a mycotoxin survey of commercial infant/toddler cereals and teething biscuits, as well as breakfast cereals in the United States. We collected 215 retail samples from 3 geographic locations and used stable isotope dilution liquid chromatography tandem mass spectrometry to analyze aflatoxins, fumonisins, deoxynivalenol, HT-2 toxin, ochratoxin A, T-2 toxin and zearalenone. Results showed the concentrations of detected mycotoxins were below the FDA action and guidance levels. One or more mycotoxins were found in 69% (101/147) of the infant/toddler foods and 50% (34/68) of breakfast cereals with deoxynivalenol being the most frequently detected. Mycotoxin co-occurrence was observed in 12% of infant/toddler foods and 32% of breakfast cereals with co-occurrence most often occurring in oat-based cereals. Rice-based cereals contained mycotoxins less frequently than other cereal types. Taken together, mycotoxins were frequently detected among food samples; however, concentrations were low.

**1634b** A Limited Survey of Corn Meal and Alkaline Cooked Products for Aflatoxin-Fumonisin Co-Contamination in the Southeastern US, 2015-2016

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Aflatoxins are mycotoxins found in corn. They exert adverse health effects in humans including liver cancer and stunted growth in young children. Fumonisins (FB) are also common mycotoxin contaminants of corn and their co-occurrence with aflatoxins has been reported. FB cause species-specific toxicities in animals, however their human health effects are until uncertain. Experimental and epidemiological evidence nonetheless suggests that FB are a risk factor for growth inhibition in children, birth (neural tube) defects, and cancer if contaminated corn is consumed in large amounts on a regular basis. Aflatoxins and FB exert their toxic and carcinogenic effects in animals through different but complementary mechanisms and co-exposure is a growing concern for human health. Corn-based food products acquired from local markets in the southeastern US were surveyed in 2015 (Survey 1) and 2016 (Survey 2) to determine the extent of co-contamination. Survey 1 included 18 alkaline cooked (nixtamalized) corn flours and 23 corn meal samples. Survey 2 included the alkaline cooked products (n=7) and grits (n=10). Aflatoxin B1 (13.4 µg/kg) was found in only one sample, an alkaline cooked corn flour from Survey 1. In contrast, FB were found in all samples surveyed. Mean total FB (FB1-24) concentrations in Survey 1 alkaline cooked corn flours and corn meals were 0.72 (±0.62 SD) mg/kg and 0.18 (±0.07 mg/kg, respectively. Total FB in Survey 2 averaged 0.23 (±0.30) mg/kg in grits and 0.43 (±0.32) mg/kg in polenta. The results of this limited survey indicate that aflatoxin-FB co-contamination of corn products purchased in the southeastern US during 2015-2016 was uncommon.

**1634c** Considerations for a Noni Juice Standard: Scopoletin Concentration


Morinda citrifolia L. (noni) fruit has been used as a food or traditional medicine in Polynesia for over 2000 years. In many parts of the world, noni juice is available as a dietary supplement or “functional food”. The FAO/WHO Coordinating Committee for North America and the Southwest Pacific is considering development of a regional commodity standard for fermented noni juice that would define essential composition and quality factors. Noni juice preparations contain varying levels of phytochemical constituents, including scopoletin (a coumarin derivative), due to variations in growth or post-growth processes. Coumarin and its derivatives are known anticlotgulants and may increase risk of bleeding in humans. A literature search was conducted to identify information about exposure, toxicity and pharmacokinetics of scopoletin to inform a specification for scopoletin in noni juice. Clinical study and adverse event information about noni juice were also examined for evidence of adverse effects related to scopoletin. Adverse effects noted in animals treated orally with scopoletin include toxicity to the brain and prolonged bleeding time. Although some people have experienced adverse events after consuming noni juice, none can be directly attributed to scopoletin exposure. A no observable adverse effect level (NOAEL) for scopoletin could not be identified from available literature. More research is needed to establish the hazard potential of scopoletin in noni juice. A 13-week, guideline, three dose toxicity study of scopoletin in rats (which includes a behavioral toxicity battery) should be conducted (at a minimum) to more fully investigate the potential for scopoletin to cause toxicity. An updated survey of scopoletin concentrations in noni juice would help inform estimated exposure levels. Studies also need to be performed to: a) identify growth and post-growth conditions which result in low concentrations of scopoletin in noni juice, and b) determine whether scopoletin peroxidase (or some other enzyme) can be employed to selectively reduce concentrations of scopoletin in noni juice.

**1634d** Scientific Analysis of Adverse Effects Associated with Kava

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Kava, derived from the roots of the plant Piper methysticum, has been traditionally used for centuries in the Pacific Islands as a recreational and ceremonial drink. It has become increasingly popular in other regions and can be found as beverages or dietary supplements marketed for relaxation and stress relief. We conducted a scientific analysis of adverse health effects associated with consumption of kava and its...
components. Kava use has been associated with adverse effects, most notably hepatotoxicity, which has been attributed to the kava cultivar, use of certain plant parts, kava constituents, extraction method, and contaminants. Considering literature highlighting the liver as a target organ for kava, we examined FDA’s CSFAN Adverse Event Reporting System (CAERS) for adverse events related to kava and kava containing products (2004-2017). CAERS identified more than 30 reported cases of adverse events from kava exposure. Of the cases reported, roughly half were associated with adverse events involving the liver. Adverse events were confounded due to poor reporting, concomitant use of other substances, and preexisting conditions. Therefore, it is difficult to determine if contamination of the reported products is directly associated with adverse liver effects. FDA will continue to monitor the literature for kava, as well as adverse events from kava exposure. In addition, we continue to provide scientific support to the development of the Codex Alimentarius Commission’s proposed regional standard for kava.

1636 The Air Pollutant Watch List as a Tool to Decrease Hazardous Air Pollutants in Texas

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The Texas Commission on Environmental Quality (TCEQ) uses air permitting, air monitoring, and the air pollutant watch list (APWL) to address areas in Texas where air monitoring data show persistent, elevated concentrations of air toxics. The APWL program allows the agency to focus its resources, notify the public, engage stakeholders, and develop strategies to reduce emissions in areas that show persistently elevated levels of toxic products. The APWL program includes a list of chemicals targeted for regulation, a list of thresholds and concentrations at which the agency will take action, and a list of areas where the concentration of a listed chemical is too high to be acceptable. The agency collects on a regular basis.

1637 Cytotoxic Flavor Chemicals in Top-Selling Electronic Cigarette Refill Fluids

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Thousands of electronic cigarettes (EC) refill fluids are sold worldwide with little understanding of their toxicology. Our purpose was to identify the top-selling EC refill fluids, quantify the presence of these products, evaluate their cytotoxicity, and identify flavor chemicals that contribute to cytotoxicity. EC users’ flavor preferences and top-selling products were evaluated in an Internet survey, by contacting local EC shops in Southern California, and by visiting online EC stores. Regulatory EC refill fluids were purchased from local shops, their flavor chemicals were identified and quantified by gas chromatography-mass spectrometry, and their cytotoxicity was evaluated using the MTT assay. Authentic standards of flavor chemicals present in the highest concentrations were screened to identify those that were the most cytotoxic.

1638 Using Systematic Review to Support the “Regulatory Intelligence” Strategy in Industry

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The rising role of systematic review on chemical risk assessment has been reshaping regulatory decisions to provide stakeholders with more precise and certain regulatory decisions. Industry continues to use systematic review methodology in risk assessment and be proactive in the agency’s decision making processes.

1639 Reduce Animal Testing for Acute Toxicity in US EPA Pesticide Programs

J. Tao, T. Keigwin, and A. Lowit. US EPA, Washington, DC.

Historically, pesticide regulations rely heavily on mammalian toxicity data derived from animal testing. US EPA’s Office of Pesticide Programs (OPP) is evaluating and adopting alternative approaches to more traditional methods of toxicity testing. OPP generally receives 200-300 6-packs per year and has set goals around significantly reducing the use of animals in acute toxicity “6-pack” studies. The current oral, acute dermal, and acute inhalation toxicity, primary eye and skin irritation, and skin sensitization. In this presentation, we are reporting on the number of alternative studies submitted to OPP for pesticide product registrations from 2010 to 2017. The Agency encourages the Up-and-Down (UAD) method for acute oral toxicity and the Local Lymph Node Assay (LLNA) for skin sensitization. As a result, the UAD method is pre-
dominate among acute oral toxicity studies submitted. The LLNA has become a preferred study over the traditional Buehler Test. The Agency has received a large number of the UADL and LLNA studies in recent years. Additionally, under the Agency’s initiative to develop non-animal alternatives for acute toxicity testing, OPP has developed the guidance document, *Updated Guidance for Testing Antimicrobial Cleaning Products (ADCPs) for Their Potential to Cause Eye Irritation*, which accepts Bovine Corneal Opacity and Permeability assay (BCOP), EpiOcular assay (EO), and Cytosensor Microphysiometer (CM) assay eye irritation testing for registration of ADCPs. We have received a limited number of in vitro alternative studies for eye irritation under this guidance but the number of submissions is slowly increasing. In 2016, OPP finalized the *Guidance for Waiving Acute Dermal Toxicity Tests for Pesticide Formulations & Supporting Retrospective Analysis*. We are beginning to see waivers submitted under this guidance.

### 1640 Evidence Supporting a Quantitative Dermal Sensitization Assessment for Methylisothiazolinone


Under 40 CFR 152.25(a), a treated article is defined as “an article or substance treated with, or containing, a pesticide to protect the article or substance itself.” Pesticides may be registered for such use, but do not bear a pesticide label or effectively use other communication methods to inform and protect people against potential hazards. Dermal sensitization is a potential hazard from exposure to treated articles. In an effort to better assess risk from such exposures, the agency has been evaluating approaches to quantitative assessment of dermal sensitization. Methylisothiazolinone (MI) is presented as a case study for quantitative assessment, since robust data from multiple lines of evidence exist. Specifically, MI is shown to be a positive dermal sensitizer in laboratory animal tests (guinea pig and local lymph node assays), from studies in human volunteers (repeat open application tests) and recently, from results of in silico assays with MI (Direct Peptide Reactivity Activation assay (DPPRA), KeratinoSens, and Human Cell Line Activation Test (h-CLAT)). Each line of evidence has strengths and uncertainties for a quantitative approach. Guinea pig assays can identify positive sensitizers but do not provide potency or dose-response information. The local lymph node assay can identify potency of a sensitizer for induction but does not provide information on elicitation thresholds. Human tests can characterize dose-response relationships for induction and elicitation and identify thresholds, but do not have large study populations and can show wide individual variation in concentrations that elicit sensitization responses. In vitro/in silico assays for identification of dermal sensitizers address animal welfare concerns and can provide information on sensitization potency as well as information on induction thresholds, but need further development for characterization of dose-response relationships and/or identification of quantitative thresholds for induction and elicitation. This abstract does not represent official US EPA policy.

### 1641 A Harmonized Global Paradigm for Crop Protection Chemical Assessment

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The collective vision and realization of Exposure-Driven Assessment of Crop Protection Chemicals requires a paradigm shift in support of globally harmonized, risk assessment-based regulatory decision-making. This requires the application of best available science, via integration of new and traditional data streams, to create tailored exposure-driven risk assessments. The International Conference of Harmonization (ICH) for pharmaceuticals can be used as a starting point for this approach. Risk assessment should be the basic operating principle for all decision-making and is essential to scientifically-defensible chemical product stewardship. To be successful this new Crop Protection Road Map for Regulatory Outcome Pathway requires appropriate communication between the regulatory community and the regulated industry. This communication is critical to enable the rapid incorporation of new technologies and advancing science. The outcome would increase the speed, efficiency and accuracy of regulatory decision-making for human health and environmental protection. Global participation and utilization are necessary in order to reap the benefits of smarter, “fit-for-concern”, integrated, and tiered testing strategies. This approach has been successful for drug development and can be successful for the development of innovative solutions for crop protection.

### 1642 Improving Scientific Peer Review by Expert Panels: Case Study of a Large, Two-Tiered Peer Review

**S. Hays, and C. R. Kirman. SciPinion, Bozeman, MT.**

A large, two-tiered peer review was conducted to assess the quality of the safety data collected for a consumer product as part of Tier 1. 5 peer review panels were convened to review various components of the data package, which consisted of 15 publications/reports covering 9 studies, with access to all underlying raw data (including histopathology slides). Tier 1 included efforts from 50 scientists from all over the world, with more than 950 combined years of experience across multiple disciplines. The Tier 1 peer reviews were robust and included a large amount of material to be reviewed by each Tier 1 panel, with unprecedented access to underlying raw data via links to an external web portal. As part of Tier 2, duplicate expert panels were convened to review the results obtained from the Tier 1 panels. Tier 2 involved 17 scientists with greater than 610 combined years in risk/safety assessment. The Tier 2 panels were intended as oversight panels, given optional access to Tier 1 data packages, for the purpose of address over-arching product safety issues. The data collected from this large peer review yielded a robust data set for additional analyses. With respect to reproducibility, the responses from the Tier 2 panels indicate that the results are highly reproducible, with similar metrics for means for codified responses (1.08 vs. 1.02) and consensus metrics (0.73 vs 0.74). With respect to employment sector bias no statistically significant differences (p>0.05) were found across employment sectors (academia, former government, industry) for science questions. Additional findings with respect to the identification of outlier reviewers, and the roles of anonymity, independence, and compensation are discussed.

### 1643 Important Differences between Summary Values Used in Scientific Studies and the Design Value of National Ambient Air Quality Standards

**S. S. Lange. Texas Commission on Environmental Quality, Austin, TX.**

Ozone and particulate matter less than 2.5 µm (PM$_{2.5}$) are 2 of the air pollutants regulated in the US Environmental Protection Agency (EPA) National Ambient Air Quality Standards (NAAQS) program. While there is a lot of focus on the level (concentration) for a NAAQS pollutant, several other factors are also crucial for setting a NAAQS, including the indicator, form, and averaging time of the pollutant, and using the highest monitor in an area. The purpose of this work was to determine how ozone or PM$_{2.5}$ summary metrics used in key epidemiology studies compare to the regulatory design value. Studies used for quantitative risk assessment in the 2015 ozone NAAQS review were evaluated. Whereas the ozone NAAQS is set at 70 ppb for the annual 4th highest 8-hour maximum daily average, averaged over three years at the highest monitor in an area, many alternative metrics were used in these studies. Most common were 1-hr maximum or 24-hr average concentrations; multi-day averages from 2 - 30 days; no consideration of the form; and averaging across all monitors in an area. Like the findings with ozone, while there is both a 12 µg/m$^3$ annual average (averaged over 3 years at the highest monitor in an area) and a 24-hour 35 µg/m$^3$ (99th percentile, averaged over 3 years at the highest monitor in an area) PM$_{2.5}$ NAAQS, many metrics have been used in epidemiology studies. Differences included the indicator itself: imputing PM$_{2.5}$ data from PM$_{10}$ data, light scattering data, clearing index, or airport visibility data; or using PM$_{10}$ or PM$_{2.5}$ data as a direct substitute for PM$_{2.5}$. Other discrepancies include using multi-day averages of 2 to 8 days; no consideration of the 24-hour form; and averaging across all monitors in an area. Therefore, many of the concentration metrics used in epidemiology studies cannot be used to directly inform regulatory metrics, and in some cases are not good representations of concentration changes over time. These summary metrics must be reconciled to the regulatory design value before any judgments are made as to the protectiveness of current and alternative NAAQS.
China has recently joined the International Conference on Harmonization (ICH) which has its set of guidelines (“Safety-ICH S” and “Multidisciplinary-ICH M”) covering nonclinical development of new drugs which are accepted by the US FDA, EMA and Japanese regulatory agencies. Chinese regulatory agency (China Food and Drug Administration, CFDA), until currently, still follows its own guidelines. These guidelines are generally similar to those of ICH but some differences exist in testing requirements. In addition, CFDA just issued a new version of GLP on Sept 2017 which is in many ways similar to international standards while some specific details need further clarifications. A drug developer needs to be aware of these different specific requirements to avoid a delay or rejection (i.e., “clinical hold”) following regulatory submission to initiate clinical trials in China. This poster will highlight similarities and differences between nonclinical requirements for both small (chemically synthetized) and biopharmaceutical molecules in terms of high level requirements and detailed study designs. Some special compounds like antibody-drug conjugate (ADC) and bio-similar drugs are good representatives of these differences and compared in this poster. Setting up a winning drug development strategy and selecting a competent lab to conduct the nonclinical studies should be considered at the initial stage to assure successful and cost-effective drug development process to win the regulatory approvals globally.

The Preclinical Innovation and Patient Safety Initiative: Recommendations for Advancing Preclinical Pharmaceutical Testing

E. Baker, Physicians Committee for Responsible Medicine, Washington, DC.

Preclinical drug testing is critical to understanding the toxicity, pharmacology and pharmacokinetics of candidate drugs. The Preclinical Innovation and Patient Safety (PIPS) Initiative fosters collaboration among drug development stakeholders - including federal agencies, patient, research and health organizations, academia, technology companies and the pharmaceutical industry - to implement modern human-focused preclinical approaches. Participants recognize that more predictive approaches are fundamental to the timelier delivery of safe and effective medicines. This presentation highlights the scientific, regulatory, policy, training and educational recommendations made by a group of stakeholders during the kick-off roundtable in January 2017, and explores three projects identified by PIPS participants as likely to provide high impact on the field of toxicology. First, modern approaches to validation should incorporate human data. This is the only way to overcome the challenge of comparing human in vitro approaches to currently available animal safety data. The legislation 21st Century Cures and the Prescription Drug User Fee Act mandate that FDA make real world human data/evidence more accessible. PIPS identified that implementation of the mandates should include making real world evidence available to method developers for validation purposes in order to better understand a method’s predictive ability. Second, researchers must have access to human cells and high-quality tissues in order to use human-focused approaches. Currently, there are no guidelines for collection and care of human cells and tissues intended to be used for research. PIPS is coordinating efforts to develop guidelines to ensure the collection and care of human cells and tissues intended to be used for human-focused approaches. Currently, there are no guidelines for the overall hazard characterization. This can facilitate characterization of biological events between MIE and effects at the cell, organ and organism level obtained from traditional ecological toxicity tests (i.e., apical effects), and enhances characterization of hazard. An example using two thiocarbamate substances (CAS RN 137-76-8 and CAS RN 120-54-7) illustrates how this approach is being used, and the potency differences within the thiocarbamate ecological hazard profiles attributable to toxicokinetics and hypothesized covalent interactions.

Cyanobacteria: Understanding the Toxicology to Communicate Risks and Support Decision Making

R. Chung, A. Leung, and R. Copes, Public Health Ontario, Toronto, ON, Canada.

Cyanobacterial blooms in recreational waters are often considered a concern for swimmers due to the potential for skin rash and gastrointestinal illness. In the summer of 2016, a conservation authority in Ontario reported a cyanobacterial bloom in a local lake. The conservation authority also reported a total microcystins concentration in exceedance of the Canadian Recreational Water Guideline of 20 µg/L.
expressed as microcystin-LR). In response, the local health unit applied the precautionary principle and posted a ‘do not enter’ warning to lake users until more samples could be taken. However, due to the number of water bodies in the area that required monitoring, a follow up visual inspection and water sample could not be taken in a short turnaround time. As a result, the health unit had to balance adequately communicating risks to public users of the lake while minimizing the decision of when to retract the warning. This common response to cyanobacterial blooms poses a challenge to public health management of recreational water activity. To assist public health’s interpretation of results and risk communication, Public Health Ontario (PHO) is undertaking a jurisdictional scan of cyanobacterial guidelines and standards. The jurisdictional scan will compare the derivations of recreational water guidelines and standards between jurisdictions to determine the interpretation and application of cyanobacterial toxicity data. Specifically, the consideration of cyanobacterial toxins such as microcystins, anatoxins, and saxitoxins and the derivation of guidelines and standards may provide public health with a better understanding of the risks posed by cyanobacterial blooms. The overall objective of this comparison is to provide an interpretation of toxicity data to inform public health action in the management of recreational waters.

1649 Margin of Exposure Approach for Interpreting Concentrations of Perfluoroalkylated Substances in Drinking Water
T. Lalvani, R. Chung, and R. Copes. Public Health Ontario, Toronto, ON, Canada.

Perfluoroalkylated substances (PFAS) were used historically in firefighting foam in Canada. While import and production of PFAS in North America has now ceased, existing stocks are still in use and international manufacturing has increased. For most Canadians, exposure to PFAS occurs largely through diet and consumer products, followed by indoor dust, and drinking water. PFAS were detected in surface water and groundwater of a Northern Ontario community, where these compounds were used during firefighting training and testing of firefighting equipment. Specifically, perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) were identified in private drinking water wells in the community, as well as the source water for the municipal system. Communicating how risks from chemical exposures is challenging, and may be further complicated when there is emerging scientific evidence. Currently, there are no established Canadian drinking water guidelines or standards for PFOS and PFOA. Health Canada has proposed drinking water guidelines for PFOS and PFOA that differ from other jurisdictions. Without an established drinking water guideline or standard, a margin of exposure (MOE) approach was undertaken to interpret concentrations of PFOS and PFOA. The MOE for a given health effect describes how close human levels of exposure are to the lowest level reported in animals for a particular health effect. It differs from a hazard quotient assessment used in setting drinking water and other standards by regulators where the hazard quotient compares a toxicological reference value (TRV) with an estimated intake. This work showcases an example of how the MOE was used to manage risk communication without selecting a particular drinking water guideline or TRV. The use of the MOE approach allowed public health officials to communicate a relative indication of health concern about the concentrations of PFAS found in the drinking water to the community. This approach also ensures that the ongoing regulatory process for establishing a drinking water guideline or standard is not overstepped, and that public health’s management of risks remains relevant even after the regulatory framework for deriving a drinking water guideline or standard has been finalized.


In June 2016 Congress passed the Frank R. Launtenberg Chemical Safety for the 21st Century Act (or the Lautenberg Act). This action amended the Toxic Substances Control Act (TSCA), which over forty years ago gave the U.S. Environmental Protection Agency (EPA) the authority to restrict the manufacture, release, use, import, and disposal of chemical substances. Among the changes included in the amendment is an express mandate that the EPA consider “potentially exposed or susceptible subpopulations” such as infants, children, pregnant women, workers, or the elderly when conducting a risk evaluation of chemical substances under TSCA. Although the susceptible subpopulation language included in several of the updated sections is new to TSCA, EPA has experience in identifying and assessing the possibility of increased risk posed by chemicals to the most susceptible groups among the public, particularly children. Amended TSCA provides an opportunity to advance this experience in the use of susceptibility concepts during the risk evaluation process. Initial steps in the effort to implement the consideration of susceptible groups under the Lautenberg Act have been undertaken. More than 20 current EPA policy and guidance documents that include discussion of susceptibility were compiled and reviewed with regard to their usefulness and potential impact under amended TSCA. This effort has resulted in the definition and scope of susceptible subpopulations found in these documents includes a wide range of considerations, with substantial information focusing on concerns for pregnant women, infants and children. Based on this review of current policy and guidance, case studies focusing on children identified were identified in an effort to illustrate how some of these key concepts may be applied to the risk evaluation process under amended TSCA. The review and case studies presented here may be helpful in implementing the potentially exposed and susceptible subpopulations provisions of amended TSCA. The findings and conclusions of this presentation do not necessarily represent the official views of US EPA or the Association of Schools and Programs of Public Health (ASPPH).
may be demonstrated by a number of means including a demonstration that the predicted ground level concentrations for the permitted emissions, evaluated on a case-by-case and chemical-by-chemical basis, do not cause or contribute to a LOC. The TCEQ’s ambient air monitoring program is extensive and provides data to help assess the potential for adverse effects from all sources (point, mobile, etc.) in an area. If air toxics are persistently monitored at a LOC, a localized APWL area is established. The purpose of the APWL is to help reduce ambient air toxic concentrations below LOCs by collaborating with stakeholders and focusing TCEQ resources in a particular area. The TCEQ Toxicology Division (TD) uses the most up-to-date methods to derive health-protection levels for chemicals in air. These methods are included in the TCEQ Guidelines to Develop Toxicity Factors, which were externally peer-reviewed by a panel of experts in inhalation toxicology and risk assessment. The Guidelines describe how the TD conducts scientifically sound toxicity assessments to establish levels of chemicals in air below which adverse health and welfare effects would not be expected (i.e., LOCs). Since the health effects review for air permitting differs from the evaluation of measured ambient air monitoring data, different risk management objectives and terminology for levels are used for the two programs. The use of different values and terminology is appropriate because the air permitting and air monitoring programs perform different functions in the protection of human health and welfare. This poster will highlight examples of decreases in air toxic levels in localized areas of Texas, resulting from the interactive nature of these programs and will illustrate the role of TD in these programs.

1653 Changes to the Common Rule Regulations and Implications for Human Research
M. Madden, US EPA, Chapel Hill, NC.

The regulations that govern research involving human subjects are known as the “Common Rule” because they are shared in common by 18 federal departments and agencies that conduct and support such research (The US Food and Drug Administration is not a signatory to the Common Rule). These regulations have not changed substantively since 1981. During that time, the research they cover has evolved considerably, with new scientific techniques and new ethical challenges that do not always fit well under the established structure. These include an evolving concept of what constitutes identifiable information and biostatistical techniques, such as whole genome sequencing, and concerns over commercialization and informed consent. A six-year rulemaking process began in 2011, with a preliminary draft notice release and more than 3,000 public comments received. This process culminated in the publication of a revised final rule on January 19, 2017. The majority of changes will take effect in January 2018, bringing sweeping changes for scientists and their institutions, funding agencies, and the Institutional Review Boards (IRBs) that oversee this work. This informational session will review the reasons for change, the rulemaking process, and the major changes in the revised regulations with a presentation by the US Environmental Protection Agency representative on the Interagency Working Group that drafted the new Common Rule. The daily experiences of implementing the Common Rule changes in a timely manner into human subject research at an academic institution and areas where the new rule is not clear will be shared by a presenter with the Office of Clinical Research at the University of Texas Health Science Center at San Antonio (UT Health San Antonio). UT Health San Antonio has several IRB authorization agreements with nearby institutions, such as Brooke Army Medical Center and the Southwest Research Institute, to serve as the single IRB of record for collaborative research. Potential issues with implementation of the new rule with studies at collaborative sites will be presented. A third speaker from the National Institute of Environmental Health Sciences IRB will discuss the potential impact of the new Common Rule on the submission and implementation of grant applications and also will provide insights on possible upcoming changes in the National Institutes of Health processes for human studies. This session will present extremely important ethics information for those who perform research with human subjects, including industry, government, and academia, and will facilitate discussions about applicability of the changes to current research and potential research designs and submissions. Disclaimer: The views expressed are those of the authors and do not necessarily represent the views or policies of the US EPA.

1654 Management of Toxic Wastes in Africa: Challenges and Opportunities
A. Kadry, US EPA, Washington, DC.

Africa is the world’s second-largest and second-most-populous continent, with a population of 1.2 billion people. In spite of its vast natural resources, Africa faces endemic poverty, food insecurity, and pervasive underdevelopment, with almost all countries lacking the human, economic, and institutional capacities to effectively and sustainably develop and manage their resources. In Africa, use of chemicals has taken a central stage in improving health, agriculture, mining, education, and many industrial processes. While African countries are heavy users of industrial chemicals, there is an absence of effective chemical waste management systems, as well as chemical safety education and rigorous enforcement of safety regulations. This absence has the potential to contribute to the exposure of a large portion of the population to toxic chemicals. Users and non-users of chemicals risk exposure to toxic chemicals as a result of ignorance of the risks, failure to employ protective measures, and ineffective implementation and enforcement of safety regulations of these chemicals. It was, thus, not surprising when the World Health Organization published alarming of the results of a survey of residents on chemicals of public health risk concern and their management. Many chemicals of public health concern that are banned, controlled, or withdrawn in the developed countries are still in use or shipped for disposal to Africa. These hazardous and toxic wastes pose risks to nearby water, soil, and air and have the potential to cause serious environmental and human health impacts. In addition, there are thousands of tons of industrial waste, containing hazardous chemicals, that are improperly discharged or emitted into the ambient. In many African countries, industrial waste in liquid form is usually discharged into sewer systems or rivers as effluent, while solid waste is either dumped in landfills or pits within workplace premises or close to residential areas. In addition, international illegal dumping remains a prevalent issue in chemicals management in Africa. Many African countries lack appropriate, cost-effective, and economically viable technology for chemical waste recovery and disposal. The purpose of this session will be to highlight examples of decreases in air toxic levels in localized areas of Texas, resulting from the interactive nature of these programs and will illustrate the role of TD in these programs.

1655 Moving Beyond Theory to the Use of Systematic Review and Evidence-Based Risk Assessment
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The use of systematic review and evidence-based methodologies in toxicology and risk assessment have evolved from theory to practice. This informational session seeks to provide an overview of the landscape of the use of systematic review in regulatory decision making. Recognizing that there are many efforts to advance the science in this arena, this session will focus on efforts specifically associated with risk-based practices, such as development of health-based benchmarks (e.g., acceptable daily intakes, reference doses, etc.) rather than characterization of potential hazard (e.g., likelihood to be a hazard to humans for a given health outcome). Tools and frameworks initially developed for the field of medicine have been adapted for applying research questions, and in many cases, new tools have been developed. The presentations will describe how the regulatory practitioners have
The obesity epidemic and associated co-morbidities are becoming an increasing concern to public health. Obesity can be generally defined by excess storage of lipids in adipose tissue. Central adipose tissue content directly correlates with the development of metabolic syndrome and cardiovascular complications that alter adipose tissue metabolism. Adipose tissue is not only a storage depot for triglycerides, but it is a metabolic active tissue that is responsible for the release of adipokines, such as liver and skeletal muscle, by hormonal-signaling pathways. In addition to lipid metabolism and mobilization, adipose tissue is intricately involved in glucose homeostasis and, therefore, contributes overall to the maintenance of systemic energy balance. It is suggested that adipose tissue plays an important role in the development of obesity-related diseases, thus requiring greater knowledge and understanding of adipose tissue development, signaling pathways, and identification of roles in systemic diseases. Current toxicological findings implicate a role for multiple persistent environmental chemicals, such as phthalates, tributyltin (TBT), and Di-(2-ethylhexyl) phthalate (DEHP) in promoting adiposity. Increases in adiposity are attributed to dysregulation of various metabolic pathways via genetic/epigenetic alterations, as well as contributing environmental factors. Recent studies have demonstrated accumulation of persistent environment toxicants due to their lipophilicity in adipose tissue, making adipocytes and preadipocytes targets of toxicity. Many of these studies have established the diverse mechanisms by which “obeses” promote adiposity. Overall, toxicants appear to have direct and indirect effects on adipose tissue homeostasis and adipose tissue dysfunction, a key characteristic of obesity with implications of metabolic disease. Many compounds have been identified via high-throughput screening programs focusing on target-specific binding. These programs include ToxCast, Tox21, and, more recently, the Endocrine Disruption Screening Program. Prioritizing these chemicals for further in vivo research is essential due to the number of identified chemicals by these programs. Discerning the cellular and molecular basis for endocrine and physiological outcomes of these chemicals is paramount in toxicological research. The objective of this symposium is to present an overview of the effect of obesogens or endocrine-disrupting chemicals on adiposity. Future research is warranted to contribute to the understanding of the contributions of other metabolically important tissues (liver, skeletal muscle, intestine) and the overall contribution to a growing obesity epidemic in both adults and children worldwide. In vitro exposure outcomes to TBT pertaining to the development of a “thrifty genotype” and phthalate exposure effects on adipocyte differentiation and maturation will be discussed, demonstrating the importance of adipocyte as a potential target of DEHP and other plasticizers. The adipocyte regulator PPARγ can be induced by environmental ligands favoring white adipocyte development, while dichlorodiphenyltrichloroethane exerts its effects on brown adipose tissue and the inhibition of thermogenesis, demonstrating the diversity of adipocyte function. Adipose tissue is responsive to endocrine disruptors; therefore, it is important to also understand the sex-dependent differences in human adipocyte function and distribution. Lastly, the implications of adipocyte research in regards to regulatory standards and the testing of compounds for adipogenic properties must be optimized and standardized utilizing specific assays that are most closely related to/adipocytes. Medium-throughput assays with greater relevance to downstream cellular outcomes in context to PPARγ and glucocorticoid signaling could be the future of prioritizing adipogenic compounds for risk assessment. The speakers will introduce the overall importance of adipose tissue homeostasis for human health and the contribution of environmental toxicants to its dysregulation.
Metabolic homeostasis is controlled, in part, by a family of proteins called nuclear receptors, which are the mechanism by which lipophilic hormones and hormone-like molecules regulate gene expression. One such nuclear receptor is peroxisome proliferator activated receptor γ (PPARγ). Its activation is essential for white, brite (brown-in-white) and brown adipogenesis, mature adipocyte maintenance, and insulin sensitivity. PPARγ activation regulates energy homeostasis by both stimulating fat mass and metabolic health, the goal of this presentation is to review current knowledge of mechanisms that determine where fat is deposited and sex differences in body fat distribution but remain poorly understood. Our data and others in the current literature provide evidence that suggests the following:

- Fat distribution in males vs. females. The world Health Organization (WHO) still recommends dichlorodiphenyldichloroethylene (DDE) for malaria control, and high body burdens of DDE, as well as other metabolites, are found in people across the world, and these levels continue to increase. The National Toxicology Program (NTP) recently used DDT. Human migration, semi-volatility, persistence, and bioaccumulation continue to globalize exposures to DDT and its more persistent metabolite dichlorodiphenyldichloroethylene (DDE) through the food supply. Further, most adults born in the US prior to the DDT ban were highly exposed to DDT during their developmental window that programs lifetime metabolic function and are now of age for heightened metabolic disease risk. Quantitative meta-analyses of seven prospective studies consistently indicate that humans exposed to elevated levels of DDE have an increased risk of obesity (beta = 0.13 BMI z-score (95% confidence interval: 0.01, 0.25) per log increase of p,p'-DDE). We exposed pregnant and nursing mice to a technical mixture of 1.7 mg DDT/kg body weight from gestational day 11.5 to postnatal day 5 and confirmed their circulating DDT and DDE levels (means: 2.2 ng p,p'-DDE/mL serum, 51.1 ng p,p'-DDT/mL serum) were within the range reported in people. Maternal DDT and DDE exposure increased the adiposity and insulin resistance of the adult mouse offspring. This long term metabolic toxicity resulted from reduced energy expenditure due to underlying impaired brown adipose thermogenesis in mice. Indeed, impaired thermogenesis was evident in mouse offspring on the last day of maternal dosing through nine months of age, when extensive transcripts involved in thermogenesis were down regulated in brown adipose. Cultured brown adipocytes also had decreased basal and uncoupled respiration in response to exposure to 1 through 1000 nM p,p'-DDE during adipogenic differentiation. These in vitro effects also coincided with increased expression of genes regulating thermogenesis, such as Ucp1. Our evidence indicates that the consistent association of DDE with human obesity can be explained by impaired brown adipose thermogenesis.

Evidence That the Pesticide DDT and Its Metabolite DDE Are Obesogens
M. A. La Merrill. University of California Davis, Davis, CA.

The developmental origins of obesity hypothesis posits a multifaceted contribution of environmental and genetic factors to the fetal origins of obesity and metabolic disease. Adipocyte hyperplasia during gestation and early childhood may result in predisposition for obesity as an adverse event later in life. The PPARG and NR3C1 (glucocorticoid nuclear receptor) pathways have a well-defined role in programming adipose-derived stem cell differentiation. For the PPARG screen, a set of 60 chemical compounds identified in ToxCast as PPARG active (49) or inactive (11) were initially evaluated for effects on adipogenesis and cytotoxicity. Appropriate hits were further tested in a series of 4 orthogonal assays representing 7 different events in PPARG-dependent adipogenesis, including gene transcription, protein expression, and adipokine secretion. Collectively, a total of 14/49 (29%) prioritized chemicals were identified with moderate-to-strong activity for human adipogenesis. A similar approach was taken to assess activity of 2 prioritized compounds identified as having NR3C1-dependent bioactivity. The universal concentration-response, multi-endpoint design of these assays provides a weight-of-evidence determination of chemical-biological activity based on hit frequency, efficacy, and potency. The approach demonstrates a process of prioritizing compounds from high-throughput screens and moving them into a mechanistic in vitro model to identify mode-of-action in context with a physiological outcome. This enables filtering of hit data to further prioritize the number of compounds deemed relevant for additional testing or risk assessment.

Screening ToxCast and Tox21-Prioritized Chemicals for Mechanistic Function in a Human Adipose-Derived Stem Cell Model of Adipogenesis
C. Deisenroth. US EPA, Durham, NC.

The developmental origins of obesity hypothesis posits a multifaceted contribution of environmental and genetic factors to the fetal origins of obesity and metabolic disease. Adipocyte hyperplasia during gestation and early childhood may result in predisposition for obesity as an adverse event later in life. The PPARG and NR3C1 signaling in context with multiple phenotypic outcomes related to adipocyte differentiation. For the PPARG screen, a set of 60 chemical compounds identified in ToxCast as PPARG active (49) or inactive (11) were initially evaluated for effects on adipogenesis and cytotoxicity. Appropriate hits were further tested in a series of 4 orthogonal assays representing 7 different events in PPARG-dependent adipogenesis, including gene transcription, protein expression, and adipokine secretion. Collectively, a total of 14/49 (29%) prioritized chemicals were identified with moderate-to-strong activity for human adipogenesis. A similar approach was taken to assess activity of 2 prioritized compounds identified as having NR3C1-dependent bioactivity. The universal concentration-response, multi-endpoint design of these assays provides a weight-of-evidence determination of chemical-biological activity based on hit frequency, efficacy, and potency. The approach demonstrates a process of prioritizing compounds from high-throughput screens and moving them into a mechanistic in vitro model to identify mode-of-action in context with a physiological outcome. This enables filtering of hit data to further prioritize the number of compounds deemed relevant for additional testing or risk assessment.

Sex and Depot Differences in Adipocyte Biology
S. K. Fried. Icahn School of Medicine at Mount Sinai, New York, NY. Sponsor: L. Armstrong

Increasing evidence suggests that chemicals present in the environment and food additives can increase risk for obesity. These so-called obesogens can act directly on adipocyte metabolism and growth, or indirectly via effects on other organ systems. As many of these compounds and their metabolic products may be conjugated within the adipocyte. For the long term goal of understanding how obesogens influence fat mass and metabolic health, the goal of this presentation is to review current knowledge of mechanisms that regulate adipocyte tissue growth and function, how these differ among different anatomical fat depots, and sex differences in adipocyte biology. Body fat is stored in highly specialized cells (adipocytes or fat cells) and organized within distinct anatomical depots. Women, compared to men, have a greater percentage of body fat, and it is distributed differently. In ~50% of women, more fat is deposited in gluteal-femoral subcutaneous depots, resulting in a pear shape. Intriguingly, the ability of these fat depots to expand is associated with metabolic health. In addition, on average, men, compared to women have more fat in the trunk, in both visceral, i.e. that associated with digestive organs, and abdominal subcutaneous depots. In both sexes, however, the size of the waist reflecting upper body or truncal fat is associated with higher risk of type 2 diabetes and cardio-metabolic diseases, and the amount of visceral fat highly correlated with metabolic dysfunction including insulin resistance and dyslipidemia. The mechanisms that determine where fat is deposited and sex differences in fat distribution but remain poorly understood. Our data and others in the literature indicate that differences in function of among adipose depots reflect distinct development origins and are in part cell-autonomous. Collectively, these observations indicate that understanding of mechanisms that by which some depots to expand or remodel is a critical to maintaining metabolic health in males vs. females.
Inhalation represents the primary route of exposure to aerosolized nanomaterials (NMs) and ultrafine particles in humans. This increasing use of NMs in consumer-based products warrants a thorough evaluation of their biological impacts and a need to test a large number of different types of NMs. Due to the substantive time, cost, and animals required to conduct traditional in vivo toxicity tests, there is much interest in developing human-relevant strategies that are less reliant on the use of animals to assess the toxicity of these materials for various risk assessment applications. This session will include presentations on in vitro systems that are currently being used to assess the inhalation toxicity of nanomaterials and ultrafine particles. Additionally, presenters will discuss the parameters that are critical to consider when designing in vitro systems and which facilitate their interpretation and application in risk assessment, including the following: dosimetry, aerosol generation, and exposure, appropriate cell types, and identification of relevant endpoints. Contribution of adverse outcome pathways (AOPs) to experimental and regulatory toxicology of NMs and strategies for the development of AOP-based computational tools and databases will also be discussed. By discussing the aforementioned parameters, this session will provide an insight into the factors that should be considered to increase the ability of in vitro models to predict human outcomes eventually leading to their use in regulatory decision making.

Integrated In Vitro-in Vivo Models for Nanomaterial and Ultrafine Particle Toxicity Testing: Moving from a Screening Hazard Tool to Predictive Models for In Vivo Adverse Effects

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Engineered nanomaterial and ultrafine materials (ENMs) are revolutionizing a diverse spectrum of commercial and industrial sectors with improved or novel technologies. Animal inhalation studies report ENM deposition in the deep lung with distinct disease risks; however, they are time- and resource-consuming and cannot be used to assess each ENM. With the advent of AOPs, and computational and computational models of ENMs, there is no consensus on how to rapidly screen associated hazards and define risks for occupational and public exposures. Several regulatory bodies have urged researchers to develop alternative approaches and integrated in vitro-in vivo effect models to press the question: Can in vitro models predict in vivo effects? Here, we examine several in vitro models that show evidence for predictability of in vivo damage, fibrosis, and tumorigenesis responses using scaled, realistic pulmonary exposure doses for several ENMs. Single- and multi-walled carbon nanotubes (CNT), and cerium oxide are known to penetrate into lung interstitium and induce interstitial fibrosis. Using scaled models of ultrasensitive per alveolar surface area, these ENMs stimulate fibroblast proliferation, collagen production, and a fibroblast stem cell-like phenotype that correlate with in vivo effects. Furthermore, the use of co-culture models has improved the understanding of how the inflammatory response mediates fibrosis development. Since some CNTs possess properties similar to known carcinogens, long-term, continuous exposures were tested in vitro and resulted in neoplastic or malignant transformation that correlated well with in vivo effects. CNT-transformed human lung bronchial epithelial cells exhibited elevated cancer hallmarks, proto-oncogene signaling, and evidence of cancer stem-like cells, consistent with known lung cancer signaling and established clinical biomarkers. Recent expansion of this approach to nano-sized metal oxides suggested that in vitro human cell models have potential as a useful tumorigenesis screening tool. In summary, development of integrated in vitro-in vivo approaches to assess ENM and ultrafine particle toxicity will fill key knowledge gaps and allow development of predictive in vitro models.

1665 Predictive 3D Lung Models to Assess the Toxicity of Inhaled Nanoparticles

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The lung is considered by far the most important portal of entry into the human body for aerosolized nanoparticles (NPs) released into the environment from combustion-derived processes, or, in an occupational setting via the use of NPs-containing consumer products, such as aerosol sprays. Although animal testing is still the most prevalent model used for risk assessment of inhaled nanoparticles, intensified efforts have been made during the last years towards a systematic development and evaluation of innovative and more reliable in vitro (human) lung cell models. Such models provide standardized and reproducible tools for high-throughput screening but also allow investigating mechanistic studies of inhaled nanoparticles at the single cell level. There are numerous in vitro lung models described to evaluate the human pulmonary epithelial tissue barrier but the choice of the model and the mode of exposure depends on the relevant scenario to be studied. These models range from simple mono-cultures to highly sophisticated 3D models, involving a combination of relevant cell types. Furthermore, air-liquid interface (ALI) exposure is more realistic towards mimicking in vivo conditions in the lung than the suspension exposure. A dose-controlled deposition of various nanomaterials at the ALI of cultured lung cells is therefore preferred. The advantage of such an approach is that the material characteristics can be fully controlled by monitoring the mass deposition on the lung cell surface on-line, allowing to produce a dose-effect correlation. Current research is ongoing to optimize such in vitro tests combining 3D lung models with ALI systems to predict the development of pulmonary diseases such as fibrosis following long-term exposures to aerosolized carbon nanotubes. Such an approach will help to address the regulatory safety testing requirements for inhaled nanomaterials while reducing the use of animals for this purpose.

1666 Contemporary Considerations in Engineered Nanomaterial Characterization, Aerosol Generation, and Exposure

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Traditional hazard testing models using animal inhalation chambers are time and financially expensive. Cell culture models have shown promise as surrogates in screening assays when multiple substances require preliminary information on toxicity. There is also a growing need for development of representative aerosol exposure systems that use a cell-based lung model to simulate inhalation exposure and assess potential hazards associated with aerosolized materials in a rapid and cost-effective manner. This talk presents two aerosol exposure apparatus: a settling chamber and a gentle impactor. Both have been demonstrated as a system capable of exposing cells in culture at the air-liquid interface using gravimetric settling and impaction as aerosol delivery mechanisms. These aerosol exposure apparatus are composed of two critical parts: (1) an aerosol concentrating chamber that delivers liquid and/or solid aerosols in the size range of 1–3 μm in mass median diameter and (2) an exposure chamber where co-cultured cells are maintained at the air-liquid interface during exposure. The aerosol used in the development of this system was mineral oil mixed with sodium fluorescein that served as a surrogate for known occupational respiratory toxicants. The settling chamber was constructed with simple design specifications but exhibits a low deposition efficiency (8%). It has also proved to be a challenge when increasing dosages of same-sized aerosols by altering aerosol input to the system; this occurred due to aggregation at high aerosol concentration in the chamber. Due to the limitations of the settling chamber an alternate system, the gentle impactor, was developed. The gentle impactor requires a more sophisticated design based on aerosol dynamics of target size for the droplets to be delivered. The delivery is more efficient which leads to a shorter exposure duration for the same dosage as compared with the settling chamber. It also enables consistent exposure to the same sized aerosols. From these results, depending on the needs of the experiment, either the settling chamber or the gentle impactor show promise as a high-throughput exposure tool that could be used in conjunction with other current cell-based biological test models.
An Integrated Methodology across the Dispersion Preparation-Colloidal Characterization-Cellular Dosimetry Continuum for Engineered Nanomaterials

P. Demokritou. Harvard School of Public Health, Boston, MA. Sponsor: M. Sharma

Due to the potential public health risk arising from exposure to engineered nanomaterials (ENMs) through consumer applications, a thorough evaluation of their safety is essential. Owing to the fast pace of ENM generation, high-throughput in vitro methods for safety assessments are sorely needed, but to date have proven unreliable with limited predictive capabilities extending to in vivo models. One major contributor to the discrepancies that exist between these models is a failure to reconcile in vitro and in vivo dosages. Despite growing evidence of the importance of ENM dosimetry for accurate hazard assessments, few toxicological studies take it into consideration. This oversight is likely due to a lack of standardization of exposure protocols, the need for additional extended preparations, characterization, and in vitro dosimetry estimation. This presentation will highlight recent advancements that strengthen our understanding on integrated approaches for calculating and predicting relevant dosages for in vitro cellular nanotoxicological studies. It will also highlight several mature, sophisticated computational tools and experimental methods for obtaining dosimetry information in vivo and extrapolating those findings to in vitro cellular systems, with specific examples related to “real world” ENM exposures. In addition to discussing the current state of the field regarding ENM dosimetry, discussions will center on the need and means for future development of this area.

Dosimetry Modeling to Aid In Vitro to In Vivo Extrapolation (IVIVE) of Inhaled Nanomaterials for Risk Assessment Applications

A. M. Jarabe, US EPA, Research Triangle Park, NC.

Accurate in vitro to in vivo extrapolation (IVIVE) of inhalation exposures for applications in support of risk assessment requires consideration of the physicochemical properties of the inhaled agent, characterization of the major mechanisms of its disposition in the respiratory tract, description of key anatomical and physiological properties in humans or other test species for which data may exist, and an adequate understanding of the target exposure scenario in humans or assumptions relevant to regulatory decision making. Dosimetry models have been developed to capture these key features and thereby afford the flexibility and versatility to support the requisite steps ranging from aiding in vitro experimental design, through translation of dose across various test species, to facilitating inferences regarding the probability of pathogenesis processes typically represented by endpoints or key events in an adverse outcome pathway (AOP) which may span a scale from genomic profiling to identifying in vivo dosimetry estimation. This presentation will describe critical concepts and key parameters of inhalation dosimetry for nanomaterials and then illustrate available model structures with key steps of IVIVE to advance the accuracy and regulatory acceptance of new testing and data streams. The impact of dosimetric adjustment on resultant assessment derivations is demonstrated. Opportunities for refining current default inhalation dosimetry algorithms used in dose-response analysis will be highlighted to show how risk assessment applications can continue to evolve as more sophisticated computational structures emerge, including a brief discussion of how ultimately computational models of inhalation dosimetry will serve as the bridge from AOP to virtual tissues and systems biology descriptions of the cardiopulmonary system is provided. Disclaimer: The views expressed in this abstract are those of the author and do not necessarily represent the views or policies of the US EPA.

Advances in Developing Adverse Outcome Pathways to Assess Inhalation Toxicity of Nanomaterials

S. V. Brule. Louvain Centre for Toxicology and Applied Pharmacology (LTAP), Brussels, Belgium. Sponsor: M. Sharma

An adverse outcome pathway (AOP) is a conceptual structure that portrays a simplified linear/sequential diagram of pathways from the molecular initiating event (MIE) induced by a chemical to the adverse outcome (AO), via a series of key events (KE). Due to the large number and types of nanomaterials (NM) with varying physico-chemical properties, there is an urgent need for strategies to predict their toxicity for regulatory purposes. By identifying causative NM characteristics and relevant intermediate toxicity pathways/endpoints, AOPs can highlight knowledge gaps and research priorities to improve predictive approaches. AOPs can help identify predictive endpoints, their respective contribution to the AO, and relevant test assays. AOPs are derived from existing knowledge based on in vitro, in vivo, or computational systems, and KE should ideally represent routinely measurable/quantifiable endpoints. In the in vivo world, AOPs are being developed to strengthen the lung fibrotic hazard of carbon nanotubes (CNTs). We will review and critically comment on these different efforts. Vietti and colleagues based their AOP on in vitro and in vivo data, emphasizing on pathways/endpoints for which correlation between in vitro and in vivo data exist. Labib et al. derived their AOP from general knowledge in lung fibrosis, including other experimental models such as bleomycin-induced lung fibrosis, and on genomics data. Both methods led to similar AOP, with most KE in common. By comparing genomics data obtained from the lung response to different types of NM, Labib et al. showed that regulations of gene expression and toxicity pathways are highly variable regardless of the NM studied, suggesting a possible common AOP for all NM. The magnitude of the effects however varies, possibly reflecting the fact that the lung responses to other NM are weaker than to CNT. By comparing different carbonaceous NM, various authors also identified common pathways (e.g., inflammation and release of growth factors) that could predict their potential to induce lung fibrosis, supporting the possibility to draw a common AOP at least for all CNT. Contribution of AOP to experimental and regulatory toxicity of NM, strategies for the development of AOP, as well as associated issues and limitations will be discussed.

Chemical Grouping for 21st-Century Toxicology, Risk Assessment, and Decision Making

J. Simmons. US EPA, Research Triangle Park, NC.

“Grouping” is a generic term describing placing chemicals in groups based on characteristics or factors that the assembled chemicals have in common to enable consideration of more than one chemical at the same time. Developing chemical groups is necessary for a variety of useful purposes, including something as seemingly simple, but as critically important, as safe chemical storage. Key uses of grouping in toxicology decision-making include grouping of co-occurring chemicals in the environment, the body, or the exposome; creating and prioritizing groups for chemical mixtures toxicology and mixtures risk assessment; and identifying chemicals that are toxicologically similar (i.e., that share the same adverse outcome pathway (AOP) or have the same molecular interaction but converge later in the pathway) or are toxicologically independent. Another important use is developing groups that facilitate the filling of data gaps by techniques such as read-across, trend analysis, extrapolation, interpolation, and QSAR; this use of grouping results in reduced experiment effort, saving time, resources, and experimental animals. Grouping also benefits green chemistry and enables the use of molecular design for reducing unwanted toxicity. Traditionally, groups have been based on exposure alone or toxicity alone. Strategies laid out in Toxicology Testing in the 21st Century: A Vision and a Strategy (National Research Council (NRC), 2010) and corresponding advances in exposure science, high-throughput toxicology, ‘omics sciences, and computational technologies have resulted in a wide array of next-generation methods and tools. The purpose of this session is to highlight how these advances are being translated and used in group decision-making approaches. In this session, experts from industry, academia, and government will present state-of-the-art insights into new methods currently being developed and employed that have current or future application to chemical groups. The importance of accurate grouping in the future development and translation of risk assessment will be illustrated. The full presentation will showcase a novel, fully-integrated text mining-based tool capable of automatically analyzing relevant literature and classifying a given chemical according to its carcinogenic mode of action based on a structured taxonomy. The second talk will focus on using a mechanistic understanding of the interaction between a chemical and the MIE within an AOP, coupled with 2D chemical structure information, group chemicals and how inclusion of chemical bioactivity profiles may then be used to refine the initial groupings. The third presentation will present the application of the concept of connectivity mapping in a predictive toxicology paradigm, where gene-expression profiles and pattern-matching software algorithms are used to find connections between chemicals, adverse events, and genes to group chemicals with similar mechanisms of action. The fourth talk will illustrate how chemical categories associated with specific MIEs can be utilized to guide higher level QSAR methods, such as comparative molecular field analysis, that enable quantitative predictions to be made regarding chemical binding affinity and/or downstream bioactivity without needing to fully elucidate the shape of the binding pocket of the biological target. The final presentation of the session will explore the influence of grouping decisions and misclassification errors that might occur on risk prediction when using
the relative potency factors method to assess mixtures risk, focusing on variability introduced if the same (or different) dose-response curve shape is assumed for all chemicals in the group when the shapes truly differ (or are the same) or when independence is assumed for convergent AOPs and the resulting uncertainty of the estimated mixture response. Disclaimer: The views expressed are those of the authors and do not necessarily represent the views or policies of the US EPA.

1671 Why Do We Need 21st-Century Grouping Methods?

J. E. Simmons. US EPA, Research Triangle Park, NC.

Historically, the lack of methods, models, and tools for grouping chemicals has limited exposure-based grouping to the chemicals or the mixture from a specific process, product, source, or chemical class. Similarly, toxicity-based groupings of chemicals, while ideally based on chemicals sharing the same mechanism/mode of action or adverse outcome pathway, have most often defaulted to the same target organ. Only recently have the advances in exposure science, high-throughput toxicology, omics sciences, and computational technologies resulting from application of the strategies laid out in in Toxicology Testing in the 21st Century (NRC, 2007) and Exposure Science in the 21st Century (NRC, 2012) begun to be applied to grouping. The importance of establishing appropriate and scientifically defensible groups and the impact of grouping choices is illustrated with an example based on grouping chemicals for chemical mixtures toxicity, risk assessment, and decision making. The views expressed in this abstract are those of the author and do not necessarily represent the views or policies of the US EPA.

1672 CRAB 2.0: A Text Mining-Based Approach for Grouping Chemicals According to Carcinogenic Modes of Action

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Searching and structuring scientific literature manually for risk assessment is a heavy time-demanding task. The number of articles of potential interest is growing exponentially, and detailed information on mechanisms and/or modes of action (MOA) is needed for characterizing the risk. An automated literature review process that could identify mechanistic information in scientific reports should have the potential to effectively support and speed up the process of chemical risk assessment. Text mining (TM), a growing field of computer science, has emerged as a potential solution for bridging the gap between free-text and structured representation of information in the scientific literature. Together with computer linguistics at University of Cambridge, we have developed a novel fully integrated TM-based tool CRAB 2.0, which automatically analyzes relevant literature and efficiently classifies a given chemical according to its carcinogenic MOA based on a structured taxonomy. The taxonomy specifies scientific data types of relevance and captures current understanding of the processes leading to cancer development. It covers both genotoxic and non-genotoxic MOA categories and sub-categories. In an effort to further facilitate risk assessment, we used the tool in order to define the groups of chemicals with homogenous or similar MOAs, for example to identify and separate dioxin-like PCBs from non-dioxin-like PCBs by automatically searching and utilizing information from the relevant text. This should be the first step toward more efficient risk assessment of such a complex group of chemicals, where the toxicity has been described in several articles of chemical groups data gap filling methods such as read-across can be used to predict provide predictions for chemicals within a chemical group that currently lack relevant toxicological data. This presentation will cover how 2D chemical structure information and bioactivity profiles can be utilized to group chemicals, and how the inclusion of bioactivity profiles may help in the refinement of these chemical groupings for chemical prioritization and/or hazard/risk assessment. Additionally, this presentation will discuss how chemical categories can be used in conjunction with AOP networks to guide various aspects of mixtures risk assessment. This is an abstract or a proposed presentation and does not necessarily reflect US EPA policy. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

1674 Use of Connectivity Mapping (CMap) as a Tool to Group Chemicals Based on Mode of Action


Perturbation of a biological system via an external insult results in a characteristic gene expression profile. This expression profile is unique to the biological system and insult under consideration. Querying a representative signature of this profile against other such profiles using pattern matching methods provides an opportunity to identify both known and unknown connections between perturbed biological systems. This concept named “Connectivity Mapping (CMap)” was first described by Lamb et al. (2006) as a means to find connections between small molecules, diseases, and drugs. The concept can be applied to a predictive toxicology paradigm to find connections between chemicals, adverse events, and genes. In regulatory risk assessment, predictive toxicology often includes read-across as the most widely used alternative to animal testing (ECHA 2014, OECD 2014). Ideally, read-across would include demonstration of biological similarity in addition to the structural similarity between the source and target chemicals. CMap can be used to probe toxicodynamic similarity between source and target chemicals. The gene profile data can be used to provide evidence of biological similarity and the potential for structurally related chemicals that are biological outliers and thus reduce the uncertainty associated with a read-across based safety assessment. This presentation will provide an overview of the CMap concept and how it can be adapted to a predictive toxicology paradigm. Data from a proof of concept study using 34 chemicals and its expansion to a further 108 chemicals will be shared. The practical applicability of the method in grouping chemicals with similar modes of action will be demonstrated. Important watchouts such as the importance of using relevant dose and its impact will be discussed. Areas for further improvement of the tool/concept will also be discussed.
Using Chemical Categories to Inform Quantitative Risk Assessment

T.E. Allen, J.M. Goodman, M.N. Grayson, S. Gutsell, and P.J. Russell. 1University of Cambridge, Cambridge, United Kingdom; and 2Unilever, Sharnbrook, United Kingdom.

Chemical categories allow us to group molecules with other similar molecules quickly and efficiently for purposes such as read-across. These chemical categories can also be used to guide higher level QSAR approaches to inform quantitative risk assessment. The aim of this research is to move such QSAR models away from a global perspective and closer to a desired target. Molecular initiating events (MIEs) provide the platform for this. MIEs are initial chemical interactions between chemicals and biological systems that can lead to a toxic outcome via an adverse outcome pathway (AOP). In one instance, chemical informatics approaches have been used to develop categories based on the chemical characteristics of molecules that interact with a wide variety of human targets. Higher level QSARs are then applied to these well-defined chemical categories to allow the prediction of the activity of some binders to human targets well within one log unit. This has been achieved using comparative molecular field analysis (CoMFA), providing both a predictive model for activity and a visual representation of the fields the test molecules sit in without any understanding of the biology of the target. CoMFA can guide molecular development, by showing the hydrogen bonds and steric interactions that cause molecules to be more or less active, allowing chemical concerns to be systematically designed out of molecules. In another instance existing chemical categories for Ames mutagens have been used to guide higher level calculations. Density functional theory transition state modelling has been used to calculate activation energies for the reactions of chemicals with a model DNA base. Chemicals with high activation energies have been found to be inactive via this MIE, while similar chemicals with lower activation energies covalently bind to DNA and are mutagenic. This model directly models the chemistry of the MIE and opens a gateway for the modelling of other similar MIEs. Predictions made at the MIE level can subsequently be used in other dose-response models, through an AOP, to understand higher level toxicological outcomes. By further developing and refining these approaches we hope to learn more about the chemical characteristics that cause MIEs to happen, and hence better understand these important molecular interactions.

Estimating the Impact of Grouping Misclassification on Risk Prediction When Using the Relative Potency Factors Method to Assess Mixtures Risk

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Environmental health risk assessments of chemical mixtures that rely on component approaches often begin by grouping the chemicals of concern according to toxicological similarity. Approaches that assume dose addition typically are used for groups of similarly-acting chemicals and those that assume response addition are used for groups of indepen- dently acting chemicals. Grouping criteria for similarity can include a common adverse outcome pathway (AOP) and similarly shaped dose-response curves, with the latter used in the relative potency factor (RPF) method for estimating mixture response. Independence of toxic action is generally assumed if there is evidence that the chemicals act by different mechanisms. Partial independence is a special case, in which the chemicals act initially by different mechanisms, but their toxicodynamic pathways eventually converge to produce or affect a common product that leads directly to the adverse outcome. Misclassification can occur when the grouping is measured if errors are made in the assignment of AOP dose-response shape, or independence. Several questions arise about the potential for misclassification error in the mixture risk prediction. If a common AOP has been established, how much error could there be if the same dose-response curve shape is assumed for all chemicals, when the shapes truly differ and, conversely, what is the error potential if different shapes are assumed when they are not? In particular, how do those concerns impact the choice of index chemical and uncertainty of the RPF-estimated mixture response? What is the quantitative impact if dose additivity is assumed when complete or partial independence is in fact the case? What is the impact if a chemical is assigned to the wrong AOP group? These concepts and implications will be presented with numerical examples in the context of uncertainty of the RPF-estimated mixture response, both regarding endpoint and numerical accuracy when representing the group. The views expressed in this abstract are those of the author and do not necessarily represent the views or policies of the US EPA.

Role for NF-κB p50 in the Regulation of Chronic Neuroinflammation by Free Radicals

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Redox-signaling is implicated in deleterious microglial activation under- lying central nervous system (CNS) diseases, but how NF-κB, a dysoxidant microglial function is poorly understood. Here, the oxidation of NF-κB p50 to a free radical intermediate is identified as a marker of dysfunctional (exaggerated and persistent) pro-inflammatory activation of microglia. Microglia exposed to steady fluxes of H2O2 showed altered NF-κB p50 protein-protein interactions, decreased NF-κB p50 DNA binding, and augmented late-stage TNFa expression indicating that H2O2 impairs NF-κB p50 function and prolongs amplified M1 activation. NF-κB p50 (+/−) mice and cultures demonstrated that loss of NF-κB p50 function disrupted M2 (alternative) response and impaired resolution of the M1 response. Treatment with the spin-trap DMPO mildly reduced LPS-induced TNFa in the brain in NF-κB p50 (+/+) mice. Interestingly, DMPO failed to reduce and strongly augmented brain TNFa production in vivo.
in NF-κB p50(-/-) mice, implicating a fundamental role for NF-κB p50 in the regulation of chronic neuroinflammation by free radicals. These data identify NF-κB p50 as a key redox-signaling mechanism regulating CNS-specific chronic inflammation. New findings demonstrating the role of environmental toxicants (for example, paraquat) and aging in this process will also be discussed.

**1679 Overcoming Bias in F2-Isoprostane Oxidative Stress Measurement: Quantifying the Contribution of Inflammation**

T. J. van’t Erve. NIEHS/NIH, Research Triangle Park, NC.

Oxidative stress is recognized to play a role in the etiology of numerous diseases as well as environmental exposures. Increases in oxidative stress are measured through biomarkers; the best recognized biomarker is the lipid oxidation product 8-isoprostaglandin F2α (8-iso-PGF2α). However, 8-iso-PGF2α is also simultaneously produced enzymatically by the prostaglandin-endoperoxide syntheses (PGHS) in vivo. This alternative pathway has been mostly ignored in the interpretation of many published studies, leading to potentially incorrect conclusions. It has now become necessary to distinguish the two sources of 8-iso-PGF2α to avoid bias in data interpretation and conclusions. We established a novel method using the 8-iso-PGF2α to prostaglandin F2α ratio. We used this approach to simultaneously assess inflammation and oxidative stress in two animal models (CCI4 and LPS) and in humans (smokers and non-smokers). Measurements from these studies show that generation of 8-iso-PGF2α involves both pathways in vivo and the predominant source changes significantly depending on the model. In conclusion, we have validated the 8-iso-PGF2α / PGF2α ratio as a method to distinguish the sources of biomarker generation in vivo. Our work necessitates re-examination and re-interpretation of previous studies claiming induction of oxidative stress. This can alter the etiology of classic oxidative stress diseases and exposures such as smoking to ones more focused on the stimulated inflammatory response.

**1680 Inflammation Differentiated from Oxidative Stress in Reproductive Epidemiology: Understanding the Environmental Impact on Birth Outcomes**

K. K. Ferguson. NIEHS/NIH, Research Triangle Park, NC. Sponsor: M. Kadiiska

Chronic exposure to elevated levels of environmental contaminants (e.g., phthalates) has been repeatedly shown to be associated with adverse pregnancy outcomes. Endocrine disruption is posited as the primary mechanism of action for these contaminants, but oxidative stress and inflammation pathways may also play a significant role. To date, little research has been performed to distinguish between these two mechanisms to adverse outcomes and complications in pregnancy such as preterm birth and fetal growth. In this analysis, we examined data from a cohort of 761 pregnant women to investigate the associations between urinary 8-isoprostane concentrations, separated into chemical and enzymatic fractions based on the novel 8-iso-PGF2α / PGF2α ratio, in relation to adverse birth outcomes. We additionally examined preliminary data on associations between urinary phthalate metabolites and these fractions. Participants were recruited early in pregnancy and provided urine samples at three study visits. Phthalate metabolites and oxidative stress measures were quantified in urine from the third visit. In preliminary analyses, the chemical fraction of 8-iso showed an inverse association with gestational age at delivery. This may be particularly important in toxicology, since some environmental contaminants are associated with an increase in this chemical fraction as well.

**1681 Emerging Indications of Biomarkers for Use in Humans within a Cluster of Redox-Related Diseases: Relevance of Biomarkers of Oxidative Stress, Inflammation, Antioxidants, and Redox Signaling**

H. H. Schmidt. Maastricht University, Maastricht, Netherlands. Sponsor: M. Kadiiska

Successful biomarker policies of both inflammation and oxidative stress are vital to the advancement of directed therapies across a variety of toxicities, exposures and diseases that can influence clinical decision and provide optimal treatment. As of today, no biomarker of oxidative stress measured in circulation provides information on the cellular source, tissue or organ involved. In addition, many markers are confounded by generation through inflammatory processes. These facts might explain why the translational shortcut of this theory, antioxidant therapy, has failed so often and has never shown enough evidence to be incorporated in guidelines, approved by regulatory agencies, or recommended and reimbursed by health insurance systems. Since ROS have a complex metabolism and are generated by different enzymes at diverse sites and at different times, it is proposed that aggregating this plurality of systems into a single theory of disease may not be the best way to develop new drugs. Future research may need to focus on specific pathways of oxidation rather than on non-specific ROS scavengers.

**1682 Estrogen Receptor Signaling as a Mechanism of Developmental Toxicity**

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Estrogens are steroid hormones that influence the development and function of nearly every major organ system in the body—from the gonads to the central nervous system. Xenobiotic agents that mimic estrogens or influence estrogen production or metabolism can exert profound effects on organismal development. Aberrant estrogen receptor signaling is classically associated with reproductive toxicity. However, in the last decade, it has become apparent that abnormal estrogen receptor signaling during development can cause widespread changes in cardiovascular, immune, and metabolic functions. With the development of new molecular tools, improved understanding of developmental biology, and the use of new model organisms, tremendous advances have been made in understanding how estrogen receptors regulate development at the molecular, cellular, and physiological levels. This session will explore cutting-edge research in developmental toxicology, revealing the mechanisms by which estrogen receptors influence organismal development. Using an Adverse Outcome Pathway (AOP) framework, the speakers will explore estrogen receptor-mediated toxicity from the molecular initiating event (receptor activation) through adverse outcomes, such as organ malformation and dysfunction, thus linking early signaling events to organismal toxicity. The first speaker will discuss novel in vitro and in silico screening approaches to predict and model estrogen receptor toxicity and how these approaches are being used for hazard assessment and testing prioritization and are leading to compound development under the new Toxic Substances Control Act guidelines. To evaluate high-throughput estrogen receptor toxicity predictions, novel in vivo models are required. The second speaker will present on a novel zebrafish model being used to explore why some embryonic tissues are more sensitive to estrogen exposure than others. The third presentation will outline the similarities and differences by which estrogens and related sex hormones influence gonad development at the molecular and cellular levels and how these mechanisms can be hijacked by exposure to xenoestrogens during critical periods of development. The final two speakers will focus on adverse outcomes and discuss how estrogen receptors influence developmental toxicity in a sexually dimorphic manner with a focus on energy homeostasis and social, sexual, or anxiety-like behaviors in offspring of exposed mothers. These five speakers, using diverse approaches and model systems, will demonstrate how understanding the detailed mechanisms by which estrogen receptors influence developmental toxicology will improve the ability to identify potentially deleterious xenoestrogens and pharmaceutical estrogens with less side effects on patients and their offspring.
Endocrine disrupting chemicals (EDCs) likely influence human and aquatic organisms by mimicking endogenous hormones and disrupting the natural balance of hormone exposure. Estrogens, steroid hormones whose signaling pathways are commonly disrupted by EDC exposures, invoke a wide range of molecular and cellular effects, where the activation or inhibition of signaling pathways depends on the types of estrogens, their levels, and the developmental stages at which exposure occurs. To study how concentration and timing of xenoestrogen exposure influence gonad development, we used the zebrafish model system. Zebrafish embryos are transparent and genetically tractable, providing a unique opportunity to study how xenoestrogens influence gonad development.

In contrast to mammals, ultrasonic vocalizations and investigation of opposite-sex and sociosexual and anxiety-like behaviors are changed by EDCs; among these, reproductive neuroendocrine systems, sex differences in gonadal hormones, especially during critical periods of life, especially in the developing fetus and infant, are particularly problematic. In the case of the brain's neuroendocrine systems, sex differences in gonadal hormones, especially estrogenic endocrine disrupting compounds (EDCs) have most recently been linked to the development of obesity and type-2 diabetes in adults. The studies have focused attention on three groups of estrogenic EDCs: Phyoestrogens/mycotoxins (zearealenone, a-zearealenol), alkylphenols (nonylphenol and flame retardant diethyl diphenyl ethers (PBDE) and organophosphate flame retardants (OPFR)). The effects of maternal exposure to zearealenone and OPFRs on adult energy homeostasis is largely unknown. In two separate studies, we characterized the effects of maternal exposures to these compounds on adult energy homeostasis and glucose homeostasis in mice. In Experiment 1, oral maternal exposures to a-zearealenol from gestational day 7 (GD7) to postnatal day (PND) 14 altered glucose tolerance and insulin sensitivity in a sex-specific manner. Female mice were more glucose tolerant and insulin sensitive. Male mice were less glucose tolerant and more insulin resistant. There was no consistent pattern of findings across parameters of energy and glucose homeostasis for nonylphenol. In Experiment 2, we exposed dams to flame retardants: The PBDE congener 2,2',4,4'-tetrabromodiphenyl ether (BDE-47, 1 mg/kg/day) and a mixture of common OPFR (triphenyl phosphate (TPP), trimethyl phosphate (TCP), tris (1,3-dichloro-2-propyl) phosphate (TDOP), 1 mg/kg/day) from gestational day 7 (GD7) to postnatal day (PND) 14. Males from OPFR-treated dams had shorter ano-genital distance indicating feminization. In adulthood, FR treatment in males fed a low-fat diet (LFD) exhibited less adiposity and activity while increasing energy expenditure. Males from OPFR-treated dams exhibited elevated heat production and lower fat diet (HFD) and increased glucose intolerance. Indeed, these males did not have a typical response to a glucose challenge when fed a HFD. Collectively, these data suggest that exposure to these estrogen EDC can alter energy homeostasis in adulthood in sex-dependent manner.

Environmental chemicals with estrogenic activity can disrupt development and programming leading to adverse physiological effects in adults. These estrogenic endocrine disrupting compounds (EDCs) have most recently been linked to the development of obesity and type-2 diabetes in adults. The studies have focused attention on three groups of estrogenic EDCs: Phyoestrogens/mycotoxins (zearealenone, a-zearealenol), alkylphenols (nonylphenol and flame retardant diethyl diphenyl ethers (PBDE) and organophosphate flame retardants (OPFR)). The effects of maternal exposure to zearealenone and OPFRs on adult energy homeostasis is largely unknown. In two separate studies, we characterized the effects of maternal exposures to these compounds on adult energy homeostasis and glucose homeostasis in mice. In Experiment 1, oral maternal exposures to a-zearealenol from gestational day 7 (GD7) to postnatal day (PND) 14 altered glucose tolerance and insulin sensitivity in a sex-specific manner. Female mice were more glucose tolerant and insulin sensitive. Male mice were less glucose tolerant and more insulin resistant. There was no consistent pattern of findings across parameters of energy and glucose homeostasis for nonylphenol. In Experiment 2, we exposed dams to flame retardants: The PBDE congener 2,2',4,4'-tetrabromodiphenyl ether (BDE-47, 1 mg/kg/day) and a mixture of common OPFR (triphenyl phosphate (TPP), trimethyl phosphate (TCP), tris (1,3-dichloro-2-propyl) phosphate (TDOP), 1 mg/kg/day) from gestational day 7 (GD7) to postnatal day (PND) 14. Males from OPFR-treated dams had shorter ano-genital distance indicating feminization. In adulthood, FR treatment in males fed a low-fat diet (LFD) exhibited less adiposity and activity while increasing energy expenditure. Males from OPFR-treated dams exhibited elevated heat production and lower fat diet (HFD) and increased glucose intolerance. Indeed, these males did not have a typical response to a glucose challenge when fed a HFD. Collectively, these data suggest that exposure to these estrogen EDC can alter energy homeostasis in adulthood in sex-dependent manner.

Environmental endocrine-disrupting chemicals (EDCs) are exogenous chemicals that perturb hormones and their actions. Exposures to EDCs during life, especially in the fetal and infant, are particularly problematic. In the case of the brain's neuroendocrine systems, sex differences in gonadal hormones, especially estrogenic endocrine disruptors, are determined by developmental EDC exposures causing the brain in a sexually dimorphic manner, manifesting later in life as a disease or dysfunction. We treated pregnant rats with low dosages of a weakly estrogenic commercial polychlorinated biphenyl (PCB) mixture, Aroclor 1221 (A1212, 0.5 or 1 mg/kg) or estradiol benzoate (EB, positive control at 50 μg/kg). Adult offspring were assessed using a battery of behavioral tests to assess functional neurobehavioral changes. The brains of these rats were subsequently used for protein and gene expression assays, and hormones were assayed. Results of behavioral testing show that social, sociosexual and anxiety-like behaviors are changed by EDCs; among these, ultrasonic vocalization and investigation of opposite-sex responses are specifically affected. Gene expression profiling of brains from these animals has identified suites of genes differ
entially affected by PCBs compared to vehicle rats, with sex-, age-, and brain-region specific differences. Interestingly, while some of the actions of PCBs are similar to those of EB, others are different, indicating both estrogenic and non-estrogenic actions. This body of work indicates that gestational exposure to PCBs has life-long effects on the developing brain, neuroendocrine systems, and reproductive and social behaviors.

1688 High-Throughput Transcript Profiling and Functional Assessment: From Screening to Systems Biology Strategies for Personal Chemical Safety Predictions

C. Corton. US EPA, Durham, NC.

High-throughput screening (HTS) assays are an important component of chemical safety evaluation programs carried out by a number of organizations. However, it is recognized that the assays do not sufficiently cover all potentially important pathways. In the last few years, adaptation of gene expression profiling to high-throughput formats has been increasingly considered an attractive alternative to individual assays due to lower costs and the ability to simultaneously measure them. While microarrays have been used extensively in more focused lower-throughput studies and comprise the bulk of large publicly-available databases, technologies that can measure the targeted expression of the entire genome are emerging as attractive alternatives. Novel computational approaches are increasingly being used to move the field from using transcript profiles as hypothesis-generation tools to accurately predicting effects. Functional genomics strategies that identify gene-chemical interactions in gene-knockdown screens have proven valuable to validate predictions from transcript profiling and to determine species-specific effects. These integrated high-throughput genomics approaches will allow identification of relevant key events that are quantifiable in high-throughput (HT) transcriptomic settings and predict cell and biological changes, as well as human-translational, implications. This session highlights major advances in the field of using transcript profiling and functional genomics in a number of areas important in risk assessment. The first presentation will highlight recent results of a large-scale HT screen of 1,000 chemicals in a human cell line, allowing dose-response modeling of biological pathways on a massive scale. The second and third presentations will describe novel computational approaches which utilize both private and publicly-available data to make predictions of molecular targets and of chemical perturbations in gene networks that lead to toxicity. The last two talks will discuss exciting work which identifies genetic modifiers of responses to chemicals, allowing assessment of individual susceptibility to chemical injury. This session will be of wide interest, including to scientists interested in the application of gene expression profiling and in vitro assays to regulatory decision making.

1689 High-Throughput Transcriptionomics: From Screening to Pathways

I. Shah. US EPA, Durham, NC.

The US EPA ToxCast effort has screened thousands of chemicals across hundreds of high-throughput in vitro screening assays. The project is now leveraging high-throughput transcriptomic (HTTr) technologies to substantially expand its coverage of biological pathways. The first HTTr screen has measured the expression of 19,290 genes in MCF7 cells for more than 1,000 chemicals in a concentration response format. We have developed a computational strategy to interpret the HTTr data in terms of pathway perturbations, the specificity of pathway perturbations and associated points of departure. A strategy for integrating these new transcriptomic technologies into high throughput toxicity testing will be presented and the challenges discussed. We believe HTTr technologies can be deployed in a tiered fashion to complement the existing suite of high-throughput in vitro screening assays to realize the vision of toxicity testing in the 21st century.

1690 Identification of Potential Chemical Carcinogens in Compendia of Gene Expression Profiles

C. Corton. US EPA, Durham, NC.

Chemicals induce cancer through partially characterized adverse outcome pathways (AOPs) that include molecular initiating events (MIEs) and downstream key events (KEs). Microarray profiling of chemical-induced effects is being increasingly used in medium- and high-throughput formats. We developed computational strategies to identify chemicals in large microarray compendia that activate MIEs/KEs. Predictive biomarkers were built from profiles of knockdown or overexpression of the transcription factor (TF) and through selection of genes consistently regulated by chemicals known to modulate the TF. Gene expression profiles were compared to biomarkers using a correlation-based method which determines the significance of the overlapping gene expression allowing for prediction of modulation of that MIE/KE. The predictive biomarkers accurately identify chemicals that modulate a number of MIEs (genotoxicity assessed using p53-dependent genes, the endocrine disruptor targets estrogen receptor (ER) and androgen receptor (AR), as well as xenobiotic receptors aryl hydrocarbon receptor (AhR), constitutive activated/androstane receptor (CAR), peroxisome proliferator-activated receptor a (PPARa)) or downstream KEs (cell proliferation and oxidative stress (assessed indirectly through Nrf2 activation)). A number of examples will be given of applications of this approach including screening in 1) ER positive breast MCF-7 cells, 2) AR positive prostate LAPC-4 cells, and 3) human primary hepatocytes. Our screen identified many unique chemicals previously unknown to affect these pathways. In the MCF-7 compendium of 1,153 unique compounds, 75 compounds were found to activate ERs and 39 suppressed ERs. ERs activators included those that are known ERs agonists as well as previously unrecognized activators. Chemicals were identified that increased cell proliferation in MCF-7 cells either dependent or independent of ER activation. Our approach highlights the value of using transcript profiling to identify chemicals that have the potential to cause cancer. This does not represent US EPA policy.

1691 Reducing Noise and Boosting Biological Signal Detection in Large Transcriptomic Datasets

I. Stevens. Lilly Research Laboratories, APex, NC.

High throughput transcriptomic data present significant challenges computationally due to the dimensionality of the data and in assigning biological interpretations necessary to construct mechanism-based risk assessments. Classification techniques, e.g. signature response profiling, were developed to allow read across from training sets of compounds to test compounds for risk characterization. Other methods focus on elucidating underlying biological response pathways that are activated or repressed by exposure to xenobiotics. Among the latter methods, unsupervised approaches that organize large datasets into a modular format based on coalescent properties of biological systems have advantages both computationally, by reducing the dimensionality of, e.g. from 104 genes to 102 modules, and for data interpretation since they reveal the underlying modular structure of the biological systems of interest. We have used one such method, weighted gene co-expression network analysis (WGCNA), to create a modular representation of gene expression for liver and kidney transcriptomic data using DrugMatrix and TG-GATES data. In this presentation, we illustrate how detailed time series of changes in modules of co-expressed genes reveal important compound-specific as well as more general organ-based adaptive responses to a drug-induced toxicity. The ability to separate compound-specific from organ-based adaptive responses provide novel insights for risk assessment.

1692 High-Throughput Identification of Genotype-Specific Vulnerabilities to Drug Treatment


Patient response to therapeutics, especially newer targeted therapies, is increasingly linked to genetic dependencies. In order to better identify clinical populations that will respond to a given therapy, we must develop robust in vitro assays using clinically relevant cellular models to study genotype-specific vulnerabilities and biomarkers of drug response. Scaling high throughput screening assays to hundreds or thousands of cell lines is critical to better cover the clinical diversity of disease and to power correlation analyses to understand specific vulnerabilities. Groups and efforts such as NC1-60, Sanger/MGH, the Cancer Cell Line Encyclopedia (CCLE) and The Broad Institute’s CTD profiler have demonstrated the utility of screening compounds against a larger axis of cell lines. However, these efforts require very large investments of both time and resources to complete, making routine screening at this scale still a challenge. To overcome this technical hurdle, we have established a high throughput cell viability screening method, PRISM, to multiplex the screening process by utilizing uniquely DNA barcoded cancer cell lines to perform drug screening in pooled cell lines. This presentation will discuss the development of a robust screening platform that allows routine and rapid screening of thousands of compounds against hundreds of genomically characterized cell lines to generate response signatures. We will show examples of how response signatures can be correlated to a variety of complementary orthogonal

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Patient response to therapeutics, especially newer targeted therapies, is increasingly linked to genetic dependencies. In order to better identify clinical populations that will respond to a given therapy, we must develop robust in vitro assays using clinically relevant cellular models to study genotype-specific vulnerabilities and biomarkers of drug response. Scaling high throughput screening assays to hundreds or thousands of cell lines is critical to better cover the clinical diversity of disease and to power correlation analyses to understand specific vulnerabilities. Groups and efforts such as NC1-60, Sanger/MGH, the Cancer Cell Line Encyclopedia (CCLE) and The Broad Institute’s CTD profiler have demonstrated the utility of screening compounds against a larger axis of cell lines. However, these efforts require very large investments of both time and resources to complete, making routine screening at this scale still a challenge. To overcome this technical hurdle, we have established a high throughput cell viability screening method, PRISM, to multiplex the screening process by utilizing uniquely DNA barcoded cancer cell lines to perform drug screening in pooled cell lines. This presentation will discuss the development of a robust screening platform that allows routine and rapid screening of thousands of compounds against hundreds of genomically characterized cell lines to generate response signatures. We will show examples of how response signatures can be correlated to a variety of complementary orthogonal

1694 High-Throughput Identification of Genotype-Specific Vulnerabilities to Drug Treatment


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data sets including baseline genomic features (CCLE), and both chemical and genetic (genome-wide shRNA and CRISPR) perturbational profiles. In particular, we will highlight our effort to assemble, annotate and screen a 5,000 compound Broad Drug Repurposing library across 500+ cell lines to provide a foundational dataset for expanded signature correlations to known and well annotated chemical entities. We will share examples of how this integrated signature based screening and analysis approach yields identification of novel genetic vulnerabilities of specific cancer cell lines. This approach can lead to rapid hypothesis generation toward mechanistic understanding of specific genetic traits to drug treatment outcome.

**Functional Genomics of Cellular Stress Pathways: Towards a Personalized Chemical Safety Assessment**

B. van de Water, Universiteit Leiden, Leiden, Netherlands.

The activation of adaptive stress response pathways is a key event in chemical-induced tissue injury. Toxicogenomics has established the variety of stress pathways and their downstream components that define cell repair and defence of different target tissues, including liver, kidney, and heart. These involve the oxidative stress response pathway, the unfolded protein response pathway, DNA damage response, and immune signalling response pathways. While the critical core regulators and transcription factors that drive these pathways have been well described, the components that control these networks and, likewise, adaptation to chemical insults, are largely unknown. We have integrated imaging-based quantitative phenotyping of adaptive stress pathway activation with large scale RNAi-based functional genomics to identify individual signalling molecules, kinases, phosphatases, ubiquitinas, and transcription factors, that define the amplitude of oxidative stress response, unfolded protein response, and cytokine immune signalling response. We have further assessed the role of these individual signalling components in the onset of cytotoxicity. Human whole genome sequencing identifies the assessment pathway number variants in P70S6k or genetic polymorphisms in these individual signalling components. This will drive our understanding of individual susceptibility to chemical injury and move the field towards personalized chemical safety assessment.

**Revisiting Biology: Using Genomic and Epigenomic Editing to Gain Novel Insight into the Molecular Mechanisms of Toxic Exposure Effects and Susceptibility**

S. McCullough, US EPA, Chapel Hill, NC.

The genome and epigenome work hand-in-hand as central regulators of cell fate and function and therefore of susceptibility and toxic exposure effects. The use of traditional molecular methods has established a foundation with respect to the molecular mechanisms underlying the adverse effects of many toxic exposures; however, their efficacy in defining causative relationships between gene products, genetic polymorphisms, and epigenetic modification states with toxic exposure effects and susceptibility has been limited. The recent development of practical applications for clustered, regularly interspaced, short palindromic repeat (CRISPR) technology permits the selective revision of both the genome and epigenome in basic science research. In our lab we have used CRISPR-Cas9 to the target DNA locus using a sequence-specific guide RNA (gRNA), which provides increased specificity and ease of design compared to previous approaches using zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs). CRISPR can also be specifically modified the genome through either the targeted disruption of CpG sites that are subject to DNA methylation, or through the expression of epigenetic modifying enzymes/domains that are fused to “deactivated” Cas9 (dCas9), such that it can be targeted to specific genomic loci by synthetic guide RNA (sgRNA) but lacks nuclease activity. This permitted the targeting of transcription factors or epigenetic modifying enzymes to specific genomic loci, thus providing the technical framework to interrogate the relationships between changes in specific epigenomic modifications and expression-associated phenotypes. Separately, a novel class of small RNAAs, known as piRNAs, has recently been shown to orchestrate the targeted methylation of DNA within the mammalian genome. In a manner similar to the capacity of sgRNA to target dCas9, synthetic designer piRNAs can recruit DNA methyltransferases to hypermethylate specific genomic loci, thus silencing associated genes. This presentation will set the stage for the session by describing the similarities and differences between the molecular mechanisms utilized by these transformative tools, discussing their potential within the field of toxicology, and demonstrating their applications in revising biology.

**Use of CRISPR-Cas9 to Elucidate the Role of Nrf2 in the Response of T Cells to Electrophilic and Oxidative Stress**

C. Rockwell, Michigan State University, East Lansing, MI.

The introduction of CRISPR-Cas9 technology for gene editing has transformed basic science research. In our lab we have used CRISPR-Cas9 gene editing to elucidate the role of nuclear factor erythroid 2-related factor 2 (Nrf2) in T cell functional and genetic immunomodulation. Nrf2 in T cell functions can be targeted to alter both DNA methylation and histone modifications at specific loci to directly link changes in epigenetic modification states to exposure outcomes and susceptibility. The application of these technologies will open the door to the next generation of precision therapeutic and revolutionary in the field of toxification by providing novel opportunities to understand and modulate exposure-related disease and susceptibility at the genetic and epigenetic level. The goal of this session is to examine the range of applications of genome and epigenome engineering from their use in molecular and mechanistic toxicology studies to their potential as therapeutic strategies and to review the inherent safety considerations that their use entails. To achieve this, the session will bring experts together to discuss the development of these technologies and their current use in toxicity studies covering cultured human cells, mouse models, and human clinical trials. The session will answer questions such as how do CRISPR-Cas9 and piRNAs work, and what are the benefits and challenges facing their integration into the field of toxicology? Can CRISPR-Cas9 genomic editing be used to explore the role of key toxicity-associated pathways, such as NF-κB and NRF2, in the response to oxidative stress? How can the targeted modification of epigenetic states with dCas9 and piRNAs be used to provide causative relationships between specific epigenetic loci and disease/exposure outcomes? What is the current state of CRISPR-based therapies, and how does the toxicity and efficacy testing of these next-generation pharmaceuticals differ from that used for traditional therapeutic agents? The session will reveal a better understanding for the benefits, challenges, and applications of genome- and epigenome-engineering approaches in toxicology studies and will provide perspective on the unique considerations required during the development and testing of these technologies as next-generation therapeutic agents.
novel methodology represents a relatively easy and inexpensive option for reliably modulating gene expression and dissecting the role of transcription factors such as Nrf2, or other genes involved in the response to toxic stimuli. Overall, our studies demonstrate an important role for Nrf2 in modulating T cell function, suggesting activation of Nrf2 could be a useful marker for predicting immunomodulatory potential of chemicals. CRISPR-Cas9 was an important tool for these studies that also has broad applicability for a variety of different mechanistic studies in toxicology.

**1697 Applications of CRISPR-Cas9-Based Epigenetic Editing Technologies in Modeling and Treating Human Disease**

I. Hilton. Rice University, Houston, TX. Sponsor: S. McCullough

The ability to define the relationships between endogenous genetic and epigenetic variation and human disease is lacking. This limitation is especially pressing for diseases that are linked to dynamic environmental and/or inflammatory signals, and therefore have multiple potential pathological drivers. The repurposing of prokaryotic CRISPR-Cas9 adaptive immune systems has revolutionized the capacity to engineer the human genome and created tools to meet these critical needs. The nuclease activity of Cas9 can be abolished to produce a “deactivated” genomic targeting platform called dCas9. The dCas9 scaffold can be combined with transcriptional or epigenetic effector domains to finely tune the expression of endogenous genes and artificially deposit epigenetic signatures. We have developed a novel epigene editing tool by fusing the catalytic core of the human EP300 acetyltransferase to dCas9 (dCas9-p300). We have used this programmable acetyltransferase to demonstrate the causal linkage between endogenous chromatin acetylation and subsequent gene expression. Moreover, we have shown that dCas9-p300 robustly activates gene expression from endogenous enhancers and promoters, thus expanding the targeting capacity of epigene editing tools. Additionally, we have developed a high-throughput epigene editing platform to functionally interrogate the noncoding human genome. Importantly, these epigene editing strategies can be used to assign the function of epigenetic signatures and to activate or suppress the transcription of genes putatively involved in disease phenotype, and have the potential to modulate exposure effects and susceptibility at the epigenetic level. For instance, our group has used these approaches to activate and suppress loci involved in human disease and inflammation, including the human globin genes, TNFR1, and IL1R1. Cutting-edge CRISPR-Cas9-based genome and epigenome engineering technologies will continue to impact basic and translational research into the foreseeable future. This talk will provide an overview of the principles that govern the use of these tools in establishing molecular mechanisms underlying human disease and in exploring cellular responses to toxic exposures and inflammation. In addition, the potential therapeutic applications of these technologies in clinical settings will be discussed.

**1698 Development of piRNAs for Target-Specific DNA Methylation**

D. C. Dolinoy. University of Michigan, Ann Arbor, MI.

Epigenetic changes to DNA are associated with age, disease, and environmental influences. Precision modification of the epigenome holds great promise for our ability to modify environmentally induced changes in gene expression, yet it is currently out of reach using common techniques (drugs, transgenics, etc.). Through a NIH Director’s Transformative Award we are developing a suite of tools, based on the Piwi-interacting RNA (piRNA) system, to advance precision epigenetic editing, while avoiding off-target effects and immunogenicity, and immunotoxicity caused by the bacterial Cas9 protein will be described and discussed in the context of US and EU authority expectations. In addition, typical gene-medicine related challenges like the definition of a safe human starting dose or identification of a useful marker for predicting immunomodulatory potential of chemicals. The 2016 Workshop Session “Cannabis in the Courtroom” explored topical areas for scientific testimony and briefly touched on the public health and safety impacts of legalized marijuana products. This session expanded upon topics raised during the 2016 session which relate to the lack of federal oversight of the cannabis industry. With the growing acceptance of marijuana use and its legalization in some form (medical, recreational) by 28 states and the District of Columbia, including new medical marijuana legislation passed in Texas in 2017, marijuana has become a booming business that essentially is unregulated at the federal level because of the conflict imposed through listing of marijuana as a Schedule I drug by the Drug Enforcement Administration (DEA). Texas has just passed medical marijuana laws in limited cases, opening up a new market for entrepreneurs in the state. Currently, the US government has relinquished authority to the states with respect to the growing, distributing, and selling of marijuana where it is legal to do so under state laws. This session will explore the impact of the current absence of federal oversight on public health and safety by presenting a number of scenarios and outcomes stemming from the widespread and legal uses of cannabis in more than half of the states and the stark differences in individual state regulations. Topics will include: 1) safety concerns for edible marijuana products; 2) regulatory status of marijuana and its implications for academic research; 3) policy implications for federal authorities in light of public perceptions of marijuana’s benefits and risks; and 4) patient safety concerns with use of non-approved drug products and access to such products. The session will include a discussion from the legal perspective on the problems encountered with the “hands-off” approach currently taken by the federal government and will conclude with a panel discussion.
1703 Ensuring the Safety of “Edibles” in West Virginia: Lessons Learned for New Medical Marijuana Legislation in Texas

E. Janus. Compassion West Virginia, Huntington, WV.

Cannabis as a medicine, as dispensed by state-authorized programs, is now available to over half the population of the United States and growing. Edible products are a rapidly growing segment of the medical and recreational cannabis programs, and the safety of these products is a concern addressed in different ways by different state regulators. A review of the regulation and safety of edible cannabis products will be provided along with what is known about exposure trends. Colorado legalized the retail sale of recreational marijuana in late 2012, and the first retail stores opened for business on January 1, 2014. Since that time, calls to the Rocky Mountain Poison Center (RMPC) for marijuana exposures have increased and subsequently plateaued. With the rising delta-9-tetrahydrocannabinol (THC) concentrations in products, much national attention has been paid to the state of Colorado for observations of epidemiologic and medical outcome following recreational marijuana use. The purpose of this presentation is to investigate and discuss the various effects of the legalization of recreational cannabis from the RMPC perspective. All calls to our poison center involving marijuana exposures from January 1, 2015 to December 31, 2016 were identified and analyzed. Data collected included age, gender, product type, referring facility, clinical outcome, signs, and symptoms. The total number of reports of exposures called to the RMPC to marijuana products increased from 225 in 2015, to 231 in 2015, and 237 in 2016. As observed in both 2014 and 2015, the most common age group involved was 13 to 19 years (N=170; 75.5%). The pediatric cases were defined as under 12 years (N=19; 9.1%). Of the pediatric cases, 6-0 years of age were responsible for 9 of the 19 pediatric cases (47.4%). Of the total pediatric cases, 107 (51.1%) were children under 5 years old, many of which were accidental child ingestions. Of the total pediatric cases, 51.1% were children under 5 years old, many of which were accidental child ingestions. Of the total pediatric cases, 51.1% were children under 5 years old, many of which were accidental child ingestions. Of the total pediatric cases, 51.1% were children under 5 years old, many of which were accidental child ingestions. Of the total pediatric cases, 51.1% were children under 5 years old, many of which were accidental child ingestions. Of the total pediatric cases, 51.1% were children under 5 years old, many of which were accidental child ingestions. Of the total pediatric cases, 51.1% were children under 5 years old, many of which were accidental child ingestions. Of the total pediatric cases, 51.1% were children under 5 years old, many of which were accidental child ingestions. Of the total pediatric cases, 51.1% were children under 5 years old, many of which were accidental child ingestions. Of the total pediatric cases, 51.1% were children under 5 years old, many of which were accidental child ingestions. Of the total pediatric cases, 51.1% were children under 5 years old, many of which were accidental child ingestions. Of the total pediatric cases, 51.1% were children under 5 years old, many of which were accidental child ingestions. Of the total pediatric cases, 51.1% were children under 5 years old, many of which were accidental child ingestions. Of the total pediatric cases, 51.1% were children under 5 years old, many of which were accidental child ingestions. Of the total pediatric cases, 51.1% were children under 5 years old

1704 RMPC Colorado Marijuana Human Exposures by Age: 2014-2016

C. Hoyte. Rocky Mountain Poison Control Center, Denver, CO.

Sponsor: S. Robst

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1705 Mechanism-Driven Computational Modeling of Hepatotoxicity Based on Chemical Information, Biological Data, and Toxicity Pathways

L. Zhao1, W. Wang1, D. P. Russo1, L. M. Aleksunes2, and H. Zhu1.
1 Rutgers, The State University of New Jersey, Camden, NJ; and 2 Rutgers, The State University of New Jersey, Piscataway, NJ.

Hepatotoxicity is a leading cause of attrition in drug development and has resulted in a considerable number of drug withdrawals from the market. Traditional preclinical and clinical studies to evaluate drug hepatotoxicity are expensive and time-consuming. With the advent of critical advancements in in vitro testing approaches, in particular High Throughput Screening (HTS), there has been a rapid accumulation of chemical toxicity data and a critical need to combine this data with available biological datasets. Together, big data offer a novel alternative approach to evaluate hepatotoxicity potential for new and existing chemicals. To this end, we curated and merged all available in vivo hepatotoxicity data obtained from the literature and public database resources, which yielded a comprehensive dataset of ~4,000 unique compounds that could be categorized as hepatotoxic or non-hepatotoxic in human. For compounds in this datasets, ToxPrint Chemotypes from ChemoTyper® were used to generate potential structural toxicophores. Next, biological responses were profiled using in-house automatic data mining tool to search against the PubChem BioAssay database. Then, the chemical-biological data were clustered based on chemical-in vitro relationships. Using the clustered bioprofiles of the training set containing over 2,500 compounds, multiple read-across models were developed that showed acceptable predictivity in the cross validation procedure (positive predictive values: 0.87 to 1.00). By integrating toxicophore information into top chemical-in vitro-in vivo relationships, the predictivity was further improved by 12.8% to 68.0% for 14 models (positive predictive values: 0.72 to 0.96). These selected models could be used to predict the hepatotoxic potential of over 1,200 external new compounds and achieved similar performance (positive predictive values: 0.64 to 1.00). The chemical-in vitro-in vivo relationships obtained from these top ranked models can
be integrated into several adverse outcome pathways (AOPs). This study developed new computational read-across models based on substantial publicly available data that can be used to predict the hepatotoxicity of new compounds and elucidate novel mechanisms of injury by integrating chemical and biological data into toxicity pathways.

### 1706 Development of an Animal Model of Abacavir-Induced HLA-Mediated Liver Injury

B. Song, S. Aoki, and K. Ito. Chiba University, Chiba, Japan. Sponsor: T. Satoh

Genome-wide association studies have indicated that adverse drug reactions are highly associated with specific Human Leukocyte Antigens (HLA) alleles. For instance, abacavir induced hypersensitivity syndrome and fluclouxacinil induced liver injury are associated with HLA-B*57:01 allele. Carbamazepine induced Stevens-Johnson syndrome/toxic epidermal necrosis is associated with HLA-B*15:02 allele. However, the mechanism of pathogenesis is still unclear due to the lack of desirable animal models. Abacavir, a human immunodeficiency virus reverse transcriptase inhibitor, can induce multi-organ toxicity exclusively in patients carrying the HLA-B*57:01 allele. Previously, we have originally developed HLA-B*57:01 transgenic mice, and found that topical application of abacavir on mice ears could provoke proliferation of CDB+ lymphocytes in the local lymph nodes. Here we challenged to reproduce abacavir-induced liver injury in these mice. However, oral administration of abacavir alone to HLA-B*57:01 transgenic mice did not elevate alanine aminotransferase (ALT, a liver injury marker). Considering the importance of innate immune activation, HLA-B*57:01 transgenic mice, their littermates and HLA-B*57:03 transgenic mice (as negative control mice) were co-treated with abacavir and CpG-oligodeoxynucleotide (a toll-like receptor 9 agonist). By the co-treatment, significant elevation of ALT, pathological change in liver, increase of activated CD8+ T cells, and tissue infiltration of immune cells were observed exclusively in HLA-B*57:01 transgenic mice. These results indicated that CpG-oligodeoxynucleotide induced inflammatory reactions or innate immune activations are indispensable for abacavir-induced HLA-mediated liver injury. In addition, abacavir-induced liver injury was mainly resulted from CD8+ T cells. Collectively, we developed for the first time HLA-mediated drug-induced idiopathic liver injury mouse model by treating HLA-B*57:01 transgenic mice with abacavir and CpG-oligodeoxynucleotide. This model has a potential use in unmasking the mechanism of HLA-mediated idiopathic adverse drug reactions, promoting drug development, and contributing to personalized medicine.

### 1707 PCBs Alter the Hepatic Phosphoproteome in TASH


Nonalcoholic fatty liver disease (NAFLD) can be caused by hyperal- loric diets in combination with environmental exposures to pollutants such as polychlorinated biphenyls (PCBs). PCBs may act as a second hit driving disease progression from steatosis to steatohepatitis. Nuclear receptor and kinase function is regulated by phosphorylation and both have been implicated in the pathogenesis of steatohepatitis. Limited data exist on the phosphoproteome alterations in NAFLD animal models. Polychlorinated biphenyl (PCB) exposure has been shown to promote liver disease in a residential human population and in diet induced obesity animal models. Identifying kinase phosphorylation alterations in the pathogenesis of toxicant-mediated NASH will elucidate therapeutic interventions to prevent steatohepatitis. C57BL/6 mice were fed either a control diet or high fat diet and treated with either corn oil or Aroclor 1260 (20mg/kg) by gavage. In this study, we used titanium dioxide phosphopeptide enrichment from mouse liver tissue and conducted LC/MS/MS analysis to measure phosphorylated proteins in each sample. Peptides sequences identified from the spectra were matched to the Mouse Protein database to identify the proteins. PEAKS Studio 8 software was used to quantitate the normalized abundance of select phosphopeptides. A total of 1765 phospho-peptides were identified in this study. Within the dietary-PCB interaction group 23.8% of the total phosphosites detected were significantly decreased. CDKS, CDC2, AKT, CK1, and ERK were among the most affected pathways. This work demonstrates that kinases are modulated in the pathogenesis of NASH. This global loss of protein phosphorylation in the liver may be due in part to multiple signalling pathways being diminished in the pathogenesis of NAFLD.

### 1708 Combined Inhibition of MET and EGFR Signaling Abolishes Hepatocyte Proliferation and Fibrosis Induced by TCPOBOP (1,4-bis [2-(3,5-Dichloropyridylxyloxy)] Benzene) in Mice

B. Bhushan, and G. K. Michalopoulos. University of Pittsburgh School of Medicine, Pittsburgh, PA.

TCPOBOP (1,4-Bis [2-(3,5-Dichloropyridylxyloxy)] benzene) is a constitutive androstane receptor (CAR) agonist that induces robust hepatocyte proliferation and hepatomegaly without any liver injury or tissue loss. A recent study demonstrated that TCPOBOP-induced regeneration can protect liver from failure even in massive tissue loss (91% hepatec- tomy), indicating great clinical potential. However, mechanisms of TCPOBOP-induced hyperplasia are highly underexplored. TCPOBOP-induced hyperplasia is known to be CAR-dependent with no evidence of involvement of cytokines and growth factors signaling. Receptor tyrosine kinases MET and epidermal growth factor receptor (EGFR) are known to play a critical role in liver regeneration after partial hepa- toctomy, but their role in TCPOBOP-induced direct hyperplasia is not yet explored, which was investigated in the current study. Since these receptor tyrosine kinases are known to compensate for each other, MET KO mice were treated with Carcaptinib, an EGFR inhibitor (EGFRI), for combined inhibition of MET and EGFR signaling. Combined inhibition of MET and EGFR signaling (MET KO + EGFRI) dramatically reduced TCPOBOP- induced hepatomegaly and remarkably attenuated hepatocyte proliferation. Effects were not significant by elimination of individual receptor tyrosine kinase signaling (MET KO or EGFRI). Effect of combined elimi- nation on hepatocyte proliferation was found to be more pronounced in females compared to males. Interestingly, nuclear translocation of CAR and expression of CAR target genes were not altered in MET KO + EGFRI mice, indicating CAR activation is not affected. However, induc- tion of Cyclin D1, a key regulator of cell cycle entry, was almost com- pletely abolished in MET KO + EGFRI mice. A similar pattern was also observed for other cyclins (Cyclin A and B) that govern cell cycle pro- gression. FOXM1 is a key transcription factor that governs transcription of genes important for DNA replication/mitosis and regulates TCPOBOP- mediated hepatocyte proliferation. TCPOBOP-driven induction of FOXM1 and its target genes was remarkably diminished in MET KO + EGFRI mice. In conclusion, our study revealed a novel role of signaling via MET and EGFR receptors in hepatomegaly and hepatocyte prolifera- tion induced by TCPOBOP.

### 1709 Comparative Analysis of Toxicokinetics and Toxicodynamics of Perclorehylene in Cytochrome P450 2E1 Knockout and Humanized Transgenic Mice

Y. Luo1, S. Furuya1, H. Yoo2, and I. Rusyn1. 1. Texas A&M University, College Station, TX; and 2. University of North Carolina at Chapel Hill, Chapel Hill, NC.

Perclorehylene (PCE), a commonly used dry-cleaning solvent, is a probable human carcinogen. Toxicity of PCE is associated with generation of oxidative and glutathione conjugation metabolites. Upon absorption, PCE can be metabolically activated by cytochrome P450s (CYPs) to trichloroacetic acid (TCA), or conjugated with glutathione to metabolites with nephrotoxic potential, S-(1, 2, 2-trichlorovinyl) glutathione (NAcTCVC). These pathways are qualitatively similar, but quantitatively different across species. It is thought that CYP2E1 is crucial to PCE oxidation, and may play a role in bio-activation of nephrotoxic metabolites; however, inter-species differ- ences in the contribution of CYP2E1 to PCE metabolism and toxicity are not well understood. Therefore, the role of CYP2E1 in metabolism and toxic effects of PCE was investigated using male and female wild-type [Sv129], Cyp2e1-null [Cyp2e1(-/-)], and humanized Cyp2e1 [hCyp2e1] mice. It was hypothesized that CYP2E1 status would determine the extent of oxidative metabolism of PCE which would be pronounced impact on the toxicity of PCE. Mice were dosed with PCE (500 mg/kg, p.o.) or vehicle (5% alkamuls EL-620 in saline) for 5 consecutive days. Liver, kidney, and serum were collected 2 hours after the last treat- ment. The amounts of TCA formed in liver of PCE-treated animals in both sexes were significantly lower in Cyp2e1(-/-) and humanized Cyp2e1 (hCyp2e1) mice. It was hypothesized that CYP2E1 status would determine the extent of oxidative metabolism via TCA pathway. PCE metabolism was significantly higher in female mice. However, PCE-induced lipid accumulation was found in livers of Sv129 mice. PCE-induced proximal tubule injury in both sexes was Cyp2e1 mouse. In conclusion, our results indicate that PCE toxicity is determined by CYP2E1 activity and that sex differences in CYP2E1 function lead to species differences in the metabolism and toxicity of PCE.
demonstrate that CYP2E1 is an important, but not exclusive player in the oxidative metabolism of PCE, as well as PCE-induced toxicity in mouse liver and kidney. Therefore, inter-species and inter-individual differences in CYP2E1 function may contribute not only to the differences in PCE metabolism, but also to differences in PCE-induced toxicity.

**1710 A High-Throughput Screen Identifies Novel Targets for Kidney Tubular Regeneration**

M. B. Monteiro, S. Ramm, V. Chandrasekaran, and V. Vaidya

Harvard Medical School, Boston, MA; and Brigham and Women’s Hospital, Boston, MA.

Acute kidney injury (AKI) is associated with substantial morbidity and mortality, and often serves as the precursor to chronic kidney disease, which can only be managed supportively, with no curative therapy. Kidney proximal tubular epithelial cells (PTECs) contribute the most towards tubule repair, playing a key role in regeneration after injury. To uncover new molecules that can promote tubular regeneration, we performed a high-throughput phenotypic screening of 1902 compounds at 11 µM, measuring increases in proliferation of primary human PTECs. After 48h of treatment, 129 compounds promoted an average increase in Normalized Cell Count (NCC) >1.1 compared to the initial count (0h). Clear separation of positive, negative and toxic controls showed sensitivity (Z-factor >0.3) and a correlation coefficient of 0.86 amongst duplicates to define a dose response (DRC) and a 2-fold increase in positive hits at 1µM, 3µM, 10µM and 30µM confirmed eight compounds as pro-proliferative based on an increase in NCC >1.1 and an increase in actively cycling Edu-positive cells >6.5%. Furthermore, we determined the impact of these compounds on tubular cell proliferation under basal conditions and after the different types of injury: hypoxia (1% O2; for 24h); drug-induced toxicity (Cyclosporin A, 7.5µM or CdCl2, 15µM) and in vitro. We have identified potential first-in-class compounds that stimulate kidney tubular epithelial cell proliferation after acute kidney injury in vitro.

**1711 2,3,7,8-Tetrachlorodibenzo-p-dioxin-Elicted Dysregulation of Bone Remodeling Produces an Osteopetrosis-Like Phenotype in Male and Female C57BL/6 Mice**

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Aryl hydrocarbon receptor (AhR) activation alters bone morphology and remodeling in a species, ligand, and regal specific manner; however, the underlying mechanisms remain poorly understood. We examined the dose-dependent effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD; 0.01-30 µg/kg) on femoral microarchitecture and gene expression in male and female mice following oral gavage every 4 days for 28 days. Micro-computed tomography revealed a dose-dependent 2.5-fold increase in trabecular bone area density (BMD) and a 2.9-fold increase in bone volume fraction (BVF) in male femurs, with similar increases in females. Bone marrow adiposity in the distal trabecular region of the femur was reduced 4.1-fold in both sexes. Decreased serum levels of bone turnover markers including tartrate-resistant acid phosphatase (TRAP), amino-terminal propeptide of type 1 collagen (P1NP), and osteocalcin, combined with femoral repression of proteases required for degradation of organic bone matrix (cathepsin K, matrix metalloproteinase 13), suggest both bone resorption and formation were affected by TCDD. Differential gene expression in the male femur was associated with bone development and regulation of mineral and structural biosynthesis, and chemotaxis. Notably, femoral expression of transmembrane glycoprotein NMB (Gpnmb), which plays a crucial role in osteoclast and osteoblast differentiation and function, was dose-dependently induced 18.8-fold. Moreover, increased serum 1,25-dihydroxyvitamin D3 levels were consistent with TCDD-elicited renal induction of 1α-hydroxylase Cyp27b1 and may contribute to impaired bone resorption. In summary, AhR activation by TCDD alters the bone resorption – formation balance, resulting in an osteopetrosis-like phenotype with increased BMD.

**1712 Role of AhR in the Susceptibility to Type 1 Diabetes**

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In recent years, there has been a steady rise in the incidence of type 1 diabetes (T1D), a childhood disease characterized by insulin-dependent hyperglycemia due to immune-mediated destruction of insulin-producing beta cells. Genetic factors only partially account for T1D risk; therefore, environmental factors must play a critical role in the disease process. Two environmental factors that have been linked to T1D susceptibility include diet and an alteration in the intestinal microbiome. Furthermore, metabolites derived from the diet and microbiome have been shown to activate the aryl hydrocarbon receptor (AhR), a ligand-activated transcription factor that regulates the immune response. In our studies, we hypothesized that these diet- and microbial-derived endogenous AhR ligands influence the development of T1D. To test this hypothesis, we generated NOD.AhR+/+ mice and compared the incidence of diabetes (BGL > 250 mg/dL) in wild-type NOD mice to their AhR-deficient littermates at 30 weeks of age. Unexpectedly, female NOD.AhR-/- mice had a decreased incidence and onset of diabetes in comparison to the NOD. AhR+/+ mice. A similar trend was observed between knockout and wild type male mice. While immune cell composition was unchanged in relation to AhR status in the pancreatic lymph nodes, the percentage of F4/80+ macrophages and CD25+Foxp3+ T cells was higher in the mesenteric LN of NOD.AhR-/- mice in comparison to NOD.AhR+/+ mice. The expression of genes that have been associated with AhR activation in the intestines was compared between NOD.AhR+/+ and NOD.AhR-/- mice. In the ileum, NOD.AhR-/- mice had a reduced expression of genes encoding the antimicrobial peptides Gzmb and Lcn2 in comparison to NOD.AhR+/+ mice, consistent with prior findings. In addition, preliminary data show that NOD.AhR-/- mice have increased Bacteroidales family bacteria (S24-7) and decreased Ruminococcus gnavus in comparison to NOD.AhR-/- mice. Interestingly, previous studies have shown that S24-7 bacteria are associated with T1D protection while Ruminococcus bacteria are associated with T1D promotion, consistent with the reduced incidence of diabetes in NOD.AhR-/- mice compared to NOD.AhR+/+ mice. Current studies are focused on determining if AhR-dependent shifts in the microbiome directly impact diabetes incidence with the goal of identifying dietary and/or bacterial interventions that could reduce susceptibility to T1D.

**1713 Live Animal Molecular Imaging Techniques Demonstrate that Anti-Tumor Necrosis Factor-α Antibody Mitigates Lung Injury Induced by Nitrogen Mustard**

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Nitrogen mustard (NM) is a cytotoxic alkylating agent that causes acute lung injury which progresses to fibrosis. This is associated with upregulation of the proinflammatory/profibrotic cytokine, tumor necrosis factor alpha (TNFα). Previously we demonstrated that anti-TNFα antibody was effective in mitigating NM-induced lung injury. In the present studies, we used high definition magnetic resonance imaging (MRI) and computed tomography (CT) to follow the progression of NM-induced injury and the effects of anti-TNFα antibody. Anti-TNFα antibody was administered i.v. to male Wistar rats (15 mg/kg, every 8 days) beginning 30 min after i.t. instillation of PBS or NM (0.125 mg/kg). CT and gradient (GRE) and fast spin echo (FSE) MRI scans were performed 1, 3, 7, 14, 21, and 28 d post exposure. CT scans showed that in control animals, total lung volume increased with time. Treatment of rats with NM resulted in a significant loss of lung volume, which was most prominent at 1 and 3 d post NM; this was ameliorated by anti-TNFα antibody. MRI imaging showed low-density shadows at 1 and 3 d post NM, indicative of fluid buildup, a response also reduced by anti-TNFα. CT scans were also used to assess the functional respiratory area of the lung (by comparing signal intensity). At 28 d, the relative respiratory area of the lung of NM treated animals was reduced when compared to control animals (0.377 - 0.532 vs. 0.965-1.027). This loss of respiratory volume was confirmed by MRI and histology. Anti-TNFα antibody treatment of the animals partially restored the decrease in respiratory volume (0.729-0.827). These data show that MRI and CT imaging efficiently track the progression of NM-induced lung injury in live animals and can be used to monitor potential therapeutics, such as anti-TNFα antibody. NIH ES04738, AR055073, ES050522, and HL086621.
Triclosan (TCS) is an antimicrobial used at high concentrations in hospitals and consumer products. Mast cells, which share signal transduction elements with numerous other cell types (such as T cells and neurons), are found throughout the body and play key roles in various physiological processes. When activated, mast cells secrete cytoplasmic granules (degranulation). Simultaneously, actin causes cellular movements (ruffling). We previously showed that TCS inhibits mast cell degranulation and membrane ruffling in antigen (Ag)-stimulated rat mast cells (RBL-2H3). We found that these effects are due, at least in part, to triclosan’s inhibition of Ag-stimulated Ca2+ influx. This result led us to hypothesize that signaling enzymes downstream of Ca2+ rise, such as phospholipase D (PLD) and protein kinase C (PKC), would be affected by TCS. We found that TCS inhibits the activity of PLD in Ag-stimulated mast cells. TCS has no effect on the activity of PKC, as measured by an ELISA which simultaneously detects activity of 8 isoforms (α, β, γ, δ, ε, η, ι, ζ). Thus, for greater specificity in assessing triclosan’s effects on PKC, we further investigated the individual PKC isoforms, in particular PKCβII and δ, which are important in mast cell degranulation and ruffling. Using PKCβII-EYFP constructs, we found rapid (within ~2 min of Ag addition) translocation of PKCβII from cytosol to plasma membrane. Surprisingly, neither translocation (%) nor time to translocation of this Ca2+-dependent PKC isoform was affected by TCS, even under experimental conditions in which TCS inhibited degranulation and ruffling. A similar lack of TCS effect on PKC translocation was found in additional experiments with 15 and 45 min antigen exposures. Overall, these ELISA and confocal results suggest that PKC activity is not a major target of TCS and that triclosan’s disruption of mast cell function is likely not mediated through PKC. We are now investigating TCS effects on PKC δ translocation. These data deepen the understanding of TCS inhibition of signal transduction in mast and related cells.
**1719 From Bedside to Bench: What Only Our Patients Can Teach Us**

S. Bradberry, National Poisons Information Service (Birmingham Unit) and West Midlands Poisons Unit, Birmingham, United Kingdom.

One of the greatest challenges faced in recent years by clinical toxicologists practising in an acute hospital setting is the diagnosis and management of individuals intoxicated with new psychoactive substances. Global legislative bodies have struggled to implement effective and workable strategies to reduce the harm caused by these agents, not least because as fast as one drug is controlled an analogue is developed to circumvent the law. The resulting rate of change of chemical structures encountered has led to a multiplicity of pharmacological and toxicological effects including an increasing number of deaths. The challenge for the clinician is increased by a paucity of toxicodynamic and toxicokinetic data linked fundamentally to the fact that even the chemicals involved may not be known. In the emergency department, physicians learn their most valuable lessons directly from their patients, building diagnostic and management expertise largely by direct experience. Rapid access to comprehensive sophisticated analytical methods (such as ultra performance liquid chromatography with exact mass time of flight/TOF) has become increasingly important in establishing an accurate diagnosis, literally clarifying what is happening at the bedside only by returning to the bench. TOF analysis typically screens qualitatively for over 1,000 drugs and metabolites and it is the norm for there to be an extremely poor correlation between what patients state or believe they have taken and what is identified in their bodily fluids. In addition, “unknown” compounds are frequently detected weeks or months before that compound is formally named and recognized as a psychoactive substance. Chemical standards or markers for these new agents are obtained only when patients are prepared to provide samples of their purchases or direct toxicologists to an appropriate website. These markers can then be used to retrospectively clarify agents taken by individuals for whom no formal diagnosis had previously been possible. Two important classes of novel psychoactive substances, the amphetamine-like cathinones and the synthetic cannabinoids admirably demonstrate the importance of this concept of bedside to bench translational toxicology.

**1720 Importance of Translation from In Vitro Testing to Approved Products and Treatments**

D. Mendrick, US FDA, Silver Spring, MD.

It is critical to be able to translate findings from the in vitro environment to the clinic and back again to make progress in developing new treatments and enabling patient care. We need more human relevant data in regulatory decision making although that can be very difficult as 1) humans do not always predict humans reliably since many drugs show toxicity issues after they are marketed and, 2) poisons and new drugs of abuse cannot ethically be tested on humans. One way to proceed is to identify biomarkers of disease and toxicity to enable translation from cell culture to animals to humans. Biomarkers of population response and individual reactions (i.e., precision medicine) are needed although, in some cases, implementation may prove to be too expensive for routine use. To accomplish this broad mission, there is a critical need for involvement of experts in all areas from the bench to the bedside to ensure public health.

**1721 Mitochondria Biogenesis and Dysfunction in Cellular Senescence in Cardiopulmonary System**

I. Rahman, University of Rochester, Rochester, NY.

Emerging evidence sheds light on new mitochondrial functions that are not related to cellular energy production, which involve mitophagy (removal of damaged mitochondria from a cell prior to cell death) and mitochondrial protein quality control. Mitochondrial function is associated with aging, cell death, and disease. Mitochondrial proteins, such as Pink1, Parkin, and Drp1, along with sub-organellar signaling by oxidant stress and redox changes, are involved in mitochondrial dysfunction. This is associated with inflammation and cellular senescence via DNA damage and alterations in telomere shortening complex (a complex which protects telomeres from DNA damage). Toxicological perspectives on mitochondrial or mitochondria toxicity (MitoTox) research include a multidisciplinary approach in different target organs in the pathogenesis of cardiopulmonary and vascular diseases. The goal of this session is to highlight the recent advances of mitochondria research in toxicology, particularly in mitochondrial biogenesis, dysfunctional mitophagy, redox changes, cell signaling, and DNA damage/repair or rejuvenation of the damaged mitochondria upon toxic chemical or environmental insults in epithelial cells, fibroblasts, and myocytes in cellular senescence (premature aging) of the cardiopulmonary system. The information in the session will share the change in paradigm of involvement of mitochondrial stress signaling that would improve the gap in understanding the mechanisms of mitochondrial dysfunction in cellular senescence in the cardiopulmonary system.

**1722 Mitochondria-Nuclear Signaling and Mitophagy by Toxicants in DNA Damage and Lung Cellular Senescence**

I. Rahman, University of Rochester, Rochester, NY.

The presentation will focus on the involvement of mitophagy in stress-induced cellular senescence in the pathogenesis of chronic inflammatory diseases in response to toxicants. Environmental tobacco smoke and toxicants/oxidants-mediated alterations of mitophagy (Pink1, Parkin) are known to occur in stress-induced DNA damage and cellular senescence. Impaired mitophagy and perinuclear accumulation of damaged mitochondria are described by redox stress during cellular senescence, and this phenomenon is due to suborganellar signaling. Current knowledge on mitophagy field in relation to stress-induced cellular senescence will be discussed.

**1723 Impairment of Mitochondrial Function by Particulate Matter and Nanomaterials in Cardiovascular and Pulmonary Diseases**

J. M. Hollander, West Virginia University School of Medicine, Morgantown, WV. Sponsor: I. Rahman.

Detrimental effects on mitochondria have been proposed to play important roles in cardiovascular disease pathogenesis. Strong evidence from a multitude of studies suggest that impairment of the mitochondrion may contribute to cardiac contractile dysfunction and atherosclerosis, both of which may be the result of disruption to critical mitochondrial processes involving various proteins and miRNA. Increasing evidence suggests that reactive oxygen species (ROS) and oxidative stress are involved in airborne particulate matter (PM), diesel exhaust, and nanoparticles-mediated lung and cardiovascular injury (endothelium, myocytes and microvascularitites). The physical characteristics and the chemical composition of PM and nanoparticles play a key role in ROS generation in vitro and in vivo. The mitochondria are major subcellular targets for PM and nanoparticles as well as a source of ROS production. Another mechanism is direct physical targeting by ambient ultrafine particles that affect redox status of the cells and mitochondrial structure. Further, nanoparticles, including nanomaterials or ambient particles, cause mitochondrial aberrations and cellular senescence. An understanding of the mitochondrial effects of these particles is key in understanding the mechanisms of particle-induced adverse health effects and exacerbations via impaired mitophagy.

**1724 Cardiovascular Mitochondrial Dynamics and Dysfunction by Exposure to Ultralite or Nano Particulate Matter**

C. J. Wingard, Bellarmine University, Louisville, KY.

Increasing evidence suggests that reactive oxygen species (ROS) and oxidative stress are involved in airborne or engineered particulate matter (PM)-mediated lung and cardiovascular injury. The mitochondria are novel subcellular targets for nanoparticles as well as a source of ROS production. Excessive ROS production with alteration of mitochondrial electron transfer and permeability transition (PT) pore opening is associated cardiovascular dysfunction. In this presentation, we will discuss evidence for a hypothesis that nanoparticles can mediate mitochondrial aberrations in driving cardiopulmonary diseases (e.g. ischemia-reperfusion, fusion, and mitotanding of the mitochondrial effects (e.g. redox changes, bioenergetics, and PT) of these particles can be critical in understanding the mechanisms of particle-induced adverse health effects and exacerbations in cardiopulmonary system.
1725 Mitochondrial DNA Damage and Dysfunction in Vascular Disease by Environmental Toxicants
J. L. Fetterman, Boston University School of Medicine, Boston, MA. Sponsor: I. Rahman

Cardiovascular risk factors increase oxidative stress within the vasculature resulting in the loss of nitric oxide bioavailability and chronic inflammation. Mitochondria are an important source of oxidants, and are particularly sensitive to oxidative damage. Evidence suggests that mitochondrial damage and abnormalities are a significant contributor to vascular dysfunction and may even precede the development of atherosclerotic plaques. We found that elevated mitochondrial DNA damage was associated with type 2 diabetes and clinical atherosclerosis even after adjustment for age and sex. Endothelial cells from type 2 diabetic patients had higher mitochondrial oxidant production compared to cells from non-diabetic patients. Endothelial cells from type 2 diabetes had lower levels of proteins involved in the regulation of mitochondrial turnover through autophagy (p62, LC3B, Fis1) and proteins involved in mitochondrial displacement through biogenesis (TFAM, NRF-1). Up-regulation of autophagy restored mitochondrial networks and lowered mitochondrial oxidant production in commercially available endothelial cells under high glucose. Additionally, autophagy up-regulation improved insulin-induced nitric oxide production and eNOS activation in both high glucose treated endothelial cells and endothelial cells from diabetic patients, suggesting improving mitochondrial turnover may be a potential therapeutic target for improving diabetic vascular disease. Further, mitochondria are highly sensitive to environmental toxicants and may serve as biomarkers of injury. Endothelial cells from traditional cigarette smokers or electronic cigarette users were associated with increased mitochondrial oxidant production and vascular dysfunction (increased inflammation and decreased nitric oxide bioavailability). This presentation by a junior/emerging toxicologist/investigator will provide recent research findings on mitochondrial DNA damage and vascular function in vascular disease by environmental toxicants.

1726 Redox Regulation of Mitochondrial Dysfunction and Cellular Senescence in Atherosclerosis
R. Asmis, University of Texas Health Science Center at San Antonio, San Antonio, TX. Sponsor: I. Rahman

This presentation will be by a local talented researcher interested in redox regulation of mitochondrial function in cardiovascular disease. Mitochondrial dysfunction leads to increased ROS production, which is a critical aspect of heart failure. Regulation by ROS and protein S-glutathionylation is an important process in mitochondrial respiration. Alteration in protein S-glutathionylation via thioltransferase glutaredoxin 2a can disrupts mitochondrial respiration and ATP production in macrophages in proatherogenic responses. The mitochondria changes in heart failure and the downstream effects of these changes will be presented.

1727 Advancing the Adverse Outcome Pathway Framework: An International Horizon-Scanning Approach
R. Conolly, US EPA, Research Triangle Park, NC.

In 2007, the US National Research Council laid out a vision and strategy for toxicity testing in the 21st century, which aspired to transform current testing approaches by making greater use of recent scientific advances in cell-based and computational methods. The adverse outcome pathway (AOP) framework, which emerged to address this vision, has since gained international traction as a systematic approach for capturing existing knowledge to transparently link mechanistic data to apical toxicity endpoints as a means to inform research and risk assessment. While the framework has evolved significantly since its introduction in 2010, it was recognized that a survey of the broader scientific community would be useful in identifying challenges and in guiding future initiatives. To that end, a horizon-scanning exercise was conducted to solicit questions from the global scientific and regulatory communities, including the SOT community, regarding the perceived challenges and/or limitations that must be addressed to realize the full potential of the AOP framework. Following this exercise, a Society of Environmental Toxicology and Chemistry (SETAC) Pellston Workshop, comprised of international participants representing industry, government, academia, and non-governmental organizations (NGOs), was held in Cornwall, Ontario, Canada, in April 2017 to begin exploring these themes and answering associated key questions. This session will serve as a podium to present the outcomes of the horizon-scanning exercise and of the Pellston Workshop and to foster discussion with attendees in order to continue advancing the AOP framework. Specifically, presentations will cover topics such as the development and application of AOP networks, qualitative AOPs and associated modeling approaches, and the status of and future needs for application of the framework in regulatory decision making. Furthermore, talks will explore a roadmap to enhance awareness of, involvement in, and acceptance of the AOP framework by regulatory agencies, scientists, and other stakeholder groups. In all, presentations will serve frequently asked questions identified during the horizon scanning and address common misunderstandings pertaining to the AOP framework. Finally, the audience will be asked to participate in a panel discussion following the presentations to build upon ideas and outcomes derived from the Pellston Workshop.

1728 Adverse Outcome Pathways: Moving from a Scientific Concept to a Globally-Accepted Framework

In 2016, we reached out to the membership of SOT, as well as other national and international scientific and regulatory communities, to collect questions and provide an opportunity to discuss key outstanding challenges that must be addressed in order to realize the full potential of the AOP framework. During this Horizon Scanning effort, a total of 340 valid questions were received and ranked by an international expert group from academia, government, industry, and NGOs. This ranking was utilized to identify the four distinct but connected core topics that were addressed during the workshop, and each of which is summarized in the subsequent presentations: 1) AOP networks and their applications (Presentation #2); 2) quantitative AOPs and their applications (Presentation #3); 3) regulatory use of the AOP framework (Presentation #4); and 4) roadmap to expand awareness of, involvement in, and application of the AOP framework and AOP knowledgebase in the broader scientific and regulatory/environmental policy communities (Presentation #5). Furthermore, this exercise provided the opportunity to identify and clarify common misperceptions, as well as bring forward new thinking to advance the science and discuss the path forward. This introductory presentation will provide an overview of the Horizon Scanning exercise, and the overall outcomes and common themes that emerged during this exercise and the subsequent Pellston Workshop during which these themes were addressed. It will set the stage for the subsequent presentations that each address one of the four main topics listed above. Common themes that spanned across these main topics included the need to simplify, translate, and better communicate the AOP framework to the broader international stakeholder community, and a consensus that the AOP framework does not represent a rigid tool but rather a knowledge repository for diverse stakeholders ranging from epidemiologists to mainstream experimental toxicologist to risk assessors and managers. Furthermore, when considering the AOP framework and its applications, the field of environmental toxicology and human health naturally merged into a continuum that is at the nexus of Toxicology in the 21st century.
The use of adverse outcome pathways (AOPs) and related concepts has increased in scientific and regulatory sectors over the past decade, coinciding with pressures to find innovative solutions for evaluating chemical safety in a more efficient and effective manner. In this context, a series of recent workshops and publications have investigated how AOPs may be applied to regulatory decision-making, concluding that in each case, the AOP must be fit-for-purpose. To enhance opportunities to utilize AOPs, there is a need to provide guidance on how to evaluate and define fit-for-purpose given a particular regulatory scenario. This presentation will provide examples for applying AOP concepts to a variety of scenarios over the life stages of chemical development (i.e., from product development/discovery, registration, to environmental regulations and stewardship activities). It is recognized that the utility of an AOP within a regulatory context is influenced by both the level of development of the construct itself in combination with the tools available to measure key events (KEs) (e.g., assays or models) and key event relationships (KERs) within the AOP. Criteria to consider when determining if the tools for measuring endpoints within the AOP of interest are sufficiently predictive (i.e., fit-for-purpose) for regulatory applications will be proposed. Considerations for evaluating the suitability of AOPs will be discussed, recognizing that the accepted level of uncertainty varies based on the nature of the decision and the context in which the AOP is being applied. Additional input from the audience will be sought to further advance the use of the concept.
to enter the skin. One of the challenges for prioritization can be potentially addressed by identifying chemicals that penetrate quickly by using the lag time parameter. This talk presents an overview for approaches taken to examine diffusion estimates based on chemical properties. This abstract does not reflect US EPA policy.

**1735 Skin Absorption of Metal Worker Fluids and Complexities Inherent in Additional Components**

R. E. Raynes. North Carolina State University, Raleigh, NC.

Occupational settings can lead to dermal exposure of workers to metalworking fluids (MWFs), which may be toxic. The fluids are mixtures of vehicles (water or oil), minerals from metal work, and additives such as corrosion inhibitors. A systematic study of dermal permeability is needed due to differing ratios of components in these metal fluids. A lack of information is especially apparent with the addition of biocides to these mixtures. The porcine skin flow-through cell system provides concentration-time profiles needed for the estimation of permeability. A large dataset is available for porcine penetration for pesticides in aqueous solution. By combining this information with the mixture data available from MWFs, computational predictions can be then used to prioritize in vivo studies. This talk will summarize our systematic effort to quantify dermal penetration for complex mixtures generated in occupational settings.

**1736 Impact of Natural Compounds on Dermal Absorption for Consumer Products and Their Computational Prediction**

J. E. Riviere. North Carolina State University, Raleigh, NC.

Natural compounds are present in increasing numbers within consumer products. This includes increased use in vehicles, adding to the complexity of the mixture used dermally. Although purported to be benign, these compounds may have the potential to affect dermal absorption of applied chemicals. In vitro experiments using porcine skin allowed for the collection of concentration-time datasets used in the calculation of permeability and flux. These dermal descriptors were then incorporated into a quantitative permeation relationships (QSPR) for different topical preparations to quantitatively assess mixture-induced interactions. Some of these natural compounds acted as permeation enhancers, leading to increased dermal penetration when used as a mixture. The study of natural compounds used in topical mixtures needs further characterization. This talk will address the need to study natural compounds and their impact on dermal penetration.

**1737 The Skin Is a Non-Homogenous Physiological Organ: What Should We Consider for Computational Predictions?**

G. B. Kasting. University of Cincinnati, Cincinnati, OH.

Although diffusion is considered the main determinant for dermal penetration, there are physiological conditions that can affect permeability. One major consideration is skin hydration, since in vitro experiments may change the normal hydration present in skin. Another physiological condition such as inflammation and swelling may affect permeability. Then there is the question about different skin components, such as nails and hair follicles. How do these skin subtypes affect absorption for a given chemical? Should computational predictions include this diverse skin landscape with different sub-types and components? Can skin condition or disease alter the normal diffusion process? This presentation will focus on the diverse skin landscape needed when considering computational predictions for dermal penetration.

**1738 In Silico-In Vitro Extrapolation for Dermal Exposure**

J. Spires. Simulations Plus, Lancaster, CA. Sponsor: M. Evans

In vitro measurements of skin penetration are often used to predict in vivo dermal exposure for a compound. However, correlations between in vitro and in vivo exposure, or correlations between different types of in vitro experiments, are often poor, and the reasons for this are often unclear. In silico modeling of transport into and between skin layers can be used to separate and quantify the mechanisms leading to different dermal exposure of diverse compounds, and may also improve extrapolations between various in vitro experiments and in vivo delivery. The GastroPlus TCAT module was developed to mechanistically model in vivo transport of compounds into and within the skin. This talk will discuss the process of adapting GastroPlus to simulate in vitro dermal experiments and explore how well it can predict in vitro dermal delivery mechanisms.

**1739 Defining Domains of Applicability for Zebrasfish within Toxicology: A Retrospective and Prospective Workshop**

J. Freeman. Purdue University, West Lafayette, IN.

Over the past 20 years, adoption and integration of the application of zebrafish as a toxicological model system has magnified in most areas of toxicology-based research. As a well-recognized biomedical research model, zebrafish presents numerous strengths that have been leveraged in many toxicity studies. Rapid ex vivo development of a small, near-transparent singular embryo permits ease for assessing chemical perturbations at all stages of early development, as well as use in high-throughput screening and automated phenotyping. In addition, a complete genome sequence, array of tools for manipulating gene function, and availability of several thousand mutant and transgenic lines provides, similarly to mouse models, readily available resources for comprehensive mechanistic studies of toxicity. Furthermore, maturation at three months of age and a shorter lifespan allow for multi- and transgenerational studies and efficient identification and evaluation of developmental origins of health and disease. As researchers continue to expand the use of the zebrafish in toxicology, limitations of this animal model also are being identified. In this session, the first presentation will highlight what has been learned from more than two decades of using zebrafish in toxicity research, which has spanned developmental mechanistic studies to current applications in high-throughput screening of chemicals and chemical mixtures. The second talk will discuss zebrafish as a comparative model to rodent neurobehavioral testing, including analysis of larval behavior outcomes with long-term neurobehavioral dysfunction in adults. The third speaker will focus on the advantages and challenges of using zebrafish to define mechanisms of immediate (larval), later in life (adult), and transgenerational consequences of a developmental toxicant exposure linking single-cell transcriptomic, epigenomic, and phenotypic outcomes. The fourth presentation will highlight the strengths and constraints for using transgenic zebrafish in drug development and therapeutics for epilepsy. The final speaker will address the benefits of using zebrafish as a replacement for mammalian toxicity testing and the importance of accounting for toxicokinetic processes and dosimetry. Overall, the session will bring together several leading research laboratories that have extensive experience with the zebrafish model in various toxicological disciplines to provide a reflection of the knowledge that has been gained over the past 20 years relative to the strengths and constraints of the model system in toxicological experiments. In addition, this session will explore a comparison of zebrafish to other animal models, best practices, current questions, and future research needs.

**1740 Utilizing the Power of High-Throughput Zebrafish Screening to Identify Hazardous Chemicals and to Help Design Safer Chemicals**

R. Tanguay. Oregon State University, Corvallis, OR.

We started over two decades ago using zebrafish as a new model system to better understand the mechanisms of TCDD developmental toxicity and these initial studies convinced us that this system could be used more broadly and across chemical space and for many applications. Since those studies in the 1990s, the field has matured into many exciting areas across the molecular and systems biology continuum. The studies utilized our rapid multi-dimensional embryonic zebrafish assay as a platform for collecting and comparing the bioactivity of hundreds of chemicals and chemical mixtures. This system has proven effective for defining chemical activity profiles and for providing the initial clues to understand the mechanisms that underlie toxic responses. Chemicals from many commercial and governmental sources for testing in our high throughput zebrafish testing facility were obtained. The identity of the compounds are coded and initially blinded to the laboratory. All chemicals were applied for developmental toxicity in zebrafish across broad concentration ranges. All exposures were initiated at 6 hours post fertilization (hpf) and were continuous until 120 hpf. At 24 hpf embryos were assessed for changes in an embryonic photomotor response (PMR) behavior, which is a sensitive early measure of neuromuscular activity. At 120 hpf the morphological endpoints were recorded. Utilizing the PMR together with the numerous visible endpoints increases the sensitivity to discern
Zebras (Danio rerio) embryos and larvae have been suggested as a vertebrate model not only to improve understanding of fish development and toxicology, but also to complement or even replace mammals for rapidly assessing chemical impact. Hundreds to thousands of chemicals can today be rapidly screened while obtaining high content information on morphological and/or physiological changes in the early life stages of this fish. Yet, the toxicokinetics—i.e., how chemicals are absorbed by the fish, distributed, metabolized, and eliminated—is far less explored. In order to shed light on the role of toxicokinetics in the response of fish to chemicals and in the comparability of the response to mammals, we characterize chemical distribution using combinations of mass spectrometry-based quantitative and imaging methods along with kinetic modeling, in addition to analyzing toxic and behavioral outcomes. Focusing, for example, on the well-known psychoactive drug cocaine, and on the new psychoactive substance, meta-chlorophenylpiperazine (mCPP), we found highest accumulation of these chemicals in the larval fish eye, while they reached much lower but comparable levels in brain and trunk. Using hypo-pigmented fish, accumulation of the drugs in the eye was confirmed to be due to melanin and can be explained by the basic chemical nature of these drugs. Though cocaine brain levels covered those known to cause hyperactivity in mammals, only mCPP (decreased locomotion) was recorded in zebrafish larvae. Results therefore point to cocaine’s anesthetic properties as the dominant mechanism of interaction in the fish: Upon entry through the fish skin and gills, it first acts on peripheral nerves, rapidly overriding any potential stimulatory response in the brain. Despite differences in effective tissue levels in zebrafish larvae and rodents with respect to mCPP, we observed similar behavioral responses—e.g., hypolocomotion—suggesting that zebrafish larvae are a useful model to study serotonin-targeting drugs. Based on these and other examples of our research, we highlight the importance of taking toxicokinetic processes into account when using zebrafish embryos and larvae in chemical risk assessment.
Get the Lead Out: The Persistent Problem of Lead Exposure from Soil, Dust, and Water

M. Hughes, US EPA, Research Triangle Park, NC.

The heavy metal lead, a known neurotoxicant, has been used for centuries in a variety of industries and household and consumer products. The choice to use lead is a reflection of its physical/chemical properties, including softness, ductility, poor conductivity, and resistance to corrosion. While a natural component of Earth’s crust, high concentrations of lead in the environment, particularly soil, have resulted from human activity and its resistance to natural degradation. From the decades-long use of lead as a fuel additive, soils in urban areas with high traffic volume have been found to have highly elevated levels of lead. In residences that pre-date 1978, when lead-based paint was banned in the United States, dust from deteriorating paint contains elevated levels of lead. The soils found on and near several industrial smelting sites, such as in East Chicago, IN, also have high levels of lead. Finally, lead is found in drinking water in homes that have water pipes containing lead, with leaching a complex function of pH, alkalinity, and source water characteristics. It is well noted that homes typically located in older urban centers have drinking water with a high risk of elevated lead levels, particularly if the water is corrosive. Elevated blood levels have been found in people, particularly children, who were exposed to soils, dust, and water containing high levels of lead. The main public health issue with lead is that it is neurotoxic, especially to children. Elevated levels of lead in children can result in behavioral disorders and impairment of intelligence and learning. There is no known biological requirement for lead, although it is absorbed fairly well following ingestion or inhalation. Lead accumulates in bone, as it has similar properties as calcium, taking its place in this organ. Lead in bone can be a long-term source of internal exposure, as it can be released from bone into the systemic circulation and reach other organs. This session will bring together experts on lead with regard to its exposure, neurotoxicological effects, and the use of models to predict blood lead levels in individuals exposed to lead in soil, dusts, and water. The first presenter will discuss the positive association between lead in soil or bioaccessible (an in vitro method simulating gastrointestinal tract) lead and blood lead levels in children in an urban area. This presentation will show the feasibility of using in vitro methods to improve child lead risk assessments in the place of total soil lead content. The second presentation will share how soil lead levels and children’s blood levels have changed pre- and 10 years post-Hurricane Katrina in New Orleans, Louisiana. It was observed that when lead soil levels decreased, the blood lead levels in the children also decreased. The third presentation will focus on the potential mechanisms of lead that result in adverse health outcomes following maternal lead exposure with the potential development of neurotoxicity in the offspring. This includes studies from both human and laboratory animal exposures. The fourth presentation will evaluate the impact of varying regional screening levels on blood lead predictions in the Integrated Exposure Uptake Biokinetic (IEUBK) model to aid in reducing uncertainty in human health risk assessments. The final presentation will describe the development of the US Environmental Protection Agency’s (EPA) SHEDS-Multimedia and IEUBK models to determine the level of lead in drinking water that should result in children’s blood lead levels that are less than specified values. The analysis reveals the importance of the soil and dust ingestion exposure pathway. The session will share information on the associations between exposure to lead in soil, dust, and water to blood lead levels, neurotoxic mechanisms of lead exposure, and modeling efforts to predict children’s blood lead levels following exposure. **Disclaimer:** The views expressed are those of the authors and do not necessarily represent the views or policies of the US EPA.

The Astonishingly Holistic Role of Soil in Lead Exposure of Children

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The long-term resilience and sustainability of community welfare is linked with its environmental quality. One impediment to community welfare is children’s chronic lead exposure and its clinical consequences including health, learning, and behavioral disorders. There is no safe level of lead exposure, and this revelation is confounded by the fact that after exposure takes place, there is no known effective intervention. In August, 2005, Hurricane Katrina flooded 80% of New Orleans. This presentation explores the natural experiment of soil and children’s blood lead changes in New Orleans before and ten years after the flood. Pre- and post-Katrina soil lead and children’s blood lead results were matched and stratified by 172 communities in New Orleans. SHEDS-Multimedia methods were used to organize, describe, and map the pre- and post-Katrina data. Comparing pre- and post-Katrina results, simultaneous decreases occurred in soil lead that also saw exaggerated declines in children’s blood lead. Health and welfare disparities continue to exist between soil lead and children’s exposure within inner-city areas and outer communities of the city. At the scale of a city this investigation demonstrates that lessening soil lead effectively reduces children’s blood lead. The lead dust deposition reservoir of soil relates to its astonishingly holistic role as both a source of ingestible and inhalable lead dust. Transporting low lead soil lead into urban communities can result in a more hopeful and economical as an effective method for primary lead prevention.

Lead-Induced Neurotoxicities From Maternal Exposure to Neurodegenerative Alzheimer’s Disease

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Lead (Pb)-induced neurotoxicities have been well documented. Children exposed to Pb have been associated with cognitive deficits and neurobehavioral alterations. This presentation will start with a recent human study conducted in China, where the researchers report that maternal exposure to Pb impairs the neurobehavioral development such as the Apgar (appearance, pulse, grimace, activity, and respiration) score in newborn babies. Recent findings from animal studies by this group on effects of Pb exposure on amyloid plaque formation, which is the initial feature of Alzheimer’s Disease (AD) pathogenesis, and the expression of calciytensin (Clstn), a protein family regulating axonal transport of amyloid precursor protein (APP), will then be discussed. In Tg-SwDI mice that carry the human APP gene, subchronic oral Pb exposure was found to increase amyloid beta (peptides found in amyloid plaques) levels in cerebrospinal fluid, brain cortex and hippocampus; there was also a significant increase of amyloid plaques in animals. Noticeably, Pb-exposed mice showed an impaired spatial learning ability. Real-time imaging of the blood-brain barrier (BBB) permeability by using a dynamic contrast-enhanced computed tomography technique further revealed reduced brain regional blood flow and increased barrier permeability following Pb exposure in the mice. Studies of offspring of pregnant rats exposed to Pb in drinking water from gestation to weaning further showed that the expression of Clstn2 in offspring brain had a Pb dose-related decrease in hippocampus and cerebral cortex at PND21. These observations suggest that Pb exposure not only disrupts the BBB function, but also disrupts the system that regulates APP axonal transport. Consolidation of these observations in the context of maternal Pb exposure and offspring neurotoxicity will be extensively discussed.
In 2012, the CDC recommended that blood lead levels (BLLs) should be benchmarked to a reference value (RV), equal to the 97.5th percentile BLL from the National Health and Nutrition Examination Survey, rather than the 10 µg/dL “level of concern.” The RV was 5 µg/dL in 2012. US EPA has not yet updated its Regional Screening Level (RSL) for soil lead to reflect the 2012 reference value, nor has it finalized revisions to other IEUBK models although it has indicated that it will do both. Use of the target BLL has important implications for managing exposures of children to lead. In this analysis, we evaluated the impact of different exposure assumptions for the soil lead RSL, assuming a target BLL of 5 µg/dL and a probability of an elevated BLL of 1 or 2%. We chose assumptions which may plausibly differ across sites, or, with the BLL geometric standard deviation (GSD), may differ at lower BLL distributions. Specifically, we varied the population BLL GSD, soil lead bioavailability, soil-dust transfer rate, and water lead concentrations. Depending upon the chosen assumptions, calculated soil lead RSLs varied several-fold, in some cases yielding median RSLs below background soil lead levels for certain urban areas. We also evaluated uncertainty in quantifying the contribution of soil lead to BLL in the IEUBK model by comparing IEUBK model estimates to those based on statistical modeling from soil lead: blood lead epidemiology studies. This comparison indicated higher blood lead concentrations with the IEUBK model than with the selected reference probabilistically-based models. While this analysis identified important challenges for remedial decisions with use of the RV target, it also identified approaches for priority setting and areas for reducing uncertainty with additional information.

**W 1750 Probabilistic Modeling of Childhood Multimedia Lead Exposures: Examining the Soil Ingestion Pathway**

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This talk describes a multimedia probabilistic exposure modeling approach to guide public health decisions related to lead, achieved by coupling US EPA’s SHEDS-Multimedia and IEUBK models. The goal of this effort was to advance a national-scale understanding of the relationship between lead concentrations in environmental media and blood lead levels (BLL) of infants and young children, with the purpose of determining what drinking water levels can keep children’s blood lead levels below specified levels. Many factors play a role in lead exposure, including media concentrations, age-dependent media intake rates, lead bioavailability, and biological variability in lead uptake. Our probabilistic modeling approach incorporated distributional assumptions for each of these factors, with the exception of bioavailability for which point estimates were applied. We observed good agreement in predicted childhood BLL (0-23% relative error) with nationally representative children’s BLL, yet somewhat decreased agreement for a regional assessment (35-43% relative error). Pathway contribution analysis (national scale) revealed soil/dust ingestion was the dominant pathway for BLL above the 80% percentile among children 2 to 7 years of age, and suggests a high value of information for determinants of this pathway (lead soil and dust concentrations, soil/dust ingestion rates, and lead soil/dust bioavailability). While considerable work has been done to assess lead bioavailability (e.g., lead species, nutritional status), making use of this data in exposure modeling is constrained by the availability of pertinent exposure information. Herein we discuss these data needs with emphasis on identifying populations most at risk from lead exposure. This abstract does not represent US EPA policy.

**W 1751 Nanotoxicology: State of the Science and the Path Forward**


The US National Nanotechnology Initiative (NNI) was established in 2001 to support the responsible development of the emerging science of nanotechnology and bring together stakeholders from the federal government, industry, and academia. The goal was to thoroughly address the potential health and safety implications of nanomaterials. Stakeholders emphasized the complexity of nanotoxicology, the importance of understanding the novel physicochemical properties of nanomaterials, and how traditional toxicity testing strategies should be modified to address these unique properties. The toxicology community has responded to this call-to-action through the emergence of nanotoxicology as a subspecialty, including the 2008 launch of the SOT Nanotoxicology Specialty Section. Over the past 10 years, thousands of peer-reviewed studies have been published in journals, including those developed specifically for nanotoxicology, in addition to numerous meetings and symposia. Currently, the nanotoxicology community is at a critical juncture where stakeholders have begun to pose serious questions regarding the achievements of this new science. Concerns include the presentation and robustness of the data in published studies and whether standardized and validated methods were used. General questions surround whether available data meet critical data gaps, whether nanotoxicology should continue to exist as a subspecialty of toxicology, and whether consolidating the toxicology community should work in tandem with other disciplines that play a critical role in understanding the relative risks associated with nanomaterials. This session will bring together researchers and scientists from the federal government, industry, and academia to provide an overview of the lessons learned and the support provided to industry for commercializing nano-enabled products. The session will present carbon nanotube toxicity as a case study of the efforts to understand whether toxicity of engineered nanomaterial exposure is adequately understood. Other topics include the state of the science in terms of human health effects, the potential for the NNI in advancing research in nanotoxicology, and the future directions of industries for incorporating nanotechnology. Lastly, the path forward for nanotoxicology, highlighting knowledge gaps and emerging research needs, will be presented, followed by an open discussion with the panel of speakers.

**W 1752 State of the Science: Nanotoxicology of Carbon Nanotubes (CNTs)**

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Carbon nanotube applications in structural materials, electronics, and medicine are expanding rapidly, leading to potential occupational exposures. Fifteen years ago, concern was raised that high aspect ratio CNTs may act as asbestos-like fibers to cause disease. Numerous studies have since shown that CNTs cause lung inflammation/damage, granulomas (due to CNT agglomerates), and fibrosis (due to deposition of smaller CNT structures in the distal lung). Although the pulmonary response, including asbestos-like, CNT pathogenesis differs from asbestos; i.e., 1) CNTs are not a single class and pose toxicity in varying ways; 2) CNTs are not single-walled; 3) CNTs are not radially rigid; and 3) lung gene expression pathways differ for MWCNTs vs. asbestos. Indeed, the fibrogenic potency of CNTs appears driven by their ability to enter the alveolar interstitium and directly activate fibroblast proliferation and collagen production. Pulmonary fibrogenic potential is single-walled CNTs > multi-walled (MWCNTs) > carbon nanofibers = asbestos. Carboxylation of MWCNTs decreases fibrotic potency, while amination enhances bioactivity. These pulmonary responses to CNTs are qualitatively similar in mice vs. rats and quantitatively similar in male vs. females. Recent results also indicate that MWCNTs can both initiate and promote lung cancer, with male rats being more susceptible to MWCNT-induced lung tumors than female rats. MWCNTs can migrate to the interpleural space, and after two years of inhalation exposure MWCNT-induced mesothelial hyperplasia was reported. However, to date, no mesotheliosis has been demonstrated following pulmonary exposure to MWCNTs. Pulmonary exposure to MWCNTs has also been reported to adversely affect cardiac performance and microvascular function. Lung sensory neurons in the lung are involved in this response. Therefore, recent expansion of nanotoxicology data allows for risk analysis for CNTs.

**W 1753 State of the Science: Human Health Effects of Engineered Nanomaterials**

M. Schubauer-Berigan. NIOSH, Cincinnati, OH. Sponsor: T. Thomas

Human (epidemiologic) studies of the health effects of engineered nanomaterials (ENM) have been few and generally confined to the materials expected to be most hazardous (e.g., carbon nanotubes (CNT) or nanofibers (CNF)) or in most common use (e.g., titanium dioxide). The occupational setting is currently the most appropriate for conducting health studies of ENM, given the low exposures and study feasibility challenges in the general population. General studies are provided to demonstrate that the small workforce sizes involved in ENM manufacturing and use, difficulty for researchers in accessing the populations, and problematic exposure assessment for most ENM; an emblematic example of the latter is uncertainty about which aspects of ENM exposure are most relevant to health. A recent review of epidemiologic studies of ENM identified 13 studies in progress (three-quarters of which were cross-sectional) of 9 unique populations of ENM workers. In addition, a relatively large cross-sectional study of US CNT and CNF workers has been completed and full publications of studies of other CNT workers have appeared in the literature. Relatively few overt health effects have been found to be
associated with CNT or CNF exposure in these epidemiologic studies. The exposures in the human studies are generally lower than those used in most toxicology studies, which hampers their comparability. Most published studies have reported some inflammatory or other biomarkers to be associated with measured exposures to ENM, although specific findings tend to be inconsistent across populations. These and other challenges in conducting epidemiologic research of ENM point to the need to pool data across studies and populations. This will require study coordination to ensure comparability of exposure and outcome measurement.

W 1754 Ensuring Responsible Development of Nanotechnology
L. Friedersdorf. National Nanotechnology Coordination Office (NNCO), Arlington, VA.

From the very inception of the US National Nanotechnology Initiative (NNI), responsible development of nanotechnology has been one of its primary goals. Responsible development encompasses support for the development of knowledge for the evaluation of the potential risks and benefits of nanotechnology to the environment and human health and safety; timely dissemination, evaluation, and incorporation of this knowledge; consideration of ethical, legal, and societal implications; and incorporation of sustainability principles. This presentation will provide an overview of the role the NNI has played in addressing this goal. Mechanisms employed to promote active discussion about nanotechnology-related environmental, health, and safety research and to leverage knowledge and resources internationally will also be discussed.

W 1755 An Industry Perspective on the Federal Role in Nanotoxicology
S. Clancy. Evonik, Parsippany, NJ.

Industry has been an active participant in the field of nanotechnology for many years. In fact, industry’s involvement precedes the use of the term “nanotechnology,” through the production and use of materials described by terms such as “ultrafine,” that we may describe as nanomaterials today. Along the way, the industry community has also taken steps to ensure the responsible development and use of nanomaterials, which has often included considering important matters such as characterization and toxicology. The US Federal government has played an important role in helping to establish more common approaches to these issues including more common terminology, metrology, and EHS practices. These improvements have facilitated the development of new materials and increased the confidence in the safe use of legacy nanomaterials. This presentation will feature examples of how the contributions from the Federal government have supported these activities and suggest opportunities for additional contributions.

W 1756 Nanotoxicology and the Path Forward
A. Elder. University of Rochester, Rochester, NY.

As the field of nanotoxicology is growing into maturity, there is a need to conduct research on the health effects of nanomaterials today. Along the way, the industry community has also taken steps to ensure the responsible development and use of nanomaterials, which has often included considering important matters such as characterization and toxicology. The US Federal government has played an important role in helping to establish more common approaches to these issues including more common terminology, metrology, and EHS practices. These improvements have facilitated the development of new materials and increased the confidence in the safe use of legacy nanomaterials. This presentation will feature examples of how the contributions from the Federal government have supported these activities and suggest opportunities for additional contributions.

P 1757 An In Vivo Model for the Simultaneous Assessment of Cardiovascular, Neuromuscular, and/or Central Liabilities of Oximes: Establishing 2PAM Liabilities and Mode-of-Death
S. Roof, R. Hamlin, and C. del Rio. QTest Labs, Columbus, OH. Sponsor: R. Hamlin

Pralidoxime (2PAM) is a clinical-stage field-ready oxime utilized as a cholinesterase reactivator. Due to its multiple on/off target actions, as well as to the tight interactions between cardiovascular, neuro-muscular, and respiratory centers, establishing its specific liabilities in vivo remains difficult. In this study, the simultaneous dose-response(s) of 2PAM on neuro-muscular and cardiovascular end-points were evaluated in vivo, in an effort to establish specific in vivo liabilities. Ten anesthetized (2.5% isoflurane) and mechanically-ventilated male Sprague Dawley rats were instrumented to simultaneously assess systemic/left ventricular hemodynamics as well as skeletal and diaphragmatic function in order to differentiate between central and direct junctional effects, diaphragmatic function was studied in both intact (n = 5, centrally driven) as well as de-centralized preparations (n = 5) where the phrenic nerve was transected cranially and electrically stimulated (4V for 10 ms at 0.2 Hz). In all cases, data were collected before and during 2PAM Administration (2.5 mg/kg/min IV) for 2 hours. 2PAM treatment dose dependently depressed skeletal and diaphragmatic muscle function. Both stimulated (femoral nerve) skeletal muscle and intact (centrally-driven) diaphragmatic twitch matched comparable time-courses, declining 50% (IC50) and 90% from baseline (IC90) at cumulative 2PAM doses of ~80 and ~160 mg/kg, respectively. Alternatively, stimulated (de-centralized) dia-phragmatic function was more resistant to inhibition (IC50:180 mg/kg, IC90: 250 mg/kg), suggesting both a direct and a central action of 2PAM. In all cases, administration of 2PAM resulted in slight vasopression (+14 ± 3 mmHg) and in an early positive chronotropic effect (+11-20 ± 3 bpm). Taken together, these data support diaphragmatic/respiratory depression as the primary liability of 2PAM in vivo (likely resulting in death via hypoventilation/asphyxia), as 2PAM lacked detrimental cardiovascular effects at the exposures studied. In addition, the results also suggest both that 1) 2PAM may directly alter central/afferent respiratory pathways and 2) skeletal muscle function may provide premonitory insights into the diaphragmatic function of oropharmacology, this study demonstrates that the potential neuro-muscular, cardiovascular, and respiratory in vivo liabilities of oximes can be simultaneously assessed pre-clinically.

P 1758 Evaluation of Agonists for the A1 Adenosine Receptor as Novel Anticonvulsant Medical Countermeasures to Soman (GD) Nerve Agent Intoxication
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Current medical countermeasures often have limited efficacy in suppressing seizure activity after organophosphorus nerve agent (NA) intoxication. Toward developing a more effective anticonvulsant treat-ment, we have shown that stimulation of the A1 adenosine (ADO) receptor (A1AR) with the agonist N6-cyclopentyladenosine (CPA) effec-tively prevents NA seizure. CPA at the effective dose (60 mg/kg) produces unwanted side effects e.g., prolonged sedation. This study aimed to determine if efficacy could be achieved at lower CPA doses, and to also determine if other agonists could potentially prevent seizure with fewer side effects. To do so, Sprague Dawley rats were surgically prepared for recording brain electroencephalographic (EEG) activity. One week later, rats received HI-6 (125 mg/kg, IP) and atropine methyl nitate (2 mg/kg, IM) to prevent peripheral cholinergic symptoms and, thus, promote survival without affecting central activity. Rats were exposed to GD (1.6 x LD50, SC) or control vehicle 30 min later. One minute after GD, rats were injected IP with one of the following A1AR agonists at increasing dose levels until anti-seize efficacy was achieved: CPA, 2-Chloro-N6-cyclopentyladenosine (CCPA), and (±)-5’-Chloro-5’-deoxy-ENBA (ENBA). Rats were prepared for neurohistopathological scoring 24 hrs later (0=no damage, 16=most severe). All A1AR agonists were effective in preventing seizure and promoting as a novel discriminative dose for the A1AR agonists were 60 mg/kg CPA, 36 mg/kg CCPA, and 62 mg/kg ENBA. Whereas saline-treated rats experienced 100% seizure and 21% survival (N=28), ADO treatments reduced seizure occurrence and improved survival rates: 8% seizure and 83% survival with CPA (60 mg/kg, N=12), 17% seizure and 75% survival with CCPA (36 mg/kg, N=12), and 8% seizure, 83% survival with ENBA (62 mg/kg, N=12). ADO also suppressed neuropathology: saline-treated rats had severe brain
damage: average score of 14.8±1.8. ADO rats appeared normal: 0.2±0.6 for CPA, 0±0.0 for CCPA, and 1.4±0.4 for ENBA. Other ADO induced physiologic effects (sedation, Bradycardia, and hypothermia) were minimized with CCPA and ENBA vs. CPA: 89% of CCPA, 100% of ENBA, and 0% of CPA rats were ambulatory at 24 hr. The data from this study suggest that CCPA and ENBA are superior candidate ADO agonists for countering NA seizure.

**1759 Temporally Altered Fecal Microbiota and Urine Metabolome following Soman Poisoning**

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The experimental pathophysiology of organophosphorus (OP) chemical exposure has been extensively reported. Here, we describe for the first time an altered fecal microbiota and urine metabolome that follows intoxication with soman, a lipophilic G class chemical warfare nerve agent. Non-anaesthetized Sprague Dawley male rats were subcutaneous-ly administered soman at 0.8 - 1.0 of the median lethal dose (LD50) and evaluated for signs of toxicity. Animals were stratified based on seizing activity to evaluate effects of soman exposure on fecal bacterial biota and urinary protein and metabolome. Soman was resuspended in the fecal biota by preferentially expanding Facklamia, Agrobacterium, Bilophila, Enterobacter, and Morganella genera of the Firmicutes and Proteobacteria phyla, some of which are known to hydrolyze OPs. However, analogous changes were not observed in the bacterial biota of the ileum, which remained the same irrespective of dose or seizing status of animals after exposure. Interestingly, when considering just the seizing status of animals, we found that the urine metabolome was markedly altered. Leukotriene C4, kynurenic acid, 5-hydroxyindoleacetic acid, norepinephrine, and aldosterone were excreted at much higher rates at 72 hrs in seizing animals, consistent with multi-organ involvement during soman poisoning. However, at 75 days post soman exposure, bacterial biota stabilized and no differences were observed. These findings demonstrate the feasibility of using the dysbiosis of fecal bacterial biota in combination with urine metabolome alterations as biosensors for OP exposure to enhance current triage standards and estimation of affected radius on existing clinical laboratory workflows. This approach will be effective at a much earlier time point that remains to be defined.

**1760 Bromine-Induced Myocardial Damage and Dysfunction**

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Inhaled halogen gases produce reactive species on the pulmonary epithelial cell surface that circulate and cause extensive cardiac injury. However, the mechanisms of such myocardial damage are not well understood. This gap in knowledge limits the potential for development of effective therapies. We sought to determine the role of bromine and brominated lipid derivatives in causing cardiac dysfunction. Bromine inhalation (600 ppm) in rats caused acute increases in circulating troponin 1, fatty acid binding protein and NT-proBNP, and ECG ST-segment elevation. Echocardiography revealed a hypercontractile left ventricle (LV), decreased heart rate, decreased mean arterial pressure (MAP), and decreased plasma and LV norepinephrine levels. LV SERCA was extensively brominated and activity was decreased by 70 percent and an increase in cytosolic calcium sensitive calpain activity. Transmission electron microscopy (TEM) demonstrated contraction band necrosis, disruption of the z-disc, and mitochondrial swelling and disorganization. Administration of MDL28170 within one hour of exposure decreased calpain activity and significantly decreased acute mortality. However, LV spherical dilatation and interstitial fibrosis remained at 7 days in survivors. That bromine reactants cause the myocardial damage was confirmed by direct injection of the brominated fatty aldehyde, 2-bromo-hexadecanal (Br-HDA) into the LV cavity of a rat. Br-HDA caused acute LV enlargement and dysfunction with extensive disruption of the mitochondrial/sarcromeric architecture and extensive myocardial damage as in the in vivo bromine inhalation. Increased trends of calpain activity and extensive neutrophil accumulation occurred in the myocardium of Br-HDA treated rats. Cardiopulmonary calpain activation mediated by both adrenergic discharge and SERCA2 inactivation may together underpin halogen (bromine)-induced myocardial damage and dysfunction.

**1761 Manipulation of Macrophage Function for Treatment of Chlorine-Induced Airway Fibrosis**


Chlorine is a highly toxic gas used in a variety of industrial processes and is considered a chemical threat agent. We showed previously that the development of airway fibrosis in mice after chlorine inhalation was associated with inflammation and inefficient epithelial repair. To assess the role of macrophage populations in chlorine-induced airway fibrosis, we treated chlorine-exposed mice with agents affecting macrophage function. FVB/NJ mice were exposed to 240 ppm-hr chlorine, and gene expression was analyzed in developing fibrotic airway lesions. To analyze myeloid cells that may be involved in the development of fibrosis, flow cytometry was performed using digested whole lungs. Macrophage ablation in chlorine-exposed mice was performed using macrophage Fas-induced apoptosis (MAFIA) transgenic mice treated with the dimerizer compound AP20187, which induces apoptosis in macrophages. To assess the contribution of the M2 macrophage marker arginase 1 (Arg1) which has been implicated as a mediator in the development of fibrosis, Arg1 conditional knockout (Arg1 flox x Tek-Cre) mice were used. Arg1 expression was examined by immunofluorescence staining. Picrosirius red staining was performed to determine collagen deposition in major airways. Quantification of collagen content was analyzed by ImageJ. Chlorine exposure resulted in upregulation of genes associated with macrophage function and increased Arg1-expressing cells in developing fibrotic lesions. Neutrophil and eosinophil macrophages increased with chlorine exposure. Corticosteroid treatment decreased Arg1-expressing cells and inhibited the development of airway fibrosis. Ablation of macrophages in MAFIA mice inhibited the development of airway fibrosis. Conditional knock out of Arg1 in Arg1 flox x Tek-Cre mice ablated Arg1 expression but did not affect chlorine-induced airway fibrosis. Taken together, these results implicate macrophages in the development of airway fibrosis and provide avenues that could prove useful for inhibiting fibrosis development after airway injury.

**1762 Nucleic Acid Scavenging Mitigates Inflammation in Rats Exposed to 2-Chloroethyl Ethyl Sulfide (CEES)**

N. Mariappan, M. Husain, I. Zafar, V. Singh, S. Ahmad, and A. Ahmad. University of Alabama at Birmingham, Birmingham, AL. Sponsor: A. Ahmad

Sulfur mustard (SM) or its analog 2-chloroethyl-ethyl sulfide (CEES) are strong alkylating agents. Its acute/chronic inhalation causes airway injury with enhanced vascular permeability and inflammation. Rats exposed to CEES showed increased levels of circulating cell free nucleic acids in the bronchoalveolar lavage fluid (BALF) and plasma. Previous studies have shown increased levels of extracellular nucleic acids (exNA) in a number of diseases. Studies have also shown that exNA can promote inflammation. Rats were exposed to 10% CEES using a nose-only aerosol inhalation system. At the end of the study (12hrs) lungs were lavaged and BALF and plasma were collected for nucleic acid measurements. An acute exposure of rats to CEES resulted in significant increase in protein and IgM in the BALF supernatant, indicating disruption of the alveolar-capillary membrane. There was also an increased neutrophil influx. Western blot analysis of BALF supernatant showed increased HMGB1 protein, a mediator of inflammation. CEES exposure also caused increase in the proinflammatory cytokines and chemokines, IL-6, IL-1α, CXCL1 and CCL2. In order to understand the role exNA in CEES induced lung injury, total NA was isolated from BALF supernatant and added exogenously to airway epithelial cells (16HBE). Addition of exNA caused increases in proinflammatory cytokines IL-6, CXCL1 and CCL2 mRNA levels. As increased circulating exNA are associated with lung injury induced by CEES, we postulated that inhibiting nucleic acids in vivo in rats could protect against CEES induced lung injury. Therefore, we used heparin/methenamine bromide (HMB) as a nucleic acid scavenging polymer or as a mitigating agent in CEES exposed rats. Two hours after CEES exposure, HMBBr (10mg/kg/bw) was administered intraperitoneally. HMBBr
Skin exposure to sulfur mustard (SM) is characterized by severe blister formation and a prolonged inflammatory response. The damage may be further exacerbated by overexpression of matrix metalloproteinases (MMPs) which involve degradation and remodeling of the extracellular matrix and basement membrane proteins. The type IV collagenase (MMP9 and MMP2) may mediate the disruption of type IV collagen (ColIV), a major component of the basement membrane zone (BMZ) in skin wounds post SM exposure. N-hydroxy-3-phenyl-2-(4-phenylbenzenesulfonamido) propanamide (BiPS) was tested as a highly selective MMP2/MMP9 inhibitor. The study aimed to evaluate the therapeutic effectiveness of topically delivered BiPS to protect against SM induced skin injury. We performed a time course study (24, 72, and 168 hr) and a single pretreatment BiPS using in vivo mouse ear skin to determine improvement using histopathologic changes, MMP9 gene expression, and BM protein expression as markers. SM induced characteristic skin structural damage including edema, inflammation, and a disrupted BMZ. The study showed significant reduction in edema and improvement in overall tissue structure by 72 hr post SM exposure. mRNA expression of MMP9 was significantly downregulated in the BiPS treated group by 168 hr post exposure. Immunofluorescent studies showed that significant reduction of MMP9 expression in the skin sections for all time points; the basement membrane (BMZ) expression appeared continuous without disruption and was similar to the unexposed control skin. These results suggest that BiPS may modulate MMP9 expression which may in turn improve base ment membrane integrity after skin exposure to SM. BiPS, the MMP2/ MMP9 inhibitor BiP may hold promise as a potential therapeutic countermeasure against SM induced skin injury. Grant funding supported by ES05022, T32ES007148, and NIAMS U54AR050573.

**1763 BiPS, a Type IV Collagenase Inhibitor Modulates Matrix Metalloproteinase 9 in Sulfur Mustard-Exposed Mouse Skin**

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Skin exposure to sulfur mustard (SM) is characterized by severe blister formation and a prolonged inflammatory response. The damage may be further exacerbated by overexpression of matrix metalloproteinases (MMPs) which involve degradation and remodeling of the extracellular matrix and basement membrane proteins. The type IV collagenase (MMP9 and MMP2) may mediate the disruption of type IV collagen (ColIV), a major component of the basement membrane zone (BMZ) in skin wounds post SM exposure. N-hydroxy-3-phenyl-2-(4-phenylbenzenesulfonamido) propanamide (BiPS) was tested as a highly selective MMP2/MMP9 inhibitor. The study aimed to evaluate the therapeutic effectiveness of topically delivered BiPS to protect against SM induced skin injury. We performed a time course study (24, 72, and 168 hr) and a single pretreatment BiPS using in vivo mouse ear skin to determine improvement using histopathologic changes, MMP9 gene expression, and BM protein expression as markers. SM induced characteristic skin structural damage including edema, inflammation, and a disrupted BMZ. The study showed significant reduction in edema and improvement in overall tissue structure by 72 hr post SM exposure. mRNA expression of MMP9 was significantly downregulated in the BiPS treated group by 168 hr post exposure. Immunofluorescent studies showed that significant reduction of MMP9 expression in the skin sections for all time points; the basement membrane (ColIV) expression appeared continuous without disruption and was similar to the unexposed control skin. These results suggest that BiPS may modulate MMP9 expression which may in turn improve basement membrane integrity after skin exposure to SM. BiPS, the MMP2/ MMP9 inhibitor BiP may hold promise as a potential therapeutic countermeasure against SM induced skin injury. Grant funding supported by ES05022, T32ES007148, and NIAMS U54AR050573.

**1764 Developmental Exposure to TCDD Has Distinct Transgenerational Effects on CD8+ T Cell Responses during Influenza Virus Infection in the Male and Female F3 Generation**

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Developmental and early life exposures can cause lasting effects on an offspring’s health and contribute to disease. The effects of some developmental exposures have even been demonstrated to persist across generations. SM exerts transgenerational effects on the immune system which have not been broadly explored. Exposure of pregnant mice to the prototype aryl hydrocarbon receptor (AhR) ligand 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) leads to a suppressed CD8+ T cell response to influenza A virus (IAV) infection in both male and female adult F1 offspring. The reduced response is characterized by fewer cytotoxic CD8+ T cells capable of producing the antiviral cytokine interferon gamma (IFNγ). The goal of this study was to determine whether the reduced CD8+ T cell response to IAV observed in male and female offspring was transmitted to the unexposed male and female generation F2. Developmentally exposed F1 offspring were generated by treating dams (F0) with 4 μg/kg body weight doses of TCDD or peanut oil vehicle control. Within each treatment group, F1 offspring were paired with non-sibling F1 offspring to create the F2 generation. The F3 generation was created by pairing F2 offspring with non-sibling, non-cousin F2 offspring from the same treatment group. Mature, 8-week-old F3 male and female vehicle and TCDD lineage offspring were infected with IAV and the CD8+ T cell response was measured using flow cytometry. The CD8+ T cell response to IAV remained suppressed in female TCDD lineage F3 offspring compared to vehicle F3 lineage offspring. In contrast, the CD8+ T cell response to IAV in male vehicle and TCDD lineage did not differ, indicating that the male lineage was no longer affected by F0 exposure to TCDD. Thus, although F0 exposure to TCDD altered the CD8+ T cell response to IAV in both male and female mice in the F1 generation, the durability of the changes across generations varied between sexes. The results of this study indicate that developmental exposures are capable of affecting the immune system across generations. This study across multiple generations also highlights the importance of including both males and females in assessments of the effects of developmental exposures on the immune system across multiple generations.

**1765 Developmental Activation of the Aryl Hydrocarbon Receptor Durably Alters the Responsive Capacity of CD4+ T Cells**


Early life environmental exposures have lasting effects on health and can contribute to disease. For example, exposure to contaminants that contain ligands of the aryl hydrocarbon receptor (AhR), including some organochlorine pesticides, has been linked to decreased immune function later in life. However, the cellular mechanisms that drive these durable changes remain poorly defined. Recently, we showed that developmental activation of the AhR using its prototype ligand 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) impairs conventional CD4+ T cell responses to influenza A virus (IAV) infection in adult offspring. Adoptive transfers revealed this defect is due to a direct effect in the CD4+ T cell lineage. This study demonstrates that the diminished response to an immune challenge, naive animals do not have detectable changes in lymphoid organ cellularity or the distribution of T cell subpopulations. This suggests that developmental exposure causes cryptic changes in CD4+ T cell function, which can contribute to disease. To identify the signaling pathways associated with altered CD4+ T cell functional capacity, we used RNA sequencing. Specifically, we compared gene expression profiles in naïve (CD44lo) and activated (CD44hi) CD4+ T cells from TCDD exposed and vehicle control offspring developmentally exposed to TCDD or vehicle control. Differentially expressed genes were identified in pathways critical for CD4+ T cell activation, proliferation, differentiation, and metabolism. Using functional bioassays, we observed that IAV infected offspring developmentally exposed to TCDD exhibit reduced CD4+ T cell proliferation and T helper differentiation. These findings suggest that AhR activation during development shapes T cell response capacity later in life by affecting numerous cellular pathways, including mitochondrial metabolism. Given that coordinated shifts in T cell metabolism are essential for T cell responses to numerous challenges, and that humans are regularly exposed to many different types of AhR ligands, this has broad reaching implications for how exposure shape T cell mediated immune responses.

**1766 Prenatal Exposure to Perfluoroalkyl Substances (PFASs) and Associations with Childhood Asthma and Allergy Related Outcomes and Infectious Diseases**


Prenatal exposure to perfluoroalkyl substances (PFASs) has been reported to be immunosuppressive in that PFAS levels have been positively associated with rates of infectious diseases in childhood. With respect to asthma- and allergy-related outcomes, reports have been inconsistent. The aim of the study was to investigate associations between prenatal PFAS exposure and asthma- and allergy-related outcomes, and infectious diseases, up to 7 years in a subcohort of the Norwegian Mother and Child Cohort Study. Nineteen PFASs were measured in plasma from a sample of 1,977 pregnant women. Health outcomes were collected from questionnaires and included doctor-diagnosed asthma, atopic eczema, food allergy, and inhaled allergy at 7 years of age and childhood infections at 3 and 7 years of age. Logistic and Poisson regression with Bonferroni corrections were applied. Most abundant PFASs were perfluorooctane sulfonic acid (PFOS), perfluorooctanoic acid (PFOA), perfluorohexane sulfonic acid (PFHxS), perfluorononanoic acid (PFNA), perfluoroundecanoic acid (PFUnDA), and perfluorohexamete sulfonic acid (PFHpS). After corrections, only a negative association between PFUnDA and atopic eczema [OR(95%CI): 0.69(0.55, 0.86)] were found between the six PFASs and the doctor-diagnosed out-
comes at age 7 years. With regard to infections, positive associations were found between PFOA and PFNA and bronchitis/pneumonia at age 3 (p<0.05): 0.22 (0.09, 0.34) and 0.17 (0.08, 0.25), respectively and gastric flu at age 7 years (0.390, 0.27, 0.51) and 0.24 (0.16, 0.32), respectively, and between PFHxS and throat infection at age 3 years (0.260, 0.11, 0.41)). Whereas, negative associations were found between PFOS and PFOA and urinary tract infection -0.24 (0.36, 0.13) and -0.25 (0.38, 0.12), respectively, and PFOS and ear infection at age 3 years (-0.13 (-0.21, -0.06)). The results show a weak support for an association between maternal PFAS levels and allergy-related outcomes. The observed associations between maternal PFAS levels and infections, support immunosuppressive effects for some, but not all, childhood infections.

1769 Difference in Immunological Characteristics between Patients with Malignant Mesothelioma and Diffuse Pleural Thickening

Our previous study has reported the immunological differences between malignant mesothelioma (MM) and pleural plaque (PL) with no tumor, malignant disease and just sign caused by inhalation exposure to asbestos respectively, in which MM showed increases in Treg markers on T helper (Th) cells and blood inflammatory cytokines, whereas PL showed increases in granulocytes with expression in cytotoxic T lymphocytes (CTL) and blood IFN-γ. The present study compared immunological characteristics between MM and diffuse pleural thickening (DP), a benign disease caused by asbestos exposure. Peripheral blood mononuclear cells (PBMCs) and plasma were prepared and assayed for cell surface markers of CD4+ (Th), CD8+ (CTL), CD56+ (NK) and monocytes by lumineux and flow cytometry respectively (FCM). The part of the four population of cells were sorted by FCM and stored in fresh or after 1-day-stimulation with PMA and ionomycin except for monocytes, followed by analysis for mRNA levels of the genes which have a role in development and functions of each population of cells. The surface expressions of GITR and CD69 on Th cells and those of CXCR3 and CD66 on CTL were high in MM compared with DP. The mRNA levels of RORC in Th and of c-Rel were high in MM, whereas the mRNAs of GATA-3 in Th and PRF1 in CTL were high in DP. There were no differences in plasma cytokine concentrations between MM and DP. Those 8 parameters showing the significant differences were statistically examined by linear regression analysis, which resulted in estimation of the two factors. The high factor loading in the first factor was shown by GITR and CD69 on Th, CXCR3 and CD66 on CTL and mRNAs of RORC in Th and c-Rel in NK, whereas mRNAs of GATA-3 and PRF1 in CTL showed high factor loading in the second factor. The 2D plot with these two factors clearly distinguished the two groups of MM and DP. These results indicate that there are obvious differences in immunological characteristics including suppressive function and target-lytic activity executed by Th and CTL between MM and DP.


During primary influenza A virus (IAV) infection, activation of the aryl hydrocarbon receptor (AHR) diminishes host responses by negatively regulating the ability of dendritic cells (DC) to prime naïve CD8 T cells, leading to reduced expansion and differentiation of CD8+ cytotoxic T lymphocytes (CTL). However, the mechanism by which AHR regulates DC function in vivo is unknown. Unbiased gene expression profiling in vivo A. M. Franchini, J. R. Myers, G. Jin, and P. Lawrence. University of Rochester, Rochester, NY.

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The prevalence of autism spectrum disorders (ASD) has increased from 1/500 in 1995 to 1/68 in 2010. While the etiology of autism is not entirely understood, it is suggested that multiple factors such as environmental, immunological, and genetic influences are involved. Anti-brain antibodies have been implicated as a possible mechanism for autism pathogenesis. Exposure to diesel exhaust particles (DEP) during pregnancy has been suggested to perturb the maternal-fetal immunologic environment. We propose that DEP exposure during pregnancy can increase the likelihood of anti-brain antibody production, resulting in an increased risk of developing ASD in the child. In vitro DEP exposure reduced neuronal viability and connectivity and the damage is suggested to be due to generation of inflammation and anti-brain antibodies. Sera of individuals with autism and with lupus display antibodies to different brain antigens as delineated by Western analysis. An organotypic culture system demonstrated that DEP inhibits development of dopamine neuron innervation from the substantia nigra into the striatum and cortex.


During primary influenza A virus (IAV) infection, activation of the aryl hydrocarbon receptor (AHR) diminishes host responses by negatively regulating the ability of dendritic cells (DC) to prime naïve CD8 T cells, leading to reduced expansion and differentiation of CD8+ cytotoxic T lymphocytes (CTL). However, the mechanism by which AHR regulates DC function in vivo is unknown. Unbiased gene expression profiling was used to identify gene and signaling pathways in DCs modulated by AHR activation in vivo. Exposure of mice to the prototypical AHR ligand 2,3,7,8-tetrachlorodibenzo- p-dioxin (TCDD) revealed 631 differentially expressed genes (DEGs) in purified lung DCs as compared to DCs from unexposed animals. This region mapped to a β-strand located at the interface between the monomer and the receptor, which recognizes a patch of overlapping peptides, compared with monomer. Antibodies from mice immunized with aggregated scFv preferentially recognize a patch of overlapping peptides, compared with monomer. This region mapped to a β-strand located at the interface between the Vh andVk domains. Further, molecular dynamics simulations indicated that the Vh domain is less stable than the Vk domain, suggesting the interface region between the two domains becomes exposed during partial unfolding of the scFv during aggregate formation. The data are consistent with the hypothesis that epitopes from partially unfolded states become revealed or more exposed in the aggregated state, and that this can augment the IgG antibody response. This observation offers the theoretical possibility that epitopes preferentially associated with aggregates can be identified from the anti-drug antibody serum IgG response which may lead to better methods for detection of anti-drug responses and informed design of therapeutic proteins to control immunogenicity.

1770 Characterization of Immunogenic Responses to Protein Aggregates by Peptide Array
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The use of therapeutic protein products or biologics has revolutionized the treatment of inflammatory conditions such as rheumatoid arthritis. Biologics are generally associated with lower onset of toxicity than low molecular weight drugs. However, a major issue is the development of immunogenicity, particularly the development of neutralizing antibodies that compromise efficacy. It has been demonstrated that aggregation of biologics is an important factor driving undesirable immunogenicity but the mechanisms are unclear. There is currently little information as to how aggregates interact with B-cell receptors or cognate antibodies at the protein sequence level and whether aggregate specific epitopes dominate these interactions. Using an antibody fragment (single chain antibody variable fragment; scFv) that forms aggregates following incubation at 40°C for 25min, anti-scFv IgG responses have been induced by intraperitoneal injection of BALB/c mice with monomer or aggregates. Aggregate specific immunogenic signatures have been identified by oligo-peptide micro array, using a series of overlapping 15mer peptides based on the linear sequence of scFv and printed onto glass slides. IgG antibodies from mice immunized with aggregated scFv preferentially recognize a patch of overlapping peptides, compared with monomer. This region mapped to a β-strand located at the interface between the Vh and Vk domains. Further, molecular dynamics simulations indicated that the Vh domain is less stable than the Vk domain, suggesting the interface region between the two domains becomes exposed during partial unfolding of the scFv during aggregate formation. The data are consistent with the hypothesis that epitopes from partially unfolded states become revealed or more exposed in the aggregated state, and that this can augment the IgG antibody response. This observation offers the theoretical possibility that epitopes preferentially associated with aggregates can be identified from the anti-drug antibody serum IgG response which may lead to better methods for detection of anti-drug responses and informed design of therapeutic proteins to control immunogenicity.
Biotherapeutics are finding increasing clinical application, particularly for the treatment of inflammatory diseases such as psoriasis. However, despite humanization, immunogenicity can limit their use due to reduced efficacy and/or associated adverse health effects. Many factors can impact on immunogenicity, and we here characterized the influence of oxidation on protein aggregation and immunogenic potential. The effects of protein oxidation on structure and folding and the quality and vigor of the in vivo immune response following immunization of BALB/c strain mice have been investigated. A model recombinant protein, HEL4, a human V δ domain, was produced in E. coli and purified using Protein A affinity chromatography. The protein was oxidized using hydrogen peroxide and heat treatment (42°C) for one hour, and then stir-stressed to achieve aggregation. Mass spectrometry, NMR, dynamic light scattering and circular dichroism were employed to characterize structural changes in the protein associated with oxidation and aggregation. Groups of BALB/c mice were injected intraperitoneally with unmodified (monomeric), oxidized or oxidized stir-stressed (aggregated) HEL4 weekly for 3 weeks, and serum antibodies were measured by ELISA. Oxidation of HEL4 resulted in loss of tertiary structure, although the protein remained monomeric, and dramatically increased the propensity of the oxidized HEL4 to aggregate. Monomeric HEL4 induced low level antibody production, whereas increased serum IgM, IgG and IgG1 antibodies were detected in mice immunized with the stir-stressed oxidized HEL4, and to a lesser extent, the oxidized HEL4. IgG2a was detected only after immunization with the aggregated protein, suggesting that aggregation triggered preferential activation of Th1 (Th1) cells. Both the Th1 skewing and the persistence of IgM antibody responses may be caused by antigen repetitiveness on the surface of antibody binding to HEL4, as it is a mutant, bovine IgG. The formation of aggregates caused IgM, IgG and IgG1 antibodies to be detected in mice immunized with the oxidized HEL4, and to a lesser extent, the oxidized HEL4. IgG2a was detected only after immunization with the aggregated protein. These data demonstrate that the loss of structure resulting from oxidation caused enhanced protein aggregation and increased immunogenicity, despite humanization, immunogenicity can limit their use due to reduced efficacy and/or associated adverse health effects. Many factors can impact on immunogenicity, and we here characterized the influence of oxidation on protein aggregation and immunogenic potential.
or MEHP supplemented cultures, showing that the neonatal ovaries are not metabolizing DEHP or MEHP. Thus, it is likely that folliculogenensis is not accelerated in neonatal ovaries exposed to DEHP because they do not metabolize it to the more toxic metabolite, MEHP. Supported by R56 ES 025147 (JAF) and T32 ES 007326 (CC).

1775 Assessment of Oxybenzone, Octylmethoxycinnamate, Octylsalate, and Octocrylene in Endocrine Disruptor Screening Panel Studies

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Oxybenzone (OXY), octylmethoxycinnamate (OCM), octylsalate (OCY), and octocrylene (OCT) are UV absorbing agents in sunscreens. Their potential estrogenic and androgenic effects were evaluated in vitro; estrogen receptor (ER) and androgen receptor (AR) binding and transcriptional activation, and in vivo; rat urotrophic and Hershberger biosassays, respectively. OXY displayed weak ERα-mediated luciferase activity with a LogEC50 of 5.5 LogMl. Using ERs isolated from rat uteri, OXY, OCT, and OCM were classified as “non-interacting” at exposure levels 10-10 to 10-4M. OXY was classified as “equivocal”. Administration of OXY, OCM, OCT, and OCY for 3 days did not alter uterine weights when assessed up to a limit dose of 1g/kg. OCM, OCT and OCY did not exhibit AR agonist or antagonist transcriptional activity. OXY did not demonstrate AR agonism, however there was an exposure-dependent increase in serum inflammatory cytokines (e.g., IFNγ, IL13, and IL6) compared to air controls. In summary, serum collected from dams after peri-implantation O2 exposure was bioactive in naïve trophoblasts and contained an altered metabolic and inflammatory profile. These findings suggest a role for O2-induced systemic factors in the impairment of placental health and vascular remodeling that can lead to IUGR. Abstract does not reflect US EPA policy.

1777 Bisphenol-Specific Effects on Gap Junction Intercellular Communication in Ovarian Theca Cells

J. Gingrich, Y. Pu, B. Upham, and A. Veiga-Lopez. Michigan State University, East Lansing, MI.

Ovarian theca cells provide structural, vascular, and endocrine support to the growing follicle. Gap junctional intercellular communication (GJIC) is essential for cell layer stability, growth, and ovulation. Bisphenols (BPs) are endocrine disrupting chemicals (EDCs) used in the production of polycarbonate plastics and epoxy resins. In particular, bisphenol A (BPA) can impair ovarian development and inhibit GJIC in mammalian epithelial cells. However, much remains unknown on the effects of BPs in ovarian cells, and if this could be a contributing factor towards impaired ovarian development reported in BPA-exposed females. We investigated if BPA, and/or its analogs bisphenol S (BPS) and bisphenol F (BPF), would inhibit GJIC in theca cells at an environmentally relevant dose. A preclinical and monovalent animal, the sheep, was used as an animal model. Primary theca cells were isolated from the ovaries of healthy sheep (n=3) and cell purity confirmed by vimentin and fibrin-5 immunostains. Passage 2 cells were grown to ~80% confluence and exposed to BPA, BPS, or BPF (1, 10, 50, 100, 200, 500, and 1,000 ng/ml) for 24h to establish a maximal response dose. This dose was then used in a time-response experiment (0, 3, 6, 12, and 24h) to determine the form fetal GJIC effect. Finally, inhibitors of the phospholipase C and MAPK pathways were used in conjunction with bisphenol exposure to elucidate a mechanism of action. GJIC was assessed using a scrape loading and dye transfer assay. BPS, but not BPA or BPF, enhanced GJIC starting at 100 ng/ml, peaked at 200 ng/ml, and began to decline at 500 ng/ml. At 200 ng/ml, BPS started to affect GJIC within 3h, but peaked and plateaued after 12h. Additionally, we demonstrate that this effect can be blocked by a PKC-dependent MAPK inhibitor (12-o-tetradecanoylphorbol-13-acetate, TPA), and rescued through pre-treatment with a panPKC-inhibitor (GF109203X). This study is the first to report that BPS can modulate GJIC. Further studies are necessary to elucidate how BPS modulates GJIC through the MAPK signaling pathway, the specificity of this effect to female reproductive tissues, and the in vivo impact enhanced GJIC in the ovary has on female fertility parameters such as steroidogenesis, folliculogenensis, and ovulation. Supported by NIEHS (1K22ES026208 to A.V-L.).
were utilized. Micro-CT imaging of juvenile animals showed that there were no differences in skeletal growth of long bones between the three groups indicating no effects of isoflurane and/or x-ray exposure on the juvenile skeletal growth. In conclusion, micro-CT can be utilized as a valuable tool for skeletal assessments in rat fetuses and for longitudinal long bone measurement in rats from birth to weaning in juvenile toxicity studies.

The Trichloroethylene (TCE) Metabolite S-(1,2-dichlorovinyl)-L-cysteine (DCVC) Alters Oxidative Stress and Apoptotic Responses During Syncytialization in a Human Placental Cell Model (BeWo)

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Syncytialization is a cellular differentiation process in which cytotrophoblasts in the placenta fuse and form multinucleated syncytiotrophoblasts. Syncytiotrophoblasts are the cells at the outermost layer of the fetal side of the placenta, exchanging waste, nutrient, and gas between the mother and fetus. They are also in direct contact with maternal blood and blood-borne toxicants. Despite the importance of syncytialization, literature is scarce about how toxicants may influence the process. This study investigated how the trichloroethylene (TCE) metabolite S-(1,2-dichlorovinyl)-L-cysteine (DCVC) impacts syncytialization and biological responses during syncytialization. DCVC is involved with antioxidant response, these results show that DCVC alters apoptotic and oxidative stress responses during syncytialization. Garcin and associates showed that regulators of cholesterol transport (e.g. STAR) that regulates cholesterol transfer from the endoplasmic reticulum to the plasma membrane were significantly upregulated in placental cells exposed to TBT. These results were replicated in all three species studied. In conclusion, TBT disrupted the ABCA1 expression in a dose-dependent manner. TBT also induced ABCA1 expression in a dose-dependent manner and increased the upregulation of ABCA1 mRNA expression relative to control. These results suggest that TBT disrupts the ABCA1 expression in a dose-dependent manner and increased the upregulation of ABCA1 mRNA expression relative to control.

Low Lead Concentrations Decrease the Sperm Function by Altering the Acrosome Reaction in Mice


The acrosome reaction (AR) is the process necessary to penetrate the oocyte zona pellucida, allowing the fertilization; it consists in the fusion of the outer acrosomal and plasma membrane to release the hydrolytic enzymes of the sperm acrosome. Before the AR occurs, the spermatozoon suffers a series of changes called sperm capacitation that include a rearrangement of the cytoskeleton, which involves the polymerization of actin that is necessary to the RA. Lead (Pb) exposure has been associated with adverse effects on the male reproductive system, including low fertilization and alterations in the acrosome in experimental models at high concentrations similar to occupational exposures. The aim of this study was to evaluate the sperm quality, the AR (initial, spontaneous and induced-ARo, sAR and iAR, respectively) using the labelling with peanut agglutinin (PNA-FITC) and fluorescence microscopy and the sperm capacitation (by Tyr phosphorylation (P-Tyr) and actin polymization) by immunodetection. Sperm cells incubated with capasitizing medium (Witten’s with 6 mg/mL BSA) of CD1-mice exposed to 0.01 % Pb2+/45 days in drinking water (a spermatogenic cycle). Blood Pb (PbB) concentration at the end of the treatment was 9.4 ± 1.6 μg/dL. The sperm quality parameters were not affected, whereas an increase in the ARo was observed compared to the controls (38.4 ± 3.7 vs 57.4 ± 2.4 %, p = 0.0038) and the iAR after the challenge with a calcium ionophore (A23187, functional AR) was decreased (29.4 ± 1.7 vs 15.1 ± 2.0 %, p = 0.001), the sAR was not affected. P-Tyr showed the same level (0.94 ± 0.2 vs 1.0 relative units) in capacitated spermatozoa compared to the controls. Finally, a significant decrease of 67 and 52 % (p<0.001) in actin polymerization was observed in Pb-treated mice in capacitating conditions, in the sperm head and tail, respectively, suggesting a molecular mechanism of Pb toxicity in sperm function. Our data suggest that exposure to environmental concentrations of Pb (PbB <10 μg/dL) decrease the acrosome function (decreased iAR), which can affect the ability of the spermatozoon to fertilize the oocyte, contributing to the male fertility problems.

Multispecies Study: Environmentally Relevant Dose of Tributyltin Impairs Theca Cell Cholesterol Homeostasis Through the Retinoid X Receptor in Pigs, Sheep, and Cows

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Tributyltin (TBT) is an organotin chemical used as a chemical catalyst and biocide with human exposure ranging between ~0.1 and 85 ng/ml in blood. TBT can stimulate cholesterol efflux in non-steroidogenic cells, suppressing steroidogenesis. Since cholesterol is the precursor for sex hormone production, we hypothesized that TBT disrupts intracellular cholesterol transport balance and impairs steroidogenesis in ovarian theca cells. We studied TBT’s effect on cholesterol trafficking, luteinization, and steroidogenesis in theca cells in three species (pig, sheep, and cow). Theca cells were isolated and purified from ovaries from all three species with collagenase and cell density gradient. Cell viability and cell death were assessed by trypan blue dye. Immunocytochemistry for impacting syncytialization because previous studies showed DCVC as bioactive in placental cells and have associated TCE exposure with adverse pregnancy outcomes. The BeWo cell line, cultured initially as cytotrophoblasts and induced to differentiate in vitro into syncytiotrophoblasts via forskolin treatment, was used to assess how DCVC affects syncytialization and biological responses during syncytialization. DCVC is involved with antioxidant response, these results show that DCVC alters apoptotic and oxidative stress responses during syncytialization. Garcin and associates showed that regulators of cholesterol transport (e.g. STAR) that regulates cholesterol transfer from the endoplasmic reticulum to the plasma membrane were significantly upregulated in placental cells exposed to TBT. These results were replicated in all three species studied. In conclusion, TBT at an environmentally relevant dose, stimulates theca cell cholesterol transport, but decreases ACAT expression, which reduces the ability of the theca cell to uptake cholesterol from the circulation, impairing the ability of the theca cells to provide cholesterol to the growing oocytes. The challenge of the ARo was observed compared to the controls (38.4 ± 3.7 vs 57.4 ± 2.4 %, p = 0.0038). Finally, a significant decrease of 67 and 52 % (p< 0.001) in actin polymerization was observed in Pb-treated mice in capacitating conditions, in the sperm head and tail, respectively, suggesting a molecular mechanism of Pb toxicity in sperm function. Our data suggest that exposure to environmental concentrations of Pb (PbB <10 μg/dL) decrease the acrosome function (decreased iAR), which can affect the ability of the spermatozoon to fertilize the oocyte, contributing to the male fertility problems.
tests. Testis T production was significantly reduced by DPeP but not by WY or clofibrate. Taken together, these results support the hypothesis that activation of the PP-α pathway is not the MIE in phthalate-mediated male reproductive toxicity in the fetal rat testis. Disclaimer: This abstract does not necessarily reflect US EPA policy.

### 1783 Biological Relevance of Key Events (KE) In Utero in the Androgen Adverse Outcome Pathway Network (AOPn) to Adverse Effects in F1 Male Rats

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We are conducting studies to evaluate the biological relevance of changes in KEs and molecular initiating events (MIEs) in AOPs to determine if these changes are predictive of the dose levels of chemicals that disrupt the androgen signaling pathway in utero. Herein, we focus on chemicals that disrupt fetal testis endocrine function or act as androgen receptor (AR) antagonists. Our objective is to determine if KE EDS or MIE EC50 can be used to predict the doses that produce adverse effects after exposure during sexual differentiation. While several MIEs for the phthalate AOP have been proposed, the data indicate that PP-α agonists (pinirinic acid, clofibrate and FRD-903, GenX), and a COX inhibitor (Paracetamol) do not reduce fetal testis testosterone production (TROD) or gene expression profiles, in contrast with the phthalate esters (PE). Because the MIE is unknown, we have developed a statistical model of the relationships between the KEs and the adverse effects of PE. This model uses data from several different PE studies and compares reductions in fetal TROD and testis gene expression with the doses that produce reproductive malformations. For AR antagonists, statistical analysis of gene data for >50 chemicals indicate that the in vitro EC50 values for AR antagonism are poorly correlated the ED50 values for effects on male rat reproductive development (R<10%). For example, potency rankings of the in vitro EC50 are negatively correlated with the in vivo EDS for BPC, BPA, vinclozolin and pynfluquinazon. In contrast, the ED50 values for the inhibition of androgen-dependent tissue growth in short-term in vivo assays are highly correlated (R>85%) with the ability of the chemical to disrupt androgen-dependent reproductive tract development in utero. At present, we are unable to entirely replace animals to interrogate the in utero effects of chemicals using the MIEs for PE or AR antagonists, these models do enable us to significantly reduce animal use by using two short term assays to identify points of departure for hazard identification. However, these KEs would not predict the adverse effects of chemicals that disrupt this AOPn through different AOPs. Research is needed to identify as yet unquantified AOPs in the AOPn to improve the predictive ability across all MIEs and to integrate these into a predictive statistical model. This abstract does not reflect Agency Policy.

### 1784 Difference in MiRNA Expression by Several Testicular Toxicants


Testicular toxicity is induced by several chemicals, such as drug or fertilizer, and the toxicity is a one of the cause of withdrawal for drug development. Recently, it was reported non-coding RNA has a role for spermatogenesis. In the present study, we investigated changes in microRNA expression by several testicular toxicants, ethylene glycol monomethyl ether (EGME, spermatocyes target compound), dimethoxybenzene (DB, Sertoli cells), 2.5-hexahexide (HD, Sertoli cell) and cyclophosphamide (CP, spermatogonia). As a first study, EGME administered to the male rats at 2000 mg/kg single orally and after 6 and 24 hr of dosing, testes were applied to histopathological examination and gene expression analysis using mRNA microarray and real-time RT-PCR. At 6hr, slight decrease of phacypetere spermatocytes with cell shrinkage and nucleus pyknosis and, at 24 hr, remarkable decrease or cell death of phacypetere spermatocytes with Sertoli cell vacuolation were observed. Gene expression analyses revealed increases in mir-700-5p expression at 6 hr and mir-134, mir-320 and mir-188-5p at 24 hr, and decreases in mir-449a and mir-92a at 24 hr. As a second study, different testicular toxicants, DB, HD or CP were given to male rats and after 24 hr, histopathological changes and above the microRNA expression were investigated. Histopathologically, decrease or cell death of spermatocytes and vacuolation of Sertoli cell was observed in DB treatment, and cell death of spermatocytes was seen in CP, but there was no change in HD. DB and HD, induced decreases in mir-134 and mir-320 expression, and there were no changes by CP, there were different changes by EGME. Taken together, the different reaction of microRNA expression may be reflected on the difference of target cells, and these data indicated these microRNAs might work as the biomarker of testicular injury.

### 1785 Evaluation of 2,3,4,5-Tetrabromo-Ethylhexylbenzoate (EHTBB)-Treated Fischer Rats in a Reproduction Screening Study


EHTBB, a common environmental contaminant in found in household dust, is a component of the flame retardant mixture Firemaster550 (FM550). FM550 replaces PBDEs and is used in the production of foam for baby chairs and mattresses and is an exposure concern for children. Routes of exposure for humans to EHTBB are unclear, although ingestion and inhalation of flame retardants has been well documented. EHTBB-related changes were monitored using apical indicators of health status in Fischer rats following oral exposure via a rodent reproductive screening study (OECD TG421). Rats were exposed in feed, ranging in dose from 0mg to 7500mg/EHTBB/kg feed. Male and female rats were on dose for 14 days prior to breeding. Male rats were 56±1 days on dose (DOD) prior to necropsy. Dams were 40-52 DOD when necropsied at postnatal day 4 (PND4). Pups were also necropsied at PND4. Body weights (BW) of rats on the 7500mg EHTBB/kg diet were significantly decreased in females compared to controls beginning on DOD3 and in males on DOD7, and remained so for the duration of the study. Animals dosed at 1875mg EHTBB/kg diet had significant decreases in BW by DOD7 in females and DOD35 in males. Food palatability was not the reason for weight loss in the high dose. Water consumption was significantly increased by DOD10 and remained so in both sexes at 7500mg EHTBB/kg diet. Excessive urination was also observed in the high dose group. Organ weight/BW ratios at necropsy were significantly changed in thymus, spleen, thyroid, kidneys and brains for females and males at 7500mg EHTBB/kg diet. Organ weights were also significantly affected for testes, heart, liver and adrenals in males, and for ovaries and uterus in females. Reproductively, no live births were observed in females at 7500mg EHTBB/kg diet and significantly reduced litter weights were found at PND4 for 1875mg EHTBB/kg diet group. Male sperm kinetic parameters at time of necropsy showed severely affected sperm motility in the highest dose group. These reproductive changes, combined with clinical chemistry and haematology endpoints, indicate that EHTBB has a number of potential target organs resulting in multiple health effects.

### 1786 The Effects of Electronic Cigarette Vapor on Placental Trophoblast Cell Function


Despite evidence that maternal smoking is associated with numerous adverse outcomes, 10-35% of women still smoke during pregnancy. Recently, electronic cigarettes (e-cigarettes) and other electronic nicotine delivery systems have become available, and are being marketed as effective smoking cessation tools. However, there is considerable uncertainty regarding their safety for use during pregnancy. The goal of this study was to examine the effects of e-cigarette vapor (EVOD KangerTech*) to vaporize 3ml of unflavored e-liquids containing 0 or 12 mg/ml nicotine (Blacklisted®) into 30ml of cell culture media. Htr-8/SVneo cells, a model of first trimester trophoblast cells, were exposed to up to 1% of the vapor conditioned media to assess cell viability (LDH release), proliferation (BrdU incorporation), migration (wound healing assay), invasion (Matrigel Transwell assay), and tube formation (a surrogate for angiogenesis). Exposure to e-cigarette conditioned media with or without nicotine did not significantly affect cell viability, proliferation or migration (all p>0.05) at any dose tested. Treatment with e-cigarette conditioned media with or without nicotine reduced trophoblast invasion by 35-40% versus control (p<0.05). E-cigarette conditioned media also had no effects on tube formation with significant reductions in total tube length, total segment length, and number of junctions (p<0.05). Exposure to e-cigarette vapor conditioned media had profound effects on normal placental trophoblast function; an effect which could not be solely attributed to the presence of nicotine. These results suggest that an evaluation of the safety of e-cigarette use during pregnancy is urgently required.
Indianapolis, IN; and decreases in induction. Dose dependent maternal and fetal endpoints were examined on GD20. TCDD tissue concentration was followed by GD9-20 daily maintenance doses to examine the potency of 2,3,7,8-tetrachlorodibenzofuran (TCDF) relative to TCDD or TCDF Effects on Pituitary and Testicular Steroidogenic Pathways

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In utero exposure to TCDD can reduce epididymal sperm counts in adult rats. A mode-of-action (MOA) involving AhR-induced suppression of fetal Leydig cell production has been proposed. Experiments were performed to evaluate fetal and in vitro activation on pituitary and testis steroidogenic genes expression and to examine the potency of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) relative to TCDD. Pregnant Sprague Dawley rats (n = 5/group) were gavaged with 30, 50, 100, 1500, 2000, 6000, or 10,000 ng/kg of TCDD or TCDF on GD8. The GD8 loading dose was followed by GD9-20 daily maintenance doses of 0, 3, 30, 220, 220, 660, 1230, and 2200 ng/kg/day for TCDD. We also examined the response to a single GD15 dose of 10,000 ng/kg for both congers. In both study designs, maternal and fetal endpoints were examined on GD20. TCDD tissue concentrations in dam liver and adipose and the whole fetus were higher than TCDF (e.g., up to approximately 4- and 6-fold, respectively). One to two orders of magnitude higher maternal TCDD and TCDF concentrations were observed in maternal tissue relative to the whole fetus. AhR activation was observed in all tissues examined (including fetal pituitary and testis) as evidenced by Cyp11a induction. Dose dependent increases in Lhb and Fshb gene expression were observed in fetal pituitary after TCDD but not with TCDF. TCDD, but not TCDF, produced a dose-dependent decrease in fetal testis steroidogenic genes (Star, Cyp11a1, Cyp17a1, and Scarb1). While the evidence suggests a potential mode-of-action for reduced epididymal sperm counts, relatively high doses of TCDD or TCDF was required. The lack of a significant effect of TCDD on fetal pituitary gonadotropin and testis steroidogenic gene expression could be secondary to limited tissue concentration accumulation caused by its self-induced metabolic clearance. TCDF’s absent response and unique kinetics should be uncertainty considerations in the development of its relative potency and toxic equivalency factor.
D(2-ethylhexyl) phthalate (DEHP) is a plasticizer used in numerous consumer products. Studies have shown that DEHP acts as an endocrine disrupting chemical and adversely affects reproductive outcomes in males. However, little is known about the effects of prenatal DEHP exposure on female reproductive outcomes and whether these effects occur in subsequent generations. Therefore, this study tested the hypothesis that prenatal exposure to DEHP accelerates the onset of puberty, disrupts birth outcomes, reduces fertility-related indices, and accelerates the onset of reproductive senescence in the F1, F2, and F3 generations of female mice. To test this hypothesis, pregnant CD-1 mice were orally dosed with corn oil (vehicle control) or DEHP (20 and 200 µg/kg/day, and 500, and 730 mg/kg/day) daily from gestation day 10.5 until birth. To produce the subsequent generations, F1 females were mated with untreated males to obtain the F2 generation and F2 females were mated with untreated males to produce the F3 generation. In the F1 generation, prenatal exposure to DEHP accelerated the onset of puberty and disrupted fertility-related indices. In the F2 generation, exposure to DEHP accelerated the onset of puberty, increased the female sex ratio, and disrupted fertility-related indices. In the F3 generation, DEHP exposure accelerated the onset of puberty, increased the female sex ratio, decreased female pup anogenital index, and reduced fertility-related indices. Collectively, these data indicate that prenatal DEHP exposure at environmentally relevant doses accelerates the onset of puberty, disrupts birth outcomes, and disrupts fertility-related indices in a multigenerational and transgenerational manner in female mice. These data also indicate that prenatal exposure to DEHP accelerates reproductive aging in a multigenerational and transgenerational manner. Supported by NIH P01 ES028484, EPA RD-83459301, T32 ES057326, and the Billie A. Field Fellowship.

**Prenatal Exposure to Di(2-ethylhexyl) Phthalate Promotes Adverse Transgenerational Effects on the Reproductive Capacity of Female Mice**

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**High-Quality Sperm RNA Isolation for Preclinical and Clinical Reproductive Toxicogenomic Studies**

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Gene expression profiling of mammalian sperm has been proposed as a novel non-invasive tool to evaluate male fertility and testicular toxicity. However, isolation of sperm RNA is a challenging procedure due to the unique biology of sperm and the heterogeneous population of cells present in the ejaculate, indicating the need for high quality control checks to ensure reproducibility of data generated from sperm RNA. Semen contains somatic cells, such as leucocytes and epithelial cells, along with spermatooza that contain a very low abundance of RNAs compared to somatic cells. Therefore, somatic cell removal is essential to avoid contamination of sperm transcripts. The present study was designed to develop a reliable and effective protocol for RNA isolation from rat and human sperm that delivers highly purified and intact RNA, verified using RNA-specific electrophoresis and molecular biology approaches such as RT-PCR and western blot analysis. To develop a standardized sperm RNA protocol, we evaluated quality and purity of rat sperm RNAs isolated using different sperm collection, purification and RNA extraction approaches. For rat, a high-quality sperm RNA extraction methodology consists of collection of epididymal fluid with repeated needle punctures of the epididymis, somatic cell elimination using detergent-based somatic cell lysis buffer (SCLB), and the use of a RNA isolation Kit. This sperm RNA isolation technique was then adapted to human sperm. The method we have optimized is suitable for comparative sperm transcriptomic analysis, such as sequencing and directed PCR. In conclusion, this RNA isolation protocol across species adaptations improves reproducibility of functional genomics studies of pharmacetical, chemical, and environmental exposures and infertility in pre-clinical and clinical setting.

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**Utilizing In Vitro Developmental Models to Predict Teratogenic Exposures**

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In vitro developmental toxicity assays have been evaluated extensively for hazard identification of compounds for potential teratogenic liability. However less is understood regarding how these assays may predict in vivo teratogenic exposures. Our laboratory combined cytotoxicity and dysmorphology in a 2 assay paradigm for predicting a range of concentrations that may correlate with teratogenic Cmax concentration in vivo. Twenty-one compounds with characterized teratogenic Cmax were used to treat mouse embryonic stem (ES) cells in a concentration range designed to identify an IC50 value over a 3-day culture period. The IC50 was used to establish a possible adverse effect exposure range in vivo (1:25 ratio of the IC50 to the IC50 value). The positive in vitro exposure concentration was captured in the predicted range in 13 out of 15 compounds (87%). The remaining 6 compounds exhibited solubility limitations with undetermined IC50 values. These compounds were assessed in rat whole embryo culture (WEC) by treating gestation day 9 embryos for 48 hours at the positive in vivo concentration of each compound. Percent effect was calculated by morphological assessment of each embryo and a 30% increase of malformations predicted in vivo teratogenic exposure. Three of 6 compounds presented teratogenicity in WEC at their reported teratogenic Cmax concentration. An expanded concentration range is under exploration to determine whether it could increase sensitivity and be applied in this paradigm. Using the ES-WEC strategy with the current methodology, predicted teratogenic Cmax concentration range was correct for 16 out of 21 compounds (76%).

**Elucidation of the Mechanism of Nitinol Toxicity in Human Fallopian Tube Cells**

B. Saritas-Yildirim. US FDA, Silver Spring, MD. Sponsor: P. Goering

Nitinol, the nickel-titanium alloy, is the major constituent of multiple medical devices including cardiovascular and reproductive stents. The cardiovascular devices have been used routinely with minimal adverse clinical effects; however, the nitinol-based devices inserted in female reproductive tracts (i.e., fallopian tubes) cause severe inflammatory and hypersensitivity responses. The reason why the reproductive tissue

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Other studies have detected TiO\textsubscript{2}-NP in the ovary, demonstrating that as well. More studies are needed. Antral follicles toxicity for each NP. These effects may be related to steroidogenic follicular internalization followed by cell death. The internalization of nickel is a function of exposure time and concentration with significant uptake observed at even the lowest concentration (0.25 mM) and the shortest exposure time (5 hours). This is linearly correlated to programmed cell death because the membrane flipping, studied with Annexin V antibody and flow cytometry, increased with nickel internalization. Expression of follicular lineage specific genes was unchanged during 5h exposures; however, majority of genes including PAX8, WDFD2, CP, CLDN3, KRT7, and MSLN were significantly down regulated (75-90%) with increasing concentrations in 24-hour exposure. Thus, the data indicate that nickel toxicity in fallopian tube cells is dependent on nickel release and that CP and PAX8 expression might serve as diagnostic biomarkers for hypersensitivity reactions.

### 1796 Effects of TiO\textsubscript{2} and ZnO Nanoparticles on Ovarian Antral Follicle Morphology and Growth


Nanoparticles (NP) are widely used because of their novel properties. NP properties and biological effects can be modified by characteristics such as the core material, size, charge, among others. Zinc oxide nanoparticles (ZnO-NP) and titanium dioxide nanoparticles (TiO\textsubscript{2}-NP) are used in several personal care products. Some studies have found that ZnO-NP and TiO\textsubscript{2}-NP exposure decrease hormone levels and pregnancy rate. Other studies have detected TiO\textsubscript{2}-NP in the ovary, demonstrating that this NP is able to circulate in the bloodstream and reach the female gonad. However, knowledge on the toxic effects or the mechanisms in the female reproductive function is limited. Follicle microenvironment closely relates to alterations on the oocyte quality. Ovarian follicles are responsible for steroidogenic hormone production and growing, housing and promoting competence of the oocyte to release a competent oocyte into the oviduct. This work aimed to evaluate ovarian follicle growth and morphology alterations after TiO\textsubscript{2} and ZnO NP exposure. Agglomeration can modify some NP properties, which can determine their transport to the ovary. Thus, we established a dispersion of the nanoparticles in water (stock suspension) and characterized their hydrodynamic diameter (D\textsubscript{h}) and zeta potential (ζ) in culture medium for both NP. Antral follicles from fresh C57BL/6J mouse ovaries were cultured for 7 days in groups: 1) control (culture medium), 2) low NP concentration (5 μg/mL), and 3) high NP concentration (50 μg/mL). Follicle growth was recorded during 7 days of culture by measuring follicle size in two perpendicular axes, and using an inverted microscope with a calibrated ocular micrometer. Follicular morphology was assessed on images captured on days 0, 3, and 7 of culture. Our results suggest that NP form agglomerates in culture medium with sizes ranging from 118 to 538 nm for TiO\textsubscript{2}-NP and 25 to 311 nm for ZnO-NP, having potential zeta values in the -14.6 to -10.0 mV range. Follicular growth was delayed by the high ZnO-NP concentration, whereas the follicular morphology was altered by the high TiO\textsubscript{2}-NP concentration, suggesting different mechanisms of toxicity for each NP. These effects may be related to steroidopgenic follicular activity and oocyte development quality alterations. Antral follicles can be affected by NP exposure, and normal functions can be damaged as well. More studies are needed.

### 1797 Environmentally Relevant Doses of Di(2-Ethylhexyl) Phthalate (DEHP) Increases the Rate of Arrest in Cell Divisions of the Zygote

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Di (2-ethylhexyl) phthalate (DEHP) is an endocrine disrupting chemical used as a plasticizer that has been related to numerous pathologies, including a negative influence in the high percentage of infertility. High doses of DEHP have been associated with detrimental effects on oocyte maturation, zygote development and implantation failures; however, the relevant doses of environmental chemicals are unknown. This study was aimed to determine the effect of environmentally relevant DEHP doses on zygote development using a murine model. Female CD1 mice older than 3 to 4 weeks with vaginal opening were administered orally with 0, 20, 200, or 2000 μg DEHP/kg of body weight (bw)/day using tocopherol-free gel as vehicle. Treatment was extended with four estrous cycles (15-20 days), from estrus to estrus. The highest dose is equivalent to an occupational exposure, whereas the intermediate and lower doses are equivalent to an environmental exposure. After the dosing period, females were induced to superovulate by injecting ip 5 IU pregnant mare serum gonadotropin (PMSG), followed by 5 IU human chorionic gonadotropin (hCG) 48 h later. After hCG injection, females were mated with a non-exposed male of proven fertility, and then sacrificed at different times to obtain zygotes in specific cell divisions, such as 1-, 2-, 4- cells, morula and blastocyst. The data showed no significant differences in estrous cycle length, however, the number of zygotes was significantly decreased by DEHP treatment at each stage of development (zygote from 1 cell to blastocyst), whereas the dose of 200 μg/kg bw/day increased cellular division arrest at the late stages of zygotic development (Morula and blastocysts). Taken together, these data suggest that an environmental dose of DEHP (200 μg/kg bw/day) may impair zygote development, but higher doses may also impair earlier events such as ovulation and fertilization.

### 1798 Mono-(2-ethylhexyl) Phthalate (MEHP) Reversely Perturbs Blood Testis Barrier (BTB) in Pre-Pubertal Rats

R. Tiwary, University of Texas at Austin, Austin, TX. Sponsor: J. Richburg

The blood testis barrier (BTB) constituted by tight junctions between Sertoli cells creates an unique microenvironment for developing spermatocytes, is a well-studied target of numerous environmental toxicants. MEHP is the active metabolite of a widely used plasticizer (DEHP) in commercial products and has been recognized as a reproductive toxicant. Here, the influence of MEHP on BTB integrity is described as well as the signal transduction pathways that underlie this effect. Treatment of Post Natal Day (PND) 27 rats with 700 mg/kg MEHP for 24 hours perturbed the BTB integrity as indicated by a biotin tracer assay. Additionally, MEHP treatment induced transient surge of p44/42 Mitogen Activated Protein Kinase (MAPK)-JNK1/2, p38 and ERK protein kinases, possibly instigated via the observed enhanced expression levels of pro-inflammatory cytokines-IL-6 and TNFα as indicated by qPCR analysis. We further investigated that MEHP treatment of PND 27 rats with 700 mg/kg MEHP followed by a recovery period of 5 weeks could reverse the BTB disruption. Taken together, these findings indicate a role for the MAPK pathway in instigating the disruption of the BTB disruption after MEHP exposure; although this effect on the BTB was reversible.

### 1799 Effects of Developmental Exposure to Bisphenol AF on Sprague Dawley Rats

M. Bharadwaj, J. Warmack, and S. E. Fenton, NIEHS, Research Triangle Park, NC.

Bisphenol AF (BPAF) is a fluorinated analogue of Bisphenol A (BPA). It is used in the manufacturing of common consumer products. The estrogenic activity of BPA has been well characterized but little is known regarding the effects of its analogues in use, such as BPAF. This study determined endocrine-related health outcomes in Sprague Dawley rats offspring following early-life exposure to BPAF. Timed-pregnant dams were randomly assigned to 6 groups (Vehicle control (VC): n = 16, 2 groups; BPAF: n = 8 or 9, 4 dose groups). Dams were gavaged, twice daily starting on gestation day (GD) 6 through postnatal day (PND) 21 in

#### References


groups that were blindly allocated: 0, 0.25, 1.0, 2.5, or 25.0 mg BPA/kg body weight (BW). Dams were weighed and dosing was adjusted daily. The VC was 5 mL refined sesame oil/kg BW. Litter size, pup BW, and live/ dead count were recorded on PND 1. Litter sizes were equalized on PND 4 (n = 8; −4 M:4 F) and BW was recorded until PND 21. On PND 4 and 21, one pup/sex and their respective dams (PND 21) were euthanized and biological samples were collected for analysis of metabolic and internal BPA dose. All remaining offspring were evaluated for pubertal timing. The dam’s percent BW gain during gestation was significantly lower in the highest dose group (p < 0.01) compared to controls. The gestational length was not significantly different from VC. There was no significant loss of fetuses during gestation (gestation loss = total implantation sites − total pups born). The loss of pups from birth until PND 4 (partition loss) was elevated in the highest dose group (18.9 ± 7.7 vs 45.8 ± 14.6%; p = 0.14). Regardless of sex, the pup BW at birth and PND 4 in the highest dose group were significantly lower than those from VC dams (p = 0.02 and 0.01; respectively). However, the pup BW at PND 21 did not show significant changes at any of the dose levels, compared to VC. The age of vaginal opening (VO) for exposed females was not significantly different from the pups born from VC dams but the BW at VO in the highest dose group was significantly lower (p < 0.01) than VC pups. Genotype effects on VO were not observed. The overall significant effects on body weight and elevated pup loss may be related to the potential disruption of normal hormonal activities. Further studies are warranted to pinpoint the exact timing and alterations of specific gene signaling pathways that may be associated with these phenotypes.

1800 A Mouse Population-Guided Approach to Identify Genetic Loci That Potentially Modulate TCDD-Mediated Gene Dysregulation in the Uterus

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2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is a ubiquitous environmental contaminant that causes a number of disease states, such as metabolic syndrome, cancer, and embryo toxicity. Most, if not all, of these toxicities are mediated through activation of a PAS-superfamily transcription factor called the aryl hydrocarbon receptor (AHR). Little is known how multiple exposures ultimately lead to TCDD-induced pathologies. Here, we used a population-based approach to identify potential genetic modifiers that may affect susceptibility to TCDD-mediated gene expression in the uterus of pregnant mice. In specific crosses (males x females), 54 genetically diverse strains of mice were dosed with 0, 1, or 100 ng/kg/day of TCDD for the first 10 days of pregnancy. Following exposure, total mRNA was extracted from the uterus and the expression of 9 AHR-target genes was assessed. Interestingly, though the 14 strains of mice varied in the AHR allele they carried, the expression of several AHR-target genes, such as cytochrome P450 1A1, did not follow the predicted pattern how such strains differ. The gene expression results were used to scan the genome for quantitative trait loci (QTL) to identify genetic modifiers that drive strain-specific expression patterns. Statistically significant QTL peaks (p<0.05) were found to overlap across multiple AHR-target genes on three chromosomes (1, 9, and 13). The overlap in significant peaks may indicate novel genetic modulators involved in driving inter-strain differences in AHR-mediated gene transcription in the uterus. Furthermore, these results may help identify genetic variants within the human population that may be at higher risk for TCDD-mediated toxicity.

1801 Chemical-Induced Facial Dismorphogenesis: A Modeling Approach to Help Understanding of the Mode-of-Action

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Oral clefts are multifactorial in origin, involving both genetic and environmental risk factors during the first trimester of pregnancy, but specific causes of mostly are unknown. The identification of the target of the different chemicals contributing to cranio-facial morphogenesis is a key step to identify the altered morphogenetic pathways and to evaluate the contribution of multiple exposures to the development of such defects. The post-implantation rat whole embryo culture (WEC) is a validated toxicological method proposed also for modelling approaches. The aim is to fit the experimental data from WEC exposed to facial morphogens, by using PROAST software, in order to model the experimental data and ascertain whether the different molecules could act via the same pathogenic pathway. The chemicals selected were: retinoic acid (RA), ethanol (EtOH), and azole antifungals (the human drug fluconazole, FLUCO and the antiprotozoal dihydrofuran, FON) and anti-epileptics (valproic acid, VPA). All selected molecules induce branchial arch defects in WEC. RA was chosen as index compound considering that all the other selected molecules are suspected to interfere with RA signaling cascade. A syndrome similar to the one described after RA, EtOH and furosemide (an anti-diuretic response exposure) was selected: the affected districts were the encephalon, the facial primordia and the auricles (branchial apparatus) and the tail. By contrast, the exposure to azoles affected only the branchial arches. The PROAST analysis was applied only on branchial outcomes, the common target for all the tested substances. Data were modelled in order to obtain the different dose-response curves (Hill model) and to evaluate the steepness confidence intervals. As the parallelism hypothesis was not rejected, data were analysed by using the exponential model normalizing the potency of each compound to that of RA. Dosages were then transformed in RA-equivalents and data was analysed dataset by dataset the data passed the goodness-of-fit test and, therefore, the assumption of a similar mode of action for the different molecules cannot be rejected. The results of the present work are consistent with the hypothesis that a common morphogenetic pathway is affected by the exposure to the tested chemicals triggering the observed craniofacial defects. Supported by H2020 Euromix.

1802 MEHP-Induced Germ Cell Apoptosis

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The testis is an immune-privileged organ that maintains an immune suppressive environment with few leucocytes in the testicular interstitium. We have previously shown that exposure of peripubertal (postnatal day) (PND) 28 Fischer rats to an acute dose of mono(2-ethylhexyl) phthalate (MEHP), a well-described Sertoli cell toxicant, leads to an accumulation of CD11b+ cells in the interstitial space of the testis that closely correlates with a robust incidence of germ cell (GC) apoptosis. CD11b is expressed on the outer membrane of many leucocytes of the innate immune system, including monocytes, macrophages, and granulocytes. Here we test the hypothesis that the infiltrating immune cells contribute to GC cell death. PND 28 Fischer rats that received 100 mg/kg MEHP showed a significant CD11b+ immune cell infiltrate consisting of both CD68+ cells, a monocyte and macrophage marker, and neutrophils, identified by morphology and the expression of myeloperoxidase. The numbers of both cell types peaked at 12 hours, but by 48 hours, the numbers of infiltrating GCs had returned to baseline. An immunofluorescence analysis of the testicular macrophages of MEHP-treated rats showed significantly upregulated expression of Tnfα and iNOS, and the Arg1/Nos2 ratio was reduced compared to macrophages from the interstitium of control-treated tests, although small increases in Il10 and Tgfβ1 were observed too. Depletion of circulating monocytes with Gd2+ liposome phosphate prior to MEHP-treatment did reduce the macrophage influx into the testis, but did not lower germ cell apoptosis. Additionally, depletion of neutrophils using an anti-polymorphonuclear cells antibody prevented both macrophage and neutrophil infiltration into the testis, but also did not affect germ cell apoptosis. These results show that exposure to MEHP leads to a rapid and temporary influx of pro-inflammatory monocytes and neutrophils in the interstitium of the testis. However, with this dosing paradigm, they do not appear to exacerbate phthalate-induced injury to the testis and their functional significance remains unknown.

1803 Dog Testis Toxicity of Glucagon Receptor Antagonist Monitored Using Monthly Sperm Assessment, Followed by Recovery

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Microscopic effects on spermatogenesis were observed in the testis and epididymis with 3 months of dosing dogs at ≥150 mg/kg/day with PF-06291874, a glucagon receptor antagonist. No effects were observed in shorter duration dog studies or in any rat studies at similar doses. To better characterize the onset and reversibility of this toxicity in dogs, sperm from normospermic dogs were euthanized, and rats were sacrificed at multiple time points. Sperm were monitored monthly during a 9-month study with a 5-month recovery period. In the 9-month study, male beagle dogs were dosed once daily by oral gavage with 0, 10, 50, 100, or 500 mg/kg with 8, 5, 5, 8, and 11 male dogs per group (female dogs were also part of the study but are not described here). Five males per group were necropsied after 9 months...
and 3 males in the control, 100 and 500 mg/kg groups were necropsied after a 5-month recovery. Post-mortem evaluation included weights and microscopic evaluation of the reproductive organs. Semen from all animals was collected approximately monthly for automated evaluation of sperm motility, density, and total counts using a Hamilton Thorne IVOS sperm analyzer. The microscopic findings in the tests at the end of the dosing phase as recorded in the group exposed to CrVI were reduced sperm count, incidence and severity of multinucleated spermatocytes at ≥100 mg/kg and degeneration of spermatocytes at 500 mg/kg. Considered secondary to the testis damage, hyposperma and cellular debris were noted in the epididymis at ≥100 mg/kg. The monthly sperm analysis resulted in test article induced reductions in sperm density and no significant differences in sperm counts during the dosing phase at ≥100 mg/kg. These effects on sperm generally started during month 3 of the dosing phase and generally correlated with the microscopic findings in the testis and epididymis. Although 2 out of 3 recovery animals at 500 mg/kg indicated lower sperm counts and motility at the end of dosing, there were no male reproductive effects observed at the end of the recovery period, including recovery of sperm endpoints. In conclusion, serial sperm evaluation provided time-course information complementary to the terminal testicular toxicity observed, as well as a potential predictive biomarker of recovery in the testis and epididymis.

1804 Temephos Impairs Ovulation, Oocyte Fertilization, and Zygote Quality in an In Vivo Model
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Temephos is an organophosphate pesticide approved by the World Health Organization (WHO) to combat vectors (Aedes aegypti) that transmit diseases, such as dengue virus and zika, and it is classified as a pesticide of low toxicity. In recent years, the toxic effects of temephos have been observed as a result of studies showing cytotoxic effects on human lymphocytes when using similar concentrations to those found in the environment. However, the potential reproductive toxicity of temephos is scarcely studied. The aim of this study was to evaluate the effect of temephos on oocyte fertilization and zygote quality. CD1 female mice received orally 15, 150 and 1500 μg/kg/day of temephos during 4 estrous cycles. To evaluate temephos toxicity, the enzymatic activity of acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) was assessed in blood and serum, respectively, by the Ellman method. At the end of exposure, females were mated with no-exposed males and 48 h later the 2-cell zygotes were collected. Our results showed that there were no significant differences in body weight gain of the treated females remained at each stage of the cycle were similar to those spent by the control group. The total number of released zygotes and the number of 2-cell zygotes were decreased in the 150 and 1500 μg/kg/day- treated groups compared to control. Furthermore, zygotes from the 150 and 1500 μg/kg/day doses had high rates of fragmented blastomeres compared to control. In conclusion, in vivo exposure to organophosphorous pesticide temephos impairs ovulation, fertilization and the zygote quality without altering AChE and BuChE activities. Supported by the Pesticide Toxicology Network-Conacyt No. 280945. [RESCM1]QUITA UNO.

1805 Vitamin C and Moringa oleifera Leaves Ameliorate Carbamazepine-Induced Reproductive Toxicity in Male Wistar Rats
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The use of antiepileptic drugs for chemotherapy has been popular for practices in decades. However, long time use of certain antiepileptic drugs such as carbamazepine (CBZ) may reduce sperm motility, induce sperm abnormalities and decrease testicular volume. The present study was aimed at evaluating the effect of vitamin C and Moringa oleifera (MO) in ameliorating adverse reproductive effect, induced by chronic CBZ administration in Wistar rats. Forty adult male wistar rats (150-250g) were randomly divided into 5 groups (8= each). Group I received distilled water (DW) at 2 ml/kg; Group II was administered vitamin C (100 mg/kg); Group III was administered Moringa oleifera (250 mg/kg), Group IV was administered CBZ only (20 mg/kg) while Group V was pretreated with vitamin C (100 μg/kg), MO (250 mg/kg) and exposed to CBZ (20 mg/kg) after 30 minutes. The regimens were administered orally once daily for 15 weeks. At the end of the treatment period, the rats were sacrificed and the pituitary glands and testicular tissues were assayed for Malondialdehyde (MDA) concentrations and Superoxide dismutase (SOD) and Catalase (CAT) activities. The serum was assayed for follicle stimulating hormone (FSH), luteinizing hormone (LH) and testosterone, epididymal sperm count and sperm motility was also determined. Data obtained were expressed as mean ± SEM and analyzed using ANOVA. The result showed that the significant increase in concentrations of pituitary and testicular MDA and a decrease in the activities of SOD and CAT exposed to CBZ were ameliorated by pretreatment with vitamin C and MO. There was significant increase in concentrations of testosterone level, while FSH and LH recorded no significant difference in the group pretreated with vitamin C and MO before exposed to CBZ. There was significant increase in epididymal sperm motility (P < 0.01) and no significant difference in the group pretreated with vitamin C and MO before exposed to CBZ. Our result indicates that vitamin C and MO ameliorate reproductive toxicity induced by chronic CBZ exposure due to their synergistic protective antioxidant activity as well as boosting the antioxidant enzyme production of the pituitary glands and the testes.

1806 Toxicity of Waltheria Indica Root
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The decreasing efficacy of anti-trypanosomal drugs and the narrow prospects of anti-trypanosome vaccine development in the nearest future have occasioned the search for plants with anti-trypanosomal potentials. Waltheria indica (Sterculiaceae) has been reported to have anti-trypanosomal, antimarial, antibacterial and antifungal effects. Most plants with antibacterial and anti-protozoan (including anti-trypanosomal) effects have been reported to adversely affect male fertility. However, there is dearth of information on the toxicological evaluation of this plant on the reproductive system. Hence, this work investigated the reproductive toxicity of Waltheria indica root extract in Wistar rats. Ethanolic extract of Waltheria indica root (200, 400 and 800 mg/kg) and distilled water (3 ml/Kg) were administered by gavage to 28 days to adult male rats (180-200 g, n=5), and thereafter sacrificed. Sperm motility, viability, count and morphology were examined microscopically. Serum from each rat was analyzed for Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH), Testosterone and prolactin by enzyme immunoassay technique. Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST) and Alkaline Phosphatase (ALP) were measured spectrophotometrically. Data were analyzed using one way analysis of variance (ANOVA) at 95% confidence interval. The result showed that Waltheria indica significantly decreased sperm motility and count and increased the percentage abnormal sperm cells. Serum FSH, LH and testosterone were also decreased. While the serum prolactin increased at the highest dose. Serum ALT, AST, LDL, HDL and total protein levels were not affected by Waltheria indica. While the cholesterol, triglyceride, urea and ALP were significantly increased at the highest dose. In conclusion, Waltheria indica root extract adversely affect the reproductive system in Wistar rats. Further investigation is ongoing to isolate the bioactive compound responsible for the reproductive toxicity and its effect on fertility specific genes such as CatSper and HongrES1 genes.

1807 Disorganization of Uterine Artery Remodeling during Pregnancy Due to Gestational Exposure to Chromium: Mediated through Matrix Distruption
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Hexavalent chromium (Cr VI) is a heavy metal environmental endocrine disruptor, used in a wide range of applications in the chemical industry, electroplating, steel alloys and stainless steels, as well as many other uses. Increased use and improper disposal of Cr in the developing countries and in the U.S. have increased Cr levels in the air, soil and drinking water. According to Environmental Working Group, Cr has been found in high levels in the drinking water sources from more than 34 states in US Women working in Cr industries and the Cr-contaminated area experience infertility problems and preterm abortions, and still birth. CrVI is rapidly transported inside the cell by anion transport mechanisms, and it leads to multiple cellular dysfunctions that include DNA adduct formation, deregulated cell cycle, apoptosis and carcinogenesis. The preterm delivery rates are 12-13% in the US, 5-9% in UK, and 11.9% in Africa. A variety of risk factors has been linked to preterm birth including medical conditions of the mothers/fetus, genetic influences, behavioral and socioeconomic factors and iatrogenic prematurity. Maternal exposure to environmental contaminations has been considered as an important contributor to preterm birth. We hypothesize that pre-natal exposure to chromium disorganized uterine artery remodeling by altering collagen, elastin, MMPs and TIMPs, as well as
Vinpocetine (VIN) is an antiandrogenic fungicide and endocrine disrupting compound reported to cause multigenerational phenotypic and epigenetic changes. However, mechanisms of perturbation and mediators of heritability remain unclear. Here, we assessed the influence of antiandrogenic activity by comparing in utero VIN exposure to the strongest antiandrogenic VIN metabolite, M2. We also assessed roles of CoA-dependent enzymes in vivo in primary rat, rabbit, human and mouse hepatocytes showed that conjugation of 4-alkyl benzoic acid to Coenzyme A (CoA) and subsequent inhibition of CoA dependent enzymes is the putative pathway leading to the reproductive effects seen in rats. Accumulation of CoA-conjugates for 4-isopropylbenzoic acid and related p-alkyl benzoic acids in plated rat hepatocytes correlates to male rat reproductive toxicity (n > 15). Studies on human plated hepatocytes provide evidence that this pathway is not relevant to humans where the accumulation of these CoA-conjugates is not seen for this class of compounds, indicating a strong species difference in metabolic fate. Additional investigation into the formation of 4-alkyl benzoic acid CoA conjugates in vivo in both rat liver and tests will provide further evidence as to this mechanistic action as well as additional studies to elucidate the direct pathway to interference with spermatogenesis. Consideration of the oral NOAEL in rats, evidence for low relevance of the rat metabolism to humans and topical exposure only from fragrance application provides for a high margin of safety in use. 

1809 Maternal Genetic Lineage and Crossing Scheme Determines Intergenerational Response to the Endocrine Disruptor Vinpocetine


Vinpocetine (VIN) is an antiandrogenic fungicide and endocrine disrupting compound reported to cause multigenerational phenotypic and epigenetic changes. However, mechanisms of perturbation and mediators of heritability remain unclear. Here, we assessed the influence of antiandrogenic activity by comparing in utero VIN exposure to the strongest antiandrogenic VIN metabolite, M2. We also assessed roles of CoA-dependent enzymes in vivo in primary rat, rabbit, human and mouse hepatocytes showed that conjugation of 4-alkyl benzoic acid to Coenzyme A (CoA) and subsequent inhibition of CoA dependent enzymes is the putative pathway leading to the reproductive effects seen in rats. Accumulation of CoA-conjugates for 4-isopropylbenzoic acid and related p-alkyl benzoic acids in plated rat hepatocytes correlates to male rat reproductive toxicity (n > 15). Studies on human plated hepatocytes provide evidence that this pathway is not relevant to humans where the accumulation of these CoA-conjugates is not seen for this class of compounds, indicating a strong species difference in metabolic fate. Additional investigation into the formation of 4-alkyl benzoic acid CoA conjugates in vivo in both rat liver and tests will provide further evidence as to this mechanistic action as well as additional studies to elucidate the direct pathway to interference with spermatogenesis. Consideration of the oral NOAEL in rats, evidence for low relevance of the rat metabolism to humans and topical exposure only from fragrance application provides for a high margin of safety in use. 

1808 Explaining the Low Relevance of the Rat Reproductive Toxicity Data on 3-(4-Isopropylphenyl)-2-Methylpropaln to Human Metabolism and Exposure

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3-(4-Isopropylphenyl)-2-methylpropanol (Cyclamen Aldehyde) is a substance used widely in fragrances for a broad range of consumer products. An extended 1-generation reproductive toxicity study in rats (OECD 415) via oral gavage was conducted at 0, 25, 75 and 150mg/kg bw/day. This study showed an increase in epididymal weights at 150 mg/kg/day, a decrease in sperm motility at 275 mg/kg/day, decreased sperm count and density at 75 mg/kg/day and moderate to marked sperm granulomas at 150 mg/kg/day in F generation males. The NOAEL for reproductive toxicity was 25 mg/kg/day. Similar effects were also seen in a short-term (14 day) oral gavage study in male rats. Further investigation has revealed that 3,4-isopropylbenzaldehyde, responsible in vitro for the reproductive effects seen, is consistent with data on other structurally similar substances, notably 4-tert-butyl benzaldehyde. An in vitro metabolism study comparing the ability to produce this metabolite in primary rat, rabbit, human and mouse hepatocytes showed that 4-alkylbenzoic acid was only detectable in rat hepatocytes which indicates that this toxic metabolite is specific to rats. Human liver metabolism in vitro was found to be closest to rabbits and a confirmatory study was conducted. A 14-day study in rabbits via oral gavage at 0, 30, 100, 300mg/kg/day showed no effects either on male reproductive organs or on sperm parameters. Mechanistic work shows that conjugation of 4-alkyl benzoic acid to Coenzyme A (CoA) and subsequent inhibition of CoA dependent enzymes is the putative pathway leading to the reproductive effects seen in rats. Accumulation of CoA-conjugates for 4-isopropylbenzoic acid and related p-alkyl benzoic acids in plated rat hepatocytes correlates to male rat reproductive toxicity (n > 15). Studies on human plated hepatocytes provide evidence that this pathway is not relevant to humans where the accumulation of these CoA-conjugates is not seen for this class of compounds, indicating a strong species difference in metabolic fate. Additional investigation into the formation of 4-alkyl benzoic acid CoA conjugates in vivo in both rat liver and tests will provide further evidence as to this mechanistic action as well as additional studies to elucidate the direct pathway to interference with spermatogenesis. Consideration of the oral NOAEL in rats, evidence for low relevance of the rat metabolism to humans and topical exposure only from fragrance application provides for a high margin of safety in use.
1812 The Role of Aryl Hydrocarbon Receptor (AHR) Signaling in Hemochorial Placentation
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AHR is a cytoplasmic ligand-dependent transcription factor controlling the biological responses to environmental pollutants. Upon binding, pollutants such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), AHR translocates into nucleus and activates transcription of AHR target genes such as Cyp1a1. The hemochorial placenta serves an essential role in fetal health and is potentially susceptible to environmental pollutants. The purpose of this study was to explore how exposure to TCDD acting through AHR shapes placental development and to determine site(s) of TCDD action. Pregnant rats were exposed to TCDD at gestational day (gd) 6.5 and placentaation sites were collected at gd 13.5. TCDD exposure resulted in a delay in upregulation of Cyp1a1 expression. Our data suggest a potential role of CYP1A1 in gd 13.5 placentaation sites revealed activation of AHR signaling in decidua/maternal gland and in mesenchymal components of the labyrinth zone but not the junctional zone of the chorioallantoic placenta. Compared to oil treated controls, TCDD treated gd 13.5 placentaation sites exhibited deep intrauterine trophoblast invasion characterized by cytokeratin positive endovascular trophoblast. We next generated an Ahr null rat model that failed to express AHR and to induce CYP1A1 enzyme expression following TCDD exposure. As a first step in determining the site(s) of TCDD actions, we evaluated the effects of TCDD administration during pregnancy in wild type females mated with wild type males, Ahr null females mated with Ahr null males, and Ahr null females mated with wild type males. Mating schemes that resulted in disruption of AHR activity in maternal tissues interfered with TCDD-activated placental site adaptations. Additionally, to explore the role of CYP1A1 in TCDD-activated placental adaptations we generated Cyp1a1 null rats using CRISPR-Cas9 genome editing. Collectively, these findings indicate that at least some of the TCDD effects on placental development are mediated through its actions on the mother. In summary, we have identified a developmental window of sensitivity to environmental pollutants affecting hemochorial placentaation, the potential of impacting fetal and postnatal health. Supported by NIH grants HD020676, HD079363; Sosland Foundation.

1813 Estrogenic Activity of Polycyclic Aromatic Hydrocarbon Ortho-Quinones in Human Endometrial Cells

Polycyclic aromatic hydrocarbons (PAHs) are pervasive byproducts of incomplete combustion of organic materials, including fossil fuels, food, and tobacco. Cigarette smoking is associated with reproductive abnormalities in women and some PAHs are endocrine disruptors. Moreover, PAHs or their metabolites can activate estrogen receptors (ER), resulting in endocrine disruption. In one pathway of PAH activation, aldo-keto reductases (AKRs) convert PAH trans-dihydrodiols into PAH ortho (o)-quinones, which are then shuttled into the nucleus by the aryl hydrocarbon receptor to modulate gene expression. Given the similarity between planar PAH o-quinones and estrogen (ER), we hypothesize that PAH o-quinones can bind and activate ERs in estrogen target tissues e.g. endometrium. This activation may modulate ER-target genes leading to cell proliferation. We tested the estrogenicity of 3 PAH o-quinones (benzo[a] pyrene-7,8-dione (BPQ), benz[a]anthracene-3,4-dione and 5-methyl-chrysene-1,2-dione) in endometrial cells. We used the inducible alkaline phosphatase activity in Ishikawa cells, a human endometrial adenocarcinoma cell-line, as the read-out for ER activation. We demonstrated that these compounds induce ER activity, and that this activation is inhibited by Fulvestrant, an ER antagonist. Western blots showed that both 17α-ethynyl estradiol (E2) and BPQ induce the translocation of ERα into the nucleus to modulate gene expression. We also found that benzo[a]pyrene (BaP), upregulates AKR1C1 and 1C3 at the mRNA, protein and functional level which would be responsible for PAH metabolism, using high performance liquid chromatography and APCI mass spectrometry in the selected reaction monitoring mode, we find that BaP and benzo[a]pyrene-7,8-dihydrodiol are metabolized to the estrogenic BPQ in Ishikawa cells. Low micromolar concentrations of BPQ increase Ishikawa cell proliferation to the same level observed with the highest concentrations of E2. Our data indicates that o-quinones may play a role in the disruption of ER signaling in the endometrium most likely through ERα. Supported by P30-ES013508 and T32-ES019851.

1814 Machine Learning-Based High-Content Analysis to Characterize Phenotypes Associated with the Reproductive Toxicity of Bisphenol A, Bisphenol S, Bisphenol AF, and Tetrabromobisphenol A in a Testicular Cell Co-Culture Model
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High-content analysis (HCA), an image-based quantitative cellular analysis, has emerged as a powerful tool for the assessment of chemical toxicity. We previously developed a multi-parametric HCA to examine the testicular toxicities of Bisphenol A (BPA) and its analogs, bisphenol S (BPS), bisphenol AF (BPAF), and tetrabromobisphenol A (TBBPA). Due to the complexity and the richness of high-dimensional HCA data, there are increasing demands for effective computational strategies to characterize and quantify phenotypic effects at a single-cell level. Our previous study established the testicular co-culture model, comprising spermatogonial cell, Leydig cell, and Sertoli cell lines, has been shown informative for assessing cellular phenotypes across the various timescales and treatment doses at single-cell based level. Our machine learning based HCA pipeline to explore complex phenotypic changes, and characterize the toxicity of BPA and its analogs in this co-culture model. Utilizing machine learning-based phenotypic analysis, we observed the treatments of BPA or its analogs resulted in the loss of spatial arrangement in the three-dimensional structure of the cytoskeleton, and induction of G2/M phase arrest in a dose- and time- dependent manner. Specifically, BPAF significantly induced multinucleated cells, which was associated with an increase of DNA damage response and impaired actin filament structure. In combination, BPA and its analogs induced different toxicities on multiple endpoints in the co-culture model, and BPAF exhibited the highest toxicity compared to TBBPA, BPA and BPS. In summary, this finding suggests that the HCA integrated machine learning algorithm powers the traditional HCA approach, and quantitatively examined the differences of cellular phenotypes across the various timescales and treatment doses at single cell-based level. Our machine learning based HCA approach provides a deeper insight into the cellular dynamics and molecular complex within an in vitro testicular co-culture model. Supported by R43 ES027374-01 funding.

1815 The Endocannabinoid Anandamide Inhibits the Fetoprotective Activity of the Placental Efflux Transporter BCRP/ABC2

The breast cancer resistance protein (BCRP/ABC2), an efflux transporter highly expressed in placental syncytiotrophoblasts that protects the fetus from exposure to a wide range of potential developmental toxins including zearalenone, zeranol, bisphenol A, and perfluorooctanoic acid. Anandamide (AEA), a lipid signaling molecule and an endogenous cannabinoid, has been shown to play a critical role in placental development and embryo development. Dysregulation of the endocannabinoid system has been implicated in gestational disorders such as spontaneous miscarriage, preeclampsia, and intrauterine growth restriction. While excess AEA has been shown to inhibit syncytialization of trophoblasts, its influence on placental transporter function has not yet been investigated. Here, we sought to identify novel mechanisms underlying placental BCRP regulation by AEA. To test this, human trophoblast BeWo B30 cells were treated with AEA and analyzed for biomarkers indicative of syncytialization and BCRP expression and activity. Treatment with AEA (0–10 μM, 3–24 h) reduced the basal syncytialization of BeWo cells, as evidenced by a 40-50% decrease in HCGβ and synectin-2 mRNAs. AEA (10 μM, 24 h) also reduced BCRP mRNA and protein expression between 35 and 50%. Down-regulation of BCRP mRNA was blocked by forskolin, an activator of adenylate cyclase, and 8-Br-CAMP, a cAMP analogue, as well as AM630, an inhibitor of the CB2 receptor. AEA (10 μM, 24 h) also reduced efflux of BCRP substrates mitoxantrone and Hoechst 33342. AEA (10 μM, 24 h) also inhibited adenylate cyclase activity as evidenced by a 75% reduction in intracellular cAMP levels and a complete inhibition of forskolin-induced phosphorylation of the cAMP response element binding protein (CREB). Syncytialization of placental trophoblasts and placental transporter function is critical in protecting the embryo from toxic insults. Our data indicate that the endocannabinoid AEA plays a mechanistic role in down-regulating placental transporter expression and activity via CB2-CAMP signaling, which may be responsible for disrupting placental barrier function during gestational disorders. Supported by R01ES20522, T22ES007148, and P30ES005022.

2018 SOT Annual Meeting
Epilepsy afflicts an estimated 65 million people worldwide and roughly 30% of patients have drug-resistant epilepsy, such as Dravet syndrome (DS), wherein seizures are not adequately controlled with existing antiepileptic drugs. DS occurs in roughly 1:16,000 individuals in the United States, most of which are diagnosed as infants. Patients often are desperate for treatment due to DS's drug-resistant characteristics; therefore, many turn to alternative medicines, such as cannabidiol (CBD). The most abundant Cannabis constituent, Δ9-tetrahydrocannabinol (THC), has been studied widely in the realm of developmental and reproductive toxicology; however, few studies have focused on CBD's toxicity. The goals of this project are to assess gene expression patterns, behavior phenotypes, and reproductive fitness of a subsequent F1 generation following an F0 developmental exposure to concentrations of THC or CBD below the lowest observed adverse effect level (0.024, 0.12, 0.6 mg/L (0.08, 0.4, 2 μM) THC, 0.006, 0.03, 0.15 mg/L (0.02, 0.1, 0.5 μM) CBD, or 0.005% DMSO control in zebrafish. During key developmental stages, THC and CBD caused differential expression of c-fos, bdnf, and dazl in the F0 generation, while only CBD differentially expressed dazl in F1 larvae at 48 and 96 hours post fertilization (hpf). In undosed F1 larvae, THC reduced F0 ovarian activity of 96 hpf larvae during light/dark cycles. Lastly, THC (0.024 and 0.12 mg/L) and CBD (0.15 mg/L) significantly reduced the number of F0 offspring, but no effect was observed on fertilization or hatching rate in the F1 generation. Behavioral assessment of F0 and F1 adults for cognitive impairment following developmental exposure to THC or CBD are underway. This research highlights the need for further understanding of the possible ramifications from early-life cannabinoid exposure.

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Cannabinoid Multigenerational Developmental and Reproductive Toxicity in Zebrafish

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Mycoxin Zearalenone (ZEA) Induces Toxicity and Alters miRNA Expression in C57Bl/6 Mouse Placenta

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Found on grain crops and grasses all over the world, zearalenone (ZEA) is a major mycoxin derived from Fusarium fungi. With potential chronic exposure, ZEA poses a great risk to the general health of both animals and humans. ZEA in feed causes reproductive disorders in domestic animals such as swine. Previously we demonstrated that ZEA (20-40 ppm) impaired female fertility in mice. Since the placenta plays an essential role in female fertility and ZEA can be metabolically activated by placental enzymes, ZEA may have adverse effects on placental development leading to impaired female fertility. To determine the effects of ZEA on placental development, young virgin female mice were mated with CD-1 males and checked for a vaginal plug the next morning. On D5.5 (post-implantation), the placenta was randomly assigned into five groups and fed with 0, 0.8, 4, 10, and 40 ppm ZEA diets. Body weight was recorded daily. Mice were dissected on D13.5. There was an increased rate of implantation site resorption in 40 ppm group, placental hemorrhage in 10 and 40 ppm groups, reduced weight of live fetus in 40 ppm group, and reduced weight of placentas with live fetus in 40 ppm group. Placental histology indicates dose-dependent changes in the labyrinth layer, most dramatically in the 40 ppm group, such as dilated fetal capillaries filled with nucleated fetal blood cells and dilated maternal blood spaces filled with enucleated maternal blood cells. These data indicate disorganized placental labyrinth upon ZEA treatment resulting in insufficient fetal-maternal interface for efficient nutrient and gas exchange, which may count for the reduced fetus weight in the 40 ppm ZEA-treated group. ZEA treatment also led to lipid accumulation in the labyrinth layer of D13.5 placentas. Placental cells have unique regular epigenetic profiles that plaque placental functions. Disrupted epigenetic profiles may lead to defective placental development. Our microRNA (miRNA) array analysis on the above D13.5 placentas in the 0, 4, and 40 ppm groups (N=4/group) reveals a unique placental miRNA profile and a set of ZEA-targeted placental miRNAs. Interestingly, several of the differentially expressed miRNAs were dysregulated in the placentas from patients with preeclampsia. We continue investigating cellular and molecular mechanisms of ZEA-induced placental toxicity.

Mycoxin Zearalenone (ZEA) Induces Toxicity and Alters miRNA Expression in C57Bl/6 Mouse Placenta

C. Andersen1, R. Li1, L. Hu2, A. E. El Zowalaty1, Z. Wang1, and X. Ye1, University of Georgia, Athens, GA; and 2South China Agriculture University, Guangzhou, China.

Pollutant-Induced Changes in the Ryanodine Receptor and the L-Type Voltage Gated Ca2+ Channel Alter DREAM-Mediated Gene Transcription

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Environmental pollutants, including non-co-planar polychlorinated biphenyl (ncPCB) congeners and triclosan, cause cellular Ca2+ signal disruptions (GDT) by altering the expression of the ryanodine receptor (RyR) or the L-type voltage gated Ca2+ channels (CaV1). These channels are important to numerous Ca2+ signaling pathways within the cell, but the extent to which CSD through RyR and CaV1 contribute to altered cellular function is unclear. We investigated whether CSD can alter gene transcription normally regulated by the Ca2+-sensitive transcriptional repressor downstream regulator element antagonist modulator (DREAM). This research utilized GTI-7 hypothalamic neuronal cells, TaT1 thyrocytic cells, and GH3 anterior pituitary cells to assess whether CSD alters transcription of gonadotropin releasing hormone (GnRH), thyroid-stimulating hormone (TSH), or prolactin (PRL), respectively, all of which are regulated by DREAM. Cells were exposed to varying concentrations of pollutants for multiple time-periods, and GnRH, TSH or PRL levels assessed using qPCR. GTI-7 cells exposed to the potent RyR activator PC-9B5 did not alter GnRH mRNA expression, which was supported by low RyR basal gene expression. In contrast, GnRH expression in GH3 cells tended to increase with increasing PC-9B5 concentrations at three hours. It is predicted that cellular exposure of TaT1 cells to PC-9B5 will increase TSH transcription in a dose-dependent manner. GTI-7 cells exposed to triclosan, a CaV1 inhibitor, decreased GnRH transcription in a dose-dependent manner and after just three hours. When CaV1 levels are inhibited or when intracellular Ca2+ concentrations are decreased, DREAM remains bound to DNA, repressing transcription, which is consistent with reduced GnRH transcription in the presence of triclosan. This work helps address whether CSD is contributing to alterations in DREAM-mediated transcription, which has been implicated in the function of the digestive system, central nervous system, and tied to pain reception, learning and memory and thyroid-gland health.
Traditional in vitro breast cancer models evaluate estrogenic compounds on breast cancer cells cultured on a flat plastic surface, and exclude the matrix proteins, tissue structure, and supportive cell types of the mammary microenvironment. Our objective was to develop a physiologically relevant in vitro breast model, characterize how the tumor microenvironment influences model sensitivity, and validate the model for chemical testing. Our base platform consists of a MCF7-derived duct surrounded by a collagen I matrix. As previous studies found that fibroblasts regulate the expression of epithelial estrogen receptor α (ER), we integrated human mammary fibroblasts (HMFs) into the matrix surrounding the duct and evaluated sensitivity to 17β-estradiol (E2). Consistent with previous studies, fibroblasts increased ER transactivation and reduced ER protein. Histological examination revealed that E2-induced hyperplasia occurred 7 days earlier when fibroblasts were included in the matrix. We found this was due to a reduction in MCF7 apoptosis, as there was reduced expression of cleaved caspase-7 protein as well as caspase-7 mRNA in our co-culture model. Next, we evaluated the sensitivity of our co-culture platform by exposing the model to 100 nM of 5 chemicals with varying levels of estrogenic activity: E2, estrone, genistein, bisphenol-A and fenamilo. Exposure to test chemicals significantly upregulated ER transactivation and increased expression of proliferation marker Ki67. Exposure to ER antagonist fulvestrant inhibited these effects. In summary, this study provides insight into how the tumor microenvironment regulates estrogenic response, and introduces a novel platform for testing estrogenic compounds in vitro.

1821 Adipogenic Activity of House Dust Extracts
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Obesity contributes to >$200 billion in annual US health care costs, and also promotes increased risks of other common health problems, chemicals, termed “obesogens”, can increase weight gain in animal models and/or triglyceride accumulation in vitro. House dust contains a wide diversity of chemicals, and we’ve previously demonstrated that dust extracts can disrupt nuclear receptors known to regulate adipogenesis (proliferation and growth of fat cells). As such, we sought to assess the extent that dust extracts could promote triglyceride accumulation and/or cell proliferation in cell culture. House dust samples (n=137) were collected from central NC households. Briefly, the main living area was vacuumed and dust was extracted with organic solvents and evaporated/reconstituted in DM50. 3T3-L1 cells were induced to differentiate and dosed with extracts over ten days, and assayed for triglyceride accumulation (normalized to max rosiglitazone (RSG)-induced response), DNA content, and cell viability. Eighty-five of the 137 dust extracts tested (62%) exhibited significant triglyceride accumulation, with 43 exhibiting >40% relative to the positive control, RSG. Twenty elicited effects at <10 μg dust/well, and 17 exhibited >100% of the maximal RSG response. Ninety-eight (72%) of the dust extracts elicited significant cell proliferation, with 12 exhibiting increases of >40% relative to vehicle controls. Sixty-three elicited effects at concentrations <1.0 μg/well, and two exhibited >80% proliferation. These data highlight the ability of house dust extracts to induce both triglyceride accumulation and pre-adipocyte proliferation at environmentally relevant levels, below the 50 mg of dust that a child is estimated to consume daily (EPA Exposure Handbook). Concentrations of several halogenated and organophosphate flame-retardants were measured in these samples and most were correlated with triglyceride accumulation, though few were independently active, suggesting that mixtures or co-occurring compounds were promoting the majority of the activity. In addition, adipogenic activity was positively correlated with serum thyroid stimulating hormone concentrations and negatively correlated with free triiodothyronine (T3) and thyroxine (T4) in matched adult household residents. This suggests that mixtures of contaminants in the house dust extracts may be disrupting molecular mechanisms that promote fat cell development and disrupt thyroid hormone homeostasis in residents.

1822 In Vitro Bioactivity of Source and Treated Water from Seven Drinking Water Treatment Plants along a Swedish River

Surface waters are contaminated with chemicals from multiple sources, including industry, agriculture, wastewater effluents, and natural toxicants. In Sweden 50% of the drinking water supply comes from surface water, which implies high demands on inlet water quality and drinking water treatment plant (DWTP) efficiency. Chemical analysis is not always sufficient to detect hazardous compounds and in vitro bioassays are useful tools to monitor potential toxic activities of the total mixture of chemicals in a water sample. The objective of this study was to compare the bioactivity of source and treated water from seven DWTPs along Göta Älv, a river in southwest Sweden. Samples were collected at the inlet and outlet of the DWTPs and concentrated by Solid Phase Extraction using Oasis HLB. Using reporter gene assays we measured the in vitro estrogen (ER), androgen (AR), aryl hydrocarbon (AhR), and peroxisome proliferator-activated alpha (PPARα) receptor activities, as well as Nrf2 and NFκB activities at non-cytotoxic concentrations of water samples concentrated 50 fold. Most of the DWTP samples exhibited AhR and PPARα activities and in a few samples ER and AR antagonistic activities were observed, with lower activities in the outlet compared to inlet samples. One DWTP exhibited a markedly different profile with higher ER, AR antagonistic and Nrf2 activities in the outlet than the inlet samples, suggesting an inefficient treatment at this specific DWTP. No samples showed any PPARα or NFκB activities. The study demonstrated that the bioactive components of the surface water was generally reduced in the DWTPs and that bioassays are useful screening tools in toxicological assessment of drinking water.
Tetrabromobisphenol A (TBBPA) is a commonly used high production volume brominated flame retardant. This study evaluated a oral TBBPA dose response exposure for endocrine disrupting activity. Timed-pregnant Wistar Han rats were exposed to TBBPA from gestation day (GD) 6 to postnatal day (PND) 21 and the resulting offspring were orally exposed from PND 22 through approximately PND 90 at concentrations of 0, 0.1, 0.5, and 200 mg/kg/day, with the same type oil vehicle control and blind dose allocation. Pups were culled to 4 per sex litter on PND 4. Serum and thyroids were collected from dams on GD20 (n=3) and PND21 (n=12), fetuses on GD20 (fetal blood pooled by sex n=3), male offspring on PND 21, 42,90 and 1 year (n=12-15), and female offspring on PND 21, 33, 90 and 1 year. Pubertal timing was assessed and vaginal smears were used to select females in estrus for necropsy at PND 90 and 1 year. Sera were evaluated for hormone concentrations of thyroid stimulating hormone (TSH), T3, T4, and free T4. Preliminary immunoassay data demonstrated the following: There were no significant changes in groups dosed with <250 mg/kg/day TBBPA at any time point or in dams at any dose. In the 250 mg/kg/day group, the females at PND 33 and the males at PND 42 had significantly lower serum T4 than controls and both males and females at PND 90 had significantly lower serum levels of T4 and free T4 compared to the controls. A recovery period was observed starting at 100 µM, where the effect peaked. This xenobiotic in the mutant strains were almost flat for most concentrations, until the treatment reached 100 µM, where the effect peaked. In contrast, NPEO9 induced a concentration-dependent response for sod-4 mutants, with greater fluorescence induction than NP at similar examined levels. In short, the toxic effects of NPEO9 and NP can be observed at low concentrations with particular responses along the concentration-effect curves. Colciencias-UniCartagena, 727-2015.
1828 Selective Biological Action of Bisphenol A, AF, and S Involves Differential Coregulator Interactions

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Bisphenol A (BPA) is an endocrine-disrupting chemical (EDC) found to be harmful to human health. Recently, widespread usage of bisphenol chemicals (BPs) such as BPAF and BPS are used as replacements for BPA. However, the potential biological action of these chemicals is poorly understood. Our objective was to examine the estrogenic effects of BPA, BPAF, and BPS and the molecular mechanisms of action in the estrogen receptor alpha (ERα) and ERβ. In this study, we performed an in vitro cell culture study, which was used to assess estrogenic effects of BPA, BPS, and BPS to estrogen. Microarray assay for real-time coregulator-Nuclear receptor interaction analysis was used to identify coregulators of BPA, BPAF, and BPS and molecular dynamic (MD) simulations were used to determine the bindings in the ERα-complex. We demonstrated that BPA and BPAF have agonistic activity for both ERα and ERβ, but BPS has ERα selective specificity. We concluded that coregulators were differentially recruited in the presence of BPA, BPAF, or BPS. Interestingly, BPS recruited more corepressors when compared to BPA and BPAF. From a series of MD analysis, we predicted concentration-addition model dose-response curve, the effect of BPA, BPAF, and BPS complexes. These findings provide an important basis for the understanding of the molecular mechanisms of EDCs, such as BPS, in ER-mediated transcriptional activation, biological activity, and their effects on physiological functions in human health.

1830 Exposure to CrVI during Early Pregnancy Increases Oxidative Stress and Disrupts the Expression of Antioxidant Proteins in Placental Compartments

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Hexavalent chromium (CrVI) is a heavy metal that is widely used in more than 50 industries such as leather and tannery, textile, metallurgical, chemical and automobile. Environmental contamination with CrVI is a growing problem both in the United States and in developing countries. Due to increased use and improper disposal of CrVI levels in the water, soil, and air continue to increase predisposing humans to several illnesses. The approved safety limit for chromium drinking levels is 0.1 ppm while levels as high as 5.28 ppm have been documented. In utero exposure to chemicals, drugs and heavy metals is well-documented to adversely affect development and biological activity and toxicological studies have demonstrated relationships between CrVI exposure and increased risk of abnormal menses, spontaneous abortion, stillbirth, preterm birth, developmental defects and neonatal death in pregnant women. These adverse outcomes in the offspring are caused as type two diabetes, stroke, heart disease and hypertension. This study was designed to understand the mechanism of CrVI toxicity on placental oxidative stress and antioxidant (AOX) machinery. Pregnant mother rats were treated with or without CrVI (50 ppm K2Cr2O7) through drinking water from gestational day (GD) 9.5-14.5, and placentas were analyzed on GD 18.5. Results indicated that maximum accumulation of Cr was measured in the merial gland compared to laryngoid and basal (junc- tional) zones of the placenta. CrVI reduced the trophoblast cell population. CrVI increased reactive oxygen species (ROS) and decreased the expression of AOX proteins. CrVI disrupts the trophoblast proliferation of the placenta. This study provides insight into the critical role of AOXs in placental function. This work was supported by National Institute of Environmental Health Sciences grants ES025234-01A1 (S.K.B.)

1831 Validation of a Glucocorticoid Receptor Effects-Based Environmental Sample Screening Tool

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Glucocorticoid receptor (GR) agonists have been detected in waste and surface waters domestically and around the world using both analytical chemistry and cell-based bioassay methods. Although a variety of GR agonists have been detected, the way in which a mixture of chemicals may contribute to a single biological endpoint e.g. additive, synergistic or antagonistic, remains unknown. Therefore, we characterized 18 known GR agonists, as well as one antagonist (mifepristone) using a transcriptional activation bioassay. CV1 cells, devoid of endoge nous GR, were virally transduced with human GR and MMTV-luciferase genes. Cells were treated with individual ligands, a fixed ratio mixture, or using a two-chemical matrix design. Agonist efficacy (maximum response relative to dexamethasone reference) varied and potency (EC50) ranged over several orders of magnitude, 48.09 to 102.5% and 1.278e-10 M to 3.93e-8 M, respectively. The IC50s for the antagonist, mifepristone, was 8.355x10^-10 M in competition with 1pM dexamethasone. Concentrations for an equipotent mixture of the twelve full agonists were determined using relative potency values (EC50/Dexam/EC50/Reference) and mean slope. Although the observed response (DexEq50) of the twelve chemical mixture fell within the 95% confidence interval of the predicted concentration-addition model dose-response curve, the response observed with two partial agonists (21-hydroxyprogesterone and corticosterone) in equipotent mixtures with dexamethasone did not. The twelve agonist mixture suggests that GR ligands activate the ability all bisphenols tested at 10 nM compared to control at early stage of differentiation, while the opposite was noted for C/EBP-α, a marker of late stage of adipogenesis. Increased PPARα caused by bisphenol correlated well with perilipin (present at the surface of lipid droplets) and lipoprotein lipase (involved in fatty acids uptake by adipocytes), both genes are regulated by PPARα. Interestingly, higher concentration of bisphenols induced downregulation of both transcription factors PPARα and C/EBP-α during the early and late stage of differentiation, which was in agreement with Red Oil R staining, suggesting inhibition of adipo genesis. Our results show that the effect of bisphenols on differentiation of adipocytes is dose-dependent and the strategy of substituting other bisphenols for BPA does not abrogate potentially dangerous health effects. This work was funded by R01 ES022759.
of the receptor to initiate gene transcription additively, however partial GR agonists may interfere with full agonist activity. This evaluation of GR-specific pharmaceuticals will enable the differentiation of GR ligands in environmental mixtures samples from future surface and waste water studies. Abstract does not necessarily reflect US EPA views or policy.

**1832 Review of Triazoles Toxicology: Mode-of-Action and Effects on the Endocrine System**

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Sponsor: L. Coney

The toxicology of triazoles was investigated in relation to potential to affect aspects of the endocrine system, from the molecular initiating event to the organism response with the aim of developing an adverse outcome pathway (AOP). Triazole compounds play a key role as antifungals in agriculture and in human mycoses and as non-steroidal anti-oestrogens in the treatment of oestrogen-responsive breast tumours in postmenopausal women. At high doses, these compounds affect the liver, the thyroid, and reproductive organs fertility, and development in several species. There is increasing evidence for adverse effects of high doses of triazole fungicides on the mammalian steroid metabolism. In the EU, specific triazoles are included in a candidate list of substances for further evaluation of their role in endocrine disruption. This review looks at the link between triazole toxicity in the female mammalian endocrine system, the CYP19 inhibition and summarizes how the available data may fit into adverse outcome pathways. Based on animal studies and human case studies, inhibition or deficiency of CYP19 causes the estrogen levels to decrease. This is the first key event of adverse outcome pathways in the female reproductive system. The estrogen profile of antifungal triazoles appears similar to the effects of CYP19 inhibitor drugs in rats and monkeys and the phenotype of CYP19 knockout mice, suggesting that the mode of action of triazoles in the female reproduction could be linked to inhibition or deficiency of CYP19 and the subsequent decrease in estrogen levels. The key events are impaired folliculogenesis and placental degeneration. The adverse outcome is reduction or loss in fertility of mammals.

**1833 Pharmacological Estrogen-17α-Ethyl Estradiol (EE)-Responsive NRF1 Gene Networks in Human Breast Cancer Cells: Its Involvement in the Carcinogenic Effect of EE**

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Lifetime exposure to elevated levels of estrogen is a risk of breast cancer. Birth-control pills containing 17α-ethinyl estradiol (EE) also increase breast cancer risk. Recently, we have shown that NRF1 (Nuclear Respiratory Factor 1) expression is upregulated in pregnancy and that this hormonally regulated gene is essential for breast tissue development. NRF1 is also involved in the development of breast cancer. In this study, we investigated the role of NRF1 in breast cancer cells. To validate findings from CTD curated data, we used Bayesian network analysis to identify molecular gene networks. NRF1 protein expression was significantly higher in clinical samples of breast cancer. Analysis of ChIP-seq data revealed ~10,000 possible NRF1 target genes (TGs) in breast cancer cells. The list of enriched pathways showed EE responsive E2 and NRF1 common TGs associated with breast cancer. Several 17β-estadiol (E2) interacting genes in breast cancer under the enrichment of the KEGG breast cancer pathway. In breast cancer, there are many E2-responsive genes that are NRF1 TGs. These genes are also responsive to EE. To validate findings from CTD curated data, we used Bayesian network (BN) analysis on RNA-seq data of breast tumor samples. We observed that both EE and NRF1 gene networks were associated with breast cancer. The breast cancer NRF1 network in human breast cancer patients was completely different from normal breast tissue samples. Breast cancer associated NRF1 regulatable genes were also regulated by both E2 and EE. Our results revealed the probabilistic graphical networks of NRF1 TG’s affected by the interaction with EE in breast cancer. These NRF1 regulatable genes alone, or in concert with others, may contribute to the breast cancer-dependent differences in the EE responsive gene networks and breast carcinogenicity in response to exposure to EE.

**1834 Tributyltin Protects against Ovariectomy-Induced Bone Loss Only in Mice on a Low Fat Diet**

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Bone quality is determined by both genetic and environmental factors. Risk factors for poor bone quality are estrogen loss at menopause, a high fat diet and exposures to drugs/chemicals that activate proinflammatory/proliferator activated receptor gamma (PPARγ). We unexpectedly observed that the environmental PPARγ/retinoid X receptor ligand, tributyltin (TBT), represses periosteal bone formation but increases trabecular bone mass in mice. This is in contrast to TBT’s known ability to suppress osteogenesis in vitro. Here, we examined the interaction of estrogen loss, diet and TBT exposure in female, C57BL/6j mice. Mice underwent sham surgery or ovariectomy (OVX) at 10 weeks of age. At 12 weeks of age, they were placed on a low (10% kcal) or high (45% kcal) fat, sucrose-matched (17% kcal) diet. Mice were treated by oral gavage 3 times per week with Vh (sesame oil, 5 µl/g) or TBT (1 or 5 mg/kg) for 14 weeks. OVX increased body weight gain in mice on either diet. TBT enhanced body weight gain in intact mice but decreased weight gain in OVX mice on a high fat diet. Bone tiss concentration increased in a dose-dependent manner; TBT resulted in a reduction of cortical thickness and bone area without a reduction in total area, regardless of diet. A significant increase in marrow area suggests that bone resorption increased on the endosteal surface. In high fat fed mice, TBT further reduced the cortical thickness, bone area and total area, without affecting marrow area, suggesting that TBT suppressed osteogenesis on the periosteal surface. OVX only decreased trabecular bone volume fraction in mice fed a high fat diet; however, trabecular number and connective density were decreased regardless of diet. Surprisingly, TBT protected against OVX-induced trabecular bone loss in low fat fed mice. TBT’s protective effect was nullified by the high fat diet. Serum CTX was reduced in TBT- treated, OVX mice, on either diet. However, serum PINP and osteocalcin were reduced by TBT only in OVX mice on the high fat diet. Gene expression analyses on cortical bone and bone marrow were being conducted and will provide information on TBT’s disparate effect between the cortical and trabecular compartments and search protective effects of TBT and why the protective effect is nullified by a high fat diet. Our novel observations will provide new information on basic bone biology, potential therapeutic targets and toxicological pathways.
Thyroid hormone (TH) homeostasis is dependent on multiple proteins for TH synthesis, transport, and peripheral metabolism and elimination. Deiodinase enzymes play an essential role in converting THs between active and inactive forms by converting the pro-hormone thyroxine (T4) to the active hormone triiodothyronine (T3) or converting both T4 and T3 to inactive forms. Chemical inhibition of deiodinase activity has been identified as an important endpoint to include in screening chemicals for thyroid hormone disruption. To address the lack of data regarding the potential of chemicals to inhibit the deiodinase enzymes, we established robust in vitro assays and screened over 1800 unique chemicals for inhibition of human deiodinase type 1, 2, and 3. The activity of the deiodinase enzymes was determined utilizing the Sandell-Kolthoff reaction for non-radioactive determination of iodide in a 96-well plate format. The majority of the chemicals did not inhibit deiodinase activity in the initial screen with a single high concentration (target concentration of 200 µM). Across the three enzymes, only 12-17% of the chemicals produced enzyme inhibition of 20% or greater. The EPA’s ToxCast phase 1, v2, phase 2, and e1k chemical libraries each included multiple chemicals that produced enzyme inhibition of 50% or greater in the three deiodinase assays; these chemicals were further tested in concentration-response mode to determine relative potency. Comparison across the three deiodinase isoforms shows similar results for over 90% of the tested chemicals; however, there are over 50 chemicals that show greater than 50% inhibition of only one of the three deiodinases. This set of three deiodinase inhibition assays is a significant contribution to the field for expanding the scope of exposure chemistry used to identify chemicals having the potential to interfere with thyroid hormone homeostasis. Further work to determine whether this in vitro activity translates to effects in vivo is needed. Additionally, this study sets the groundwork for development and evaluation of structure-activity relationships for deiodinase inhibition in the context of deiodinase chemicals for further testing to identify adverse outcomes of deiodinase inhibition. This abstract does not necessarily reflect US EPA policy.

Benign prostate hyperplasia (BPH) affects 40% of men over the age of 50 and is characterized by enlargement of the prostate gland. BPH is a multifactorial disease and several mechanisms such as aging, proliferation, hormones, and fibrosis have been proposed. While the etiology of BPH is still poorly understood, estrogens have been implicated in the development and maintenance of BPH. Estrogens primarily signal via classical estrogen receptors ERα and ERβ through ligand activated nuclear development and maintenance of BPH. Estrogens can bind to ERs to facilitate genomic and nongenomic responses. A proposed mechanism is that when estrogens mediate their effects through GPER, this can cause transactivation of EGFR. Activation of EGFR can stimulate signaling cascades that regulate cell functions such as proliferation, meaning this mechanism could be a contributing cause of BPH. BPA’s transactivation of EGFR is of concern as it is used to harden plastics in household products, and repeated exposure can be considered a public health concern for consumers.

In vitro based assays are increasingly being used to identify potential endocrine disrupting chemicals. Thyroperoxidase (TPO), an enzyme essential for thyroid hormone (TH) synthesis, is a target site for disruption of the thyroid axis for which a high-throughput screening (HTS) assay has been developed. Physiologically-based pharmacokinetic/pharmacodynamic (PBPK/PD) models can aid in translating findings from in vitro assays to in vivo effects. This study used known TPO inhibitors, methimazole (MMI) and 6-propylthiouracil (PTU), to provide in vivo data for PBPK/PD modeling. Adult male LE rats were exposed to 0, 1, 3, and 10 ppm PTU or 0, 3, 30 or 200 ppm MMI in drinking water for 4, 7, and 14 days. Serum and thyroid glands were collected to assess PTU, MMI, and TH levels. Serum and gland PTU and MMI levels increased in a dose-dependent manner. Serum T3 and T4 were reduced by PTU in a dose- and time-dependent manner, with larger decrements seen at all time-points with exposure to 3 and 10 ppm. TSH was elevated after 7 days of exposure, largest increases observed with 14-day exposure to 10 ppm PTU. However, the low dose group did not differ from controls, despite declines in serum T3 and T4. Serum T3 and T4 decreased with 4-day exposure to MMI. TSH increased with 7 and 14-day exposure to 30 and 200 ppm MMI. T3, T4, rT3, and mono- and di-iodothyronines (MIT, DIT) were also measured in the glands of rats exposed to PTU for 4 days. TH levels decreased in a dose-dependent manner with greater decreases observed in D1 (53%, 73%) and T4 (40%, 80%) following exposure to 1 and 10 ppm PTU, respectively. Glandular TH and PTU measured permitted the estimation of in vivo TPO inhibition using a PBPK/PD model. The in vivo derived concentration constant (IC50) was 1.55 µM which was slightly higher than the in vitro IC50 value of 1.24 µM. Moreover, measured serum PTU and TH concentrations were adequately simulated by PBPK/PD modeling. Analyses of additional time-points are underway for gland concentrations of chemical and TH as input for TPO inhibition in the context of exposed animals. These data provide a framework to translate the magnitude of TPO inhibition obtained from HTS assay relative to the degree of TH perturbation in vivo. This abstract does not necessarily reflect US EPA policy.

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The hypothalamus regulates many important biological functions such as reproduction, thermoregulation, and energy balance. Orexigenic NPY neurons located in the arcuate nucleus (ARC) of the hypothalamus play an essential role in energy homeostasis. Disruption of this balance can cause metabolic disease states such as obesity and anorexia. EDGs such as organophosphate flame retardants (OPFR) are a potential cause of hypothalamic dysfunction as they accumulate in human tissues and impinge on endogenous nuclear receptors such as ERα and PPARy. Previously, we demonstrated that OPFR oral treatment in adult mice decreased ARC expression of neuropeptide Y and increased growth hormone secretagogue receptor (GHSR) and KCNQ channel subunit expression in a sex-dependent manner. We hypothesized that these effects would augment the activity of the M-current in NPY neurons leading to a suppression of NPY excitability and a reduction in food intake. We will use voltage clamp, whole-cell patch-clamp to record the M-current in NPY neurons utilizing selective KCNQ channel blocker XE991 with standard activation and deactivation protocols from male and females after OPFR treatment. In males, OPFR treatment increased the peak current (84±15) in NPY neurons at -35 mV compared to control (59±10). These data correlated with an increase in expression of KCNQ-3 in male NPY neurons (1.63±0.16 compared to 0.97±0.14, p < 0.05). Our data suggest that serial exposures to EDC, especially flame-retardants may impact the hypothalamic melanocortin neurocircuitry leading to dysregulation of energy homeostasis.
Few ligands for thyroid hormone receptor (TR) have been identified beyond endogenous ligands and pharmaceuticals, suggesting TR is a very selective nuclear receptor (NR). However, diverse chemical libraries, particularly of environmental chemicals, have not been tested for TR activity. We evaluated the hypothesis that TR is highly selective and that modulation of TR activity is unlikely to be a key mechanism for thyroid hormone disruption by developed environmental chemicals. A TR luciferase reporter gene assay in the rat pituitary-derived GH3 cell line was used for quantitative high-throughput screening in agonist and antagonist format against the 10K Tox21 chemical library. In agonist mode, 28 active compounds were identified with potencies from 0.01-91 µM. To confirm these as TR agonists, a series of additional assays were performed: 1) the GH3 cell line; 2) a second TR reporter gene assay—human TRβ in a mammalian one-hybrid format; and 3) a mammalian one-hybrid assay for the retinoid X receptor (RXRα), a potential activator of this receptor through a permissive heterodimer effect; and, 4) a TR:coactivator recruitment assay using the human TRβ ligand-binding domain and SRC-2 peptide. Results showed all 28 compounds repeated as actives in the GH3 assay, with 22 direct TR ligands and 6 via RXR activity. The antagonist assay mode identified 2352 actives of which 1488 had an AC50 for cytotoxicity within 3-fold or less of the corresponding reporter gene AC50, and 868 were considered false positives. We confirmed activity in the 2 reporter gene assays; however, the coactivator recruitment assay failed in antagonist mode. As an alternative, we tested 285 available putative antagonists in a NR translocation assay using GFP-tagged chimeric molecules of glucocorticoid receptor and the TRβ ligand-binding domain known to be responsive to a known TR antagonist, 1-850. Of these, 43 exhibited significantly increased NR translocation activity at the highest concentration tested, 100 µM, but only 17 demonstrated activity at lower concentrations. Fourteen of these were pharmaceutical compounds not previously shown to have TR activity. Overall, this work supports the hypothesis that thyroid hormone disruption via TR perturbation by environmental chemicals is a rare event, and that other modes of action are likely of higher importance for screening this chemical space. This abstract does not necessarily represent the views of the US EPA.
Incidence and prevalence of reproductive disorders have significantly increased over the past three decades. This fact has led to the study of endocrine disruptors (ED), ubiquitous chemical compounds present in the environment and in the human diet, able to interact with hormones, disrupting among others, reproductive function. ED act at very low doses, exert different mechanisms of action and comprehend a great number of substances. Genistein is a phytoestrogen classified as so-called endocrine disruptor (EED) and its human exposure occurs primarily through the consumption of soy-based food and beverages products, which are becoming more popular as nutritional supplements and are frequently used in baby’s formulas, juices and milk substitutes. At certain age periods, like development and early childhood, there is a window in which certain substances exert higher susceptibility to toxic effects. This study aimed to use as a biological model the nematode Caenorhabditis elegans to assess the effect of genistein as an endocrine disruptor using reproduction related endpoints such as brood size and egg production. As lethal concentration was determined at 1000 μM of Genistein previously dissolved in Dimethyl sulfoxide (DMSO), exposures were carried out at sub-lethal concentrations of 0.1, 1, 10, and 100 μM. DMSO 1% was used as control. About ten nematodes were exposed to genistein solutions for 24 h, and both the number of eggs and the number of worms born per nematode exposed were counted. This procedure was carried out three times. It was concluded that genistein increases the reproduction behavior on C. elegans augmenting brood size and raising egg production at low concentrations, but this effect is inverse at high concentrations, evidencing its endocrine disruption capability. GRANT 155-2017 Investigation Vice-rectory of the University of Cartagena.

Bisphenol A (BPA) is used in the production of polycarbonate plastics, epoxy resins and thermal paper, among others. Its exposure to humans through food, drinks and dermal contact makes BPA an ubiquitous molecule. BPA binds to several proteins associated with the endocrine system, and some reports suggest it increases the risk of obesity, among other metabolic diseases in humans. The objective of this work was to determine the obesogenic action of BPA on human adipoblasts. A sample of peripheral white adipose tissue was taken from a female volunteer. Adipocyte derived stem cells (ADSCs) were isolated, exposed to vehicle control or different BPA concentrations, and assayed their proliferative activity. Viable cells were counted by trypan blue staining exclusion method. To determine adipogenic differentiation, confluent cultures were incubated for four weeks with DMSO or BPA, and stained with Oil Red O. Pigment was extracted with isopropanol and its absorbance quantified by spectrophotometry. The proliferation of adipoblasts increased with BPA concentration, a process that was also observed for lipid accumulation. This study demonstrated the obesogenic action of BPA and the need to regulate the use of this xenobiotic to eliminate human exposure.
1852a Exposure to Low Levels of Triclosan Reduces Estrogen Sulfotransferase Activity in Fetal Liver and Placenta of Pregnant Ewes

M. O. James, E. N. Jackson, L. Rowland-Faux, and C. E. Wood. University of Florida, Gainesville, FL.

Sulfonation is an important reaction in the biotransformation and homeostasis of estrogens. Previous in vitro studies found that triclosan is an especially potent inhibitor of ovine placental estrogen sulfotransferase, with $K_c$ of less than 0.1 nM. As the placenta is the main organ responsible for estrogen synthesis in pregnancy in sheep as well as women, and the liver is another site of estrogen biotransformation, this study examined the effects of triclosan exposure of pregnant ewes on fetal placental and hepatic sulfotransferase activity. Triclosan, 0.1 mg/kg/day, or saline vehicle were administered to late gestation fetal sheep for two days either by direct infusion into the fetal circulation or infusion into maternal blood. On the third day, fetal liver and placenta were harvested and analyzed for triclosan and for cytosolic estradiol sulfotransferase activity. Placenta contained higher concentrations of triclosan than liver in each individual sheep in both treatment groups. In both placenta and liver there was a negative correlation between triclosan tissue concentration (pmol/g tissue) and cytosolic sulfotransferase activity (pmol/min/mg protein) towards estradiol. These findings demonstrated that in the sheep exposed to very low concentrations of triclosan, this substance is taken up into placenta and inhibits estrogen sulfonation. Supported in part by the US Public Health Service, NIH R21 ES020545.

1853 Exposure to PCB126 during the Nursing Period Significantly Impairs Early-Life Glucose Tolerance

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Polychlorinated Biphenyls (PCBs) are persistent environmental organic pollutants that are known to have detrimental health effects. In a mouse model in our laboratory, PCB126 exposure during pregnancy and nursing alters offspring body composition and glucose tolerance. This purpose of this study was to expose dams to PCB126 during the nursing period only. Female ICR mice were bred and half of the dams were exposed to either vehicle (safflower oil) or 1 mol% PCB126 per kg of body weight via oral gavage on postnatal days 3, 10, and 17 (n = 9/group). Offspring body weight, lean and fat mass, and glucose tolerance were measured. Both male and female offspring displayed normal body weight as well as body composition (p > 0.05). However, both male and female offspring that were exposed to PCBs during the nursing period had significantly impaired glucose tolerance at 3 weeks of age (p < 0.05). This persisted until 9 weeks of age in the male offspring (p < 0.05), but the difference disappeared as the male offspring aged (p > 0.05). Our earlier work suggests that in utero and postnatal PCB126 exposure predisposes offspring to having lower lean mass and impaired glucose tolerance later in life. However, our current study shows that exposure to PCB126 through the mother's milk impairs glucose tolerance in the short-term and is likely caused by impairments in insulin receptor signaling in the periphery as others have shown with direct PCB exposures in adult mice. Future experiments will investigate the mechanisms of dysfunction caused by in utero PCB126 exposure, which may be driving the increased risk of obesity and insulin resistance in adult offspring.

1854 In Utero and Lactational TCDD Exposure Causes Lower Urinary Dysfunction in Adult Male C57BL/6J Mice


Benign prostatic hyperplasia (BPH) and other lower urinary tract factors such as increased prostatic collagen content have been associated with voiding dysfunction (increased frequency, urgency, and incomplete bladder emptying) in aging men. The causes of BPH and mechanisms for voiding dysfunction are not fully known. IUL TCDD exposure exacerbates urinary dysfunction in Tg(CMV-cre);Nkx3-1(+/-);Pten(fl/+). The purpose of this study was to test the hypothesis that IUL TCDD exposure alone or in conjunction with adult T+E exposure also causes urinary dysfunction in wild type (C57BL/6J) male mice. Mice received sham surgery or were subcutaneously implanted with T (25mg) and E2 (2.5mg) slow-release implants to drive voiding dysfunction and were evaluated 8 weeks later. Anesthetized cystometry was used to measure non-voiding contractions and intervoid interval (IVI). Prostatic collagen content was assessed by quantitative analysis of picrosirius red staining. IUL TCDD alone, T+E alone, and IUL TCDD combined with T+E, all significantly increased non-voiding contractions compared to control, T+E alone increased IIVI compared to untreated control mice, and combining IUL TCDD exposure with T+E appeared to protect against this increase. Prostate collagen density was unchanged by IUL TCDD alone, evidence that it is not sufficient to drive prostate fibrosis. This study is the first to demonstrate that IUL TCDD exposure changes lower urinary tract function in adult male wild type (C57BL/6J) mice. Supported by NIH Grants U54 DK099328, R01 ES001332 and T32 ES007015.

1855 Metabolic Impacts of Developmental Exposures to Phthalates and Phthalate Mixtures: Early Evidence for Persistent PPAR Activation

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Exposures to phthalates, ubiquitous plasticizing chemicals, have been associated with an increased risk for metabolic syndrome and obesity. Phthalates' impacts on metabolism are thought to be due to peroxisome proliferator-activated receptor (PPAR) activation; however, this mechanism has not yet been investigated with respect to developmental exposures. Furthermore, little is known with respect to phthalate mixtures and PPAR activation. To test the hypothesis that developmental exposures to phthalates and phthalate mixtures result in PPAR activation that persists through adulthood, we used an ovine genetic mouse model. Two weeks prior to mating, dams were fed 1 of 6 experimental diets: 1) 7% corn oil control, 2) 25 mg DEHP/kg chow, 3) 25 mg DBP/kg chow, 4) 75 mg DINP/kg chow, 5) 25 mg DEHP + 75 mg DINP/kg chow, and 6) 25 mg DBP + 25 mg TEBP + 75 mg DINP/kg chow. At weaning, approximately 1 male and 1 female were sacrificed for tissue collection, and 1 male and 1 female were followed until 10 months of age, with metabolic phenotyping occurring across the life course. Sex-specific differences in metabolic parameters were observed in phthalate-exposed offspring. Males exposed to DEHP or DINP only had increased body weight compared to controls (p=0.06 & 0.04, respectively), but did not exhibit significant alterations in body composition. Females exposed to DEHP or DINP only had increased body fat (p=0.04 & 0.08, respectively), decreased lean mass (p=0.02 & 0.04, respectively), and decreased glucose oxidation (p=0.04 & 0.10, respectively) compared to controls; females exposed to DEHP also exhibited increased fat oxidation (p=0.04). Females exposed to a mixture of all three phthalates had increased water weight at 8 months (p=0.07), and had increased plasminogen activator inhibitor 1 (PAI-1) (p=0.09) and improved insulin sensitivity (p=0.08) at 10 months relative to controls. Obese metabolic effects were not evident in offspring fully exposed to phthalates are consistent with PPAR activation, suggesting PPARs may be persistently activated. Interestingly, mice exposed to phthalate mixtures showed protection against obesity and insulin resistance. Ongoing analyses will further characterize PPAR activation in liver and adipose using RNA-seq, DNA methylation, and oxidative stress analyses.

1856 Developmental Exposure to Brominated Flame Retardant 2,2',4,4'-Tetrabromodiphenyl Ether (BDE-47) Permanently Alters Liver Lipid Metabolism in Mice

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Tight regulation of fatty acids uptake by the liver is the major process that contributes to a healthy balance of lipids between blood and the liver. Abnormal shifts in this balance in either direction result in increased morbidity and mortality risks. An increased uptake of fatty acids results in accumulation of triglycerides in hepatocytes and is referred to as hepatic steatosis or nonalcoholic fatty liver disease (NAFLD). The most common form of chronic liver disease, NAFLD increases the risk of type 2 diabetes, dyslipidemia, hypertension, cardiovascular and kidney disease, liver cirrhosis, hepatocellular carcinoma, and mortality. On the other hand, a decreased uptake of fatty acids by the liver may result in hyperlipidemia and atherosclerosis, the primary risk factors for heart attack. Thus, improving our understanding of preventable causes of lipid imbalance may have significant consequences for public health. Polybrominated diphenyl ethers (PBDEs) were used as flame-retardant additives in a wide range of polymers and were recently withdrawn from commerce in North America and Europe. Generations that were born
when environmental concentrations of PBDE reached their maximum have now reached 0-15 years of age and in the US account for 1/5 of the total population. Our experimental data demonstrate that exposure to 2.2',4,4'-tetrabromodiphenyl ether (BDE-47), the most prevalent PBDE congenor in human samples, during sensitive developmental windows may result in permanent changes in liver lipid metabolism. Developmental exposure to 0.2 mg/kg body weight of BM-A77 in CD-1 mice results in a permanent 2.5-fold suppression of the fatty acid influx pump CD36 in the liver, which is associated with significant increases in circulating triglycerides in blood. Exposure of CD-1 mice to 1 mg/kg BDE-47 produces an opposite effect on CD36 expression and triglyceride balance, and results in more than a 5-fold increase in expression of CD36 and an associated increase in lipid deposition in the liver. Many other lipid metabolism genes undergo similar changes in expression in liver in response to low and moderate doses of BDE-47. Similarly, low and moderate/high doses of PBDE have opposite effects on mTOR regulated processes. Our evidence suggests that developmental exposure to PBDE may be an important environmental factor of lipid imbalance programming and that this programming may be mTOR dependent.

This study tests the hypothesis that embryonic exposure to ATZ results in age and sex-specific changes in behavior, the adult brain transcriptome, and adult body and brain size, according to the Developmental Origins of Health and Disease (DOHaD) hypothesis. Zebrafish (Danio rerio) embryos were treated with 0, 0.3, 3, or 30 ppb (ppb; μg/L) ATZ immediately after fertilization and exposed through 72 hours post-fertilization (hpf). At 120 hpf, a visual motor response (VMR) test examined larval behavior. Larvae were also grown to 9 months post fertilization (mpf) or 14 mpf. At 9 mpf, a novel tank test, a light-dark box, and an open field test evaluated adult behavior. Microarray analysis investigated ATZ related differences in gene expression. At 14 mpf, the body length, weight, and brain weight was measured to evaluate effects of ATZ on mature body and brain size. For the larval VMR, only the 30 ppb treated larvae were hypactive. However, the 9 mpf adult behavioral tests found non-monotonic, sex-specific behavior changes, with male zebrafish having decreased activity and female zebrafish having increased signs of anxiety. Microarray analysis identified sex-specific transcriptomic alterations, with females having altered expression of genes in pathways related to sexual injury, neurodevelopment, and system disorders and males having altered gene expression in endocrine and reproductive system disorder and nervous system development and function pathways. Adult zebrafish also had non-monotonic, sex-specific alterations in body length, body weight, and brain weight. This study suggests that developmental exposure to ATZ does cause sex-specific alterations in adult neural function.

Trans-Generational Consequences of Lead Exposure, Prenatal Stress, and the Combination

Fixed interval schedule of food reward in F3 offspring. Subsequently, changes in anxiety-like behavior were examined using an elevated plus maze (EPM), as were striatal protein levels of tyrosine hydroxylase (TH), glucocorticoid receptor (GR) and brain derived neurotrophic factor (BDNF) and serum corticosterone levels in F3 offspring. EPM behavior was significantly altered by Pb and P5 in a lineage specific fashion: Pb increased center and closed arm entries and times of F3 MMM females, Pb increased center arm duration of F3 MMM females, and Pb reduced center and closed arm entries/duration of F3 MMM males. Serum corticosterone was altered only in F3 females, with Pb-related reductions in FMF females, and P5-related reductions in MFF females. Changes in striatal protein levels early in F3 were consistent across both sexes and included Pb- and P5-based increases in striatal BDNF protein in FFF females, Pb and P5-induced alterations in TH, GR and BDNF in MFF females, and altered TH protein levels in MMM, MFM and MFF males. These studies further confirm transgenerational effects of Pb/P5 in mice and show that they can exhibit lineage-dependency; they also underscore the need for human studies of Pb-based transgenerational effects. Supported by P30 ES00247.
female C57BL/6J mice on defined, low-fat diets were exposed to control Cd for >2 weeks before being established into breeding pairs (F0). Pregnant dams and offspring were continuously exposed to the same toxicant as their parents after weaning. At weaning, offspring (F1) were collected and portions of the livers were fixed in 10% formalin for histology or processed for metals analysis. Liver to body weight ratios were calculated and liver damage was assessed by pathology (ALT, AST) and histology (HE). Metal exposure did not significantly change any variable in the F1 group. Hepatomegaly and histology; females appeared to be less responsive to HFD than males. All toxicant exposures exacerbated the effect of HFD on variables of liver damage; the effect became more severe the longer the animals were exposed and was more pronounced in males than females. The initial results suggest that these toxicants enhanced liver injury caused by obesity. Future work will build on these findings and examine other target organs of metabolic syndrome.

**1863 In Utero Exposure to Ultralow Particles Induces Placenta Stress in Mice**

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Recent studies have shown that the exposure to ambient airborne matter (PM) with an aerodynamic diameter of ≤100 nm, also called incidental ultrafine particles (UFP), during sensitive developmental stages may predispose to disease later in life. In addition, epidemiological studies suggest that exposure to airborne particulate matter is associated with adverse gestational outcomes, such as premature birth, reduced birth number, and small size for gestational age. Furthermore, placenta stress may influence these adverse outcomes. We tested if the hypothesis of in utero exposure to UFP affects stresses in the pregnant mouse and if the placenta affects gestational outcomes.

Our results show that the collected UFP exhibited nanometric diameters (60% < 100 nm) and irregular morphology, measured by DLS and SEM. Organic carbon, elemental carbon, metals (Ti, Cr, Fe, Ni, Cu, and Zn) and PAHs (naphthalene, phenanthrene, and benzo[a]pyrene) were detected in the lungs of exposed dams to UFP. After pregnancy, plasma BPA was measured from newborns post waning. First, we evaluated the indirect or direct contact of UFP on fetuses by the increase of pigmented cells (eye-sprouts) on the RPE (unpigmented retinal pigment epithelium) in the exposure group. In the lungs of exposed dams to UFP, we observed an increase in pro-inflammatory cytokine (IL-6, IL-10, and TNF-α), expression of MCP-1, and MIP-2 as a pulmonary response to UFP. On the other hand, the placental stress by in utero UFP exposure was shown by an increase of 8-hydroxy-2-deoxyguanosine (8-OHdG) as well as an increase in IL-6, KC, and MCP-1. Also, an increase of 8-OHdG, IL-6, KC, MCP-1 and a decrease of the fetus. Our results support that the in utero exposure to UFP of Mexico City promotes adverse gestational outcomes involving placental stress.
Multidrug resistance protein 4 (Mrp4; encoded by Abcc4) is a basolateral efflux pump that transports several xenobiotics and endogenous molecules such as prostaglandins and cyclic nucleotides. Mrp4 is highly expressed in kidney and bladder, with minimal expression in liver. Recently, our laboratory determined that mice lacking Mrp4 develop hepatic steatosis during compensatory tissue regeneration following partial hepatectomy. These studies indicate that Mrp4 may play a significant role in lipid homeostasis. Recent studies have also showed that Mrp4 is expressed in adipose tissue, yet the role of Mrp4 in adipose tissue physiology and lipid metabolism is unknown. To begin addressing this, WT and Mrp4 knockout (Mrp4-/−) mice were subjected to 24-hour fasting. The mouse embryonic fibroblast cell line 3T3-L1 was also used to investigate the role of Mrp4 in adipogenesis. The results showed that both male and female Mrp4-/− mice have increased adipose tissue to body weight ratio and adipocyte cell size in both fed and fasted conditions. Additionally, the lack of Mrp4 is associated with increased serum glucose levels and impaired glucose tolerance in mice challenged with the glucose tolerance test. In parallel to increased adipose tissue weights, Mrp4-/− mice also showed increased leptin gene expression. In vitro studies using 3T3-L1 cells showed that pharmacological inhibition of Mrp4 function using either MK571 or C1 increases adipogenesis and expression of adipose tissue markers such as fatty acid binding protein 4 (Fabp4), lipoprotein lipase (Lpl), glucose transporter type 4 (Glut4) and peroxisome proliferator-activated receptor gamma (Ppar-γ). In conclusion, our studies show that the absence of Mrp4 or chemical inhibition of Mrp4 function using either MK571 or C1 increases adipogenesis and the total sum of metabolism. The findings suggest that exposure to VOCs could disrupt cardiometabolic functions. While VOC exposures have been linked to several outcomes of cardiometabolic dysfunctions, the impact of VOCs on pediatric obesity remains unclear. Exposure to VOCs may contribute to the development of obesity in children and adolescents. The association of urinary PAH with obesity and overweight in children and adolescents is particularly high within 200 m of homes. Further studies are needed to determine the mechanisms underlying this association and to develop strategies to attenuate the perturbed gene expression patterns in response to VOC exposure in both mother and offspring.

Polycyclic aromatic hydrocarbons (PAHs) are known carcinogens and suspected endocrine disruptors. Exposure to PAHs has been associated with obesity in children and adolescents. The association of urinary PAH metabolites with adiposity outcomes (BMI z-score and rate of obesity) in children and adolescents, the last National Health and Nutrition Examination Survey (NHANES). 2009-2014 cycles were analyzed. Multivariate linear and logistic regression to analyze the association of BMI z-score, and obesity with concentrations of the molar sum of total PAHs metabolites, and the sum of the metabolites based on their family structure were performed in 2241 individuals 6-19 years of age who participated in the 2009-2014 NHANES. Furthermore, the analyses were stratified by developmental stage (e.g. children 6-11 and adolescents 12-19). BMI z-score, and obesity were positively associated with the molar mass sum of the PAHs and the total sum of PAHs metabolites with increasing quartiles of exposure among children 6-11 years of age and adolescents (12-19 years of age) with evidence of a dose-response. The sum of polycyclic aromatic hydrocarbons were associated with obesity and increased BMI z-score in children, but not in adolescents. Total urinary PAH metabolites and naphthalene metabolites were associated with higher leptin gene expression in children, but not in adolescents if NHANES 2009-2014. Furthermore, this is the first report of an association of the sum of polycyclic aromatic hydrocarbons with obesity in children and of an association of the molar mass sum of PAH with obesity in adolescents in a nationally representative cross-sectional survey.

Disclaimer: The findings and conclusion in this report are those of the author and do not necessarily represent the views of CDC/ATSDR.

The general public is frequently exposed to high levels of volatile organic compounds (VOCs) derived from a variety of sources. VOC exposures have been linked to acute myocardial infarction, heart rate variability, hospital admissions and the prevalence of type 2 diabetes (T2D). This finding suggests that exposure to VOCs could disrupt cardiac processes and induce or exacerbate obesity, T2D and their resultant cardio-vascular complications. While VOC exposures have been linked to several outcomes of cardiometabolic dysfunctions, the impact of VOCs on cardiometabolic risk factors such as serum lipids has not been examined, and which VOCs exert specific health effects have not been identified. This study will use the 2011/2012 National Health and Nutrition Examination Survey (NHANES) to cross-sectionally examine the associations between urinary metabolites of VOC (U-VOC) exposures and serum lipid levels using survey weighted regression models. The findings suggest that exposure to VOCs showed more negative associations with high-density lipoproteins (HDL) and more positive associations with circulating triglycerides. Of the 26 UM-VOCs examined, 13 were negatively associated with HDL and positively associated with triglycerides in smokers, while none showed such a pattern of association in non-smokers. In smokers; negative associations with HDL ranged from -0.3 to -3.6 percent change per VOC inter-quartile range (IQR) and positive associations with triglycerides ranged from 0.5 to 8.3 percent. In smokers, MHBMA1, a metabolite of 1,3-butadiene, was associated with a 1.4% decrease in HDL and a 5.7% increase in triglycerides per 0.2 ng/mg increase. Both metabolites of trichloroethylene showed negative associations with HDL and xylene, and xylene (-15.4%) change. Associations between peak greenness and overall VOC exposure, based on primary PCA classification, were significant at 25m, 50m, 100m, 200m, and 300m radii. This association was observed to be highest within 200m radii, with an increase of 22% with a 0.1 unit change in NDVI. The interquartile range of NDVI standard deviation within a 200m radius was significantly associated with urinary metabolites of acrylamide, benzene, 1,3-butadiene, styrene, and xylene (10.7 to 48.3% change). Associations between NDVI standard deviation and principal component analysis of VOC compounds were observed to be highest within a 200m radii, with a significant increase of 40%. Results suggest that residential vegetation, particularly within 200m of homes, is associated with lower exposure to anthropogenic VOCs.

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and positive associations with triglycerides with increasing UM-VOCs in smokers. In non-smokers increased odds of dyslipidemia was associated with increasing levels of two 1,3-butanediol metabolites (DHMBMA OR: 1.003, MHMBMA OR: 1.25) and two xylene metabolites (2MHA OR: 1.007, 3MHA, 4MHA OR: 1.001). Collectively VOCS, particularly 1,3-butanediol and trichloroethylene, at concentrations found in conventional tobacco smoking exposures, as well as 1,3-butanediol and xylene at concentrations found at general environmental levels, are associated with cardiovascular disease risk by inducing dyslipidemia.

**1869 Phthalate Exposure and Pediatric Asthma Exacerbation: Reviewing Evidence of an Association and Future Directions**

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Phthalates are a class of high-production volume chemicals with applications in consumer products, building materials, and medical devices. Phthalates migrate from polymers and are present in the US in indoor dust at higher concentrations than any other consumer product chemical. Their prevalence in home, daycare, and school environments poses a high risk of exposure for children, and over 90% of children in the US have detectable concentrations of phthalate metabolites in their urine. Emerging evidence suggests these exposures may promote complex pathological mechanisms in the lung which ultimately lead to common pathways of cellular inflammation, enhanced bronchial responsiveness, and greater obstruction of airflow - the hallmarks of asthma. Most investigations of the association between phthalate exposure and pediatric asthma focus on developmental effects by examining gestational exposure, early-life exposure, or both, and the attendant health effects are becoming major public health concerns. In order to investigate the ameliorative effects of ascorbic acid as an antioxidant and intervention therapy in cases of occupational exposure especially when the subjects cannot be removed from the source of exposure.

**1871 Plasma Fluoride and Renal Function in Adolescents (12-19 Years): An Analysis of NHANES 2013-2016**

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Fluoride has an important role in bone mineralization and the formation of dental enamels; it is frequently added to drinking water, toothpaste, and mouth rinse to prevent dental decay. The major route for excretion of fluoride is the kidney, and fluoride nephrotoxicity has been reported in animal studies and in humans undergoing administration of halothane anesthetics. To investigate whether there is an association between plasma fluoride (PF) and markers of renal function—estimated glomerular filtration rate (eGFR), serum uric acid (SUA), and hyperuricemia—in US adolescents (12-19 years of age) of the National Health and Nutrition Examination Survey (NHANES) 2013-2016 (n=2096) who are exposed to low levels of fluoride. Multivariate linear regression was used to analyze the association of PF with eGFR (both the original and Schwartz formula – eGFRschwartz) and the updated eGFR(urea) and SUA. Both eGFR and SUA were natural log-transformed in the analyses; the results were re-transformed and presented as percent differences. Multivariate logistic regression was used to analyze the association of PF with hyperuricemia (defined as SUA ≥6 mg/dL). PF was analyzed via weighted quartile and as natural log-transformed. Participants in the 3rd and 4th PF quartiles had decreased eGFR - using either formula. These decreases remained when analyses excluded individuals with eGFR ≤60 mL/minute/1.73 m2. Furthermore, individuals in the 4th PF quartile had significantly increased SUA (% difference [95% CI]: 5.74 [2.54, 9.04] compared to the referent PF quartile, with evidence of dose-response trend (p-trend =0.008). Compared to the referent PF quartile, participants in the 2nd 3rd, and 4th PF quartiles had higher odds to have hyperuricemia (OR [95% CI]: 1.78 [1.22, 2.60]; 1.59 [1.03, 2.45]; and 2.25 [1.47, 3.34], respectively). Sensitivity analyses using natural log-transformed PF confirmed the associations with eGFR, SUA, and hyperuricemia. This exploratory study is the first to look at markers of renal function associated with PF following low-level exposure in an adolescent population. Reverse causation, where decreased eGFR may lead to increased PF, may not be excluded. However, analyses restricted to individuals with normal eGFR (>60 mL/minute/1.73 m2) suggests that PF may affect renal function, as indicated by decreased eGFR and increased SUA. Further studies, especially a prospective cohort study, are necessary to evaluate the association between PF and renal function, considering the public health implications of renal dysfunction in adolescents.

**1872 Toxicological Effects of Lead in Children from a Fishing Community on the Atlantic Coast of Colombia**


Lead (Pb) is a multifaceted and complex environmental pollutant that affects childhood health. In Colombia Pb is a threat for vulnerable children. The aim of this research was to evaluate possible relationships between blood lead levels (BLL) and IQ, hematological and biochemical parameters, and gene expression in children from Tasajera, a fishing town at the Colombian Caribbean. A total of 133 blood samples were collected from children 5-16 years old. This research was approved by the ethics committee of the University of Cartagena and each study participant’s parent provided written informed consent. BLL were measured by atomic absorption; the Kaufman Brief Intelligence Test (K-BIT) was administered to measure the IQ; whole blood was used for hematology; plasma was utilized to analyze hepatic toxicity markers (ALT, AST, ALP); and gene expression was quantified from blood RNA using RT-PCR. The ALAD rs 1800435 polymorphism was characterized in children who had BLL ≥20 μg/dL, employing RFLP. The mean BLL was 20.6±9.4 μg/dL. BLL >10 μg/dL were found in 29% of children. The mean BLL of 10 μg/dL was used as the cut-off to separate children with Pb exposure from those without. The mean BLL of the group with BLL ≥10 μg/dL was 20.6±9.4 μg/dL, while the other group had a mean BLL of 4.6±2.8 μg/dL. The BLL ≥10 μg/dL group had significantly lower IQ scores compared to the BLL <10 μg/dL group. Regarding ALAD
variants, ALAD 1-1 was predominant, whereas ALAD 1-2 had little prev-
ance. In short, children who live in fishing areas present a high risk of exposure to Pb that affects their physical and neurological develop-

1873 Behaviors, Motivations, Health Effects, and Biomarkers among Smoking and Non-Smoking E-Cigarette Users

T. Sussan1, M. Patatanian2, G. Roy2, J. Meinent2, F. Shahzad2, E. Tabassum2, J. Cohen2, R. Wise3, M. Blaha3, C. Fraser4, J. Holbrook2, Atlanta, GA; Colciencias-UniCartagena, Grants of exposure to Pb that affects their physical and neurological develop-
ment, as well as hematological status. Colciencias-UniCartagena, Grants

The use of electronic cigarettes (EC) has risen exponentially, and ECs are now the most popular tobacco product among teenagers in the US. EC manufacturers utilize marketing strategies to target both smokers and non-smokers, but it is unclear how perceptions, behaviors, and health effects differ between these groups. We conducted a survey of 320 adult EC users to determine demographics, behaviors, perceptions, and moti-
vations underlying use. Based on these results, a longitudinal trial of non-smoking EC users and non-users is currently being conducted to
determine oral, respiratory, and cardiovascular health effects and oral and
urinary biomarkers of use. Our survey respondents were predominantly
young adults, 74% were identified to be former smokers, while 20% identified themselves as current smokers and 6% were never smokers. Former smokers reported a longer history of EC use and higher nicotine concentrations than current smokers. For former and
current smokers, the primary motivation for EC use was to quit smoking, and nearly half indicated in the plan to reduce their nicotine concen-
tration and quit using ECs. Among former smokers, self-reports on use
and measures of dependence were consistent with nicotine replacement
as their primary motivation. The majority of former and current smokers also reported that their respiratory health had improved as a result of EC use, although this effect was stronger for former smokers. Never smokers reported less frequent EC use and dependence compar-
ted to former and current smokers. Their primary motivations for use were enjoyment and popularity, and they displayed a reduced desire to eventually quit using ECs. As of October 2017, 20 EC users and 20 non-
users were recruited in the VAPing Observational Research Study (VAPORS), and analysis of oral microbiome and urinary biomarkers is ongoing. These responses provide insight into the underlying behav-
iors of smoking and non-smoking EC users. Ongoing analysis of health effects and biomarkers among non-smoking EC users will improve our understanding of the physiological effects of chronic EC use.

1874 Dioxin-Like Compounds, Cytokines, and Hypertension in the Anniston Community Health Survey Follow-Up

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In 2014, we conducted the follow-up study (ACHS II) of the Anniston Community Health Survey (ACHS in 2005-7) where residents’ polychlo-
rinated biphenyls (PCBs) concentrations were 2-3 times higher than in the general US population. Results from ACHS showed significant associations between PCBs and hypertension/blood pressure. When endothelial cells are exposed to PCBs or other dioxin-like compounds in animal studies, inflammatory pathways may be activated and lead to increased expression of cytokines, which can result in the develop-
ment of atherosclerosis and hypertension. ACHS II included measure-
ments of dioxins in addition to PCBs as well as measurements of serum cytokine in order to further study the connection between these chem-
icals and hypertension. Serum samples and covariate information were available for 338 participants. Hypertension status was defined as being on antihypertensive medication or having a systolic/diastolic blood pressure greater than 140/90 mmHg; 262 (77.5%) participants were hypertensive. Cytokines measured included interleukin IL-1β, IL-2, IL-
4, IL-5, IL-12, IL-13, plasminogen activator inhibitor-1 (PAI-1), and tumor necrosis factor alpha (TNFα) using two separate multiplex bead arrays (HADK2MAG-61K and HADK1MAG-61K; EMD Millipore, Billerica, MA). The polychlorinated dibenzo-p-dioxins (PCDD), dibenzofurans (PCDF), and non-ortho PCBs were measured using high-resolution gas chroma-
tography/high-resolution mass spectrometry and expressed as dioxin
equivalent toxins (TEQs, pg/g lipid). To analyze the connection between cytokines and the various chemicals among hypertensive individuals we used multivariate linear regression, adjusting for age, sex, BMI, family history of high blood pressure, and smoking status. Among hyper-
tensive individuals, we found significant associations between PCDD TEQ and TNFα and PAI-1 (β=0.14, p=0.010). These relationships were not observed among non-hypertensive individuals. The associations observed here among the hypertensive participants support the findings that the chemicals may contribute to endothe-

1875 Perfluoroalkyl Substances in the Fernald Community Cohort: Exposure Distributions and Associations with Thyroid and Kidney Function

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Perfluoroalkyl substances (PFAS) are a diverse class of chemicals used in industry and consumer products to make materials resistant to friction, water, and oil. Exposure is ubiquitous in industrialized populations and those whose drinking water comes from contaminated groundwater, and previous human studies suggest exposure to PFAS is associated with adverse health effects. Associations between eight PFAS and in-
dicators of thyroid disruption and kidney function were examined using repeated measures of both PFAS and outcomes. Participants were selected from the Fernald Community Cohort based on household water supply from a PFAS-contaminated water source. Subjects had up to 3 repeated measures of serum PFAS (N=210 participants, N=517 total measurements) and several health exams. Kidney function (estimated glomerular filtration rate, eGFR) and thyroid stimulating hormone (TSH) were the focus endpoints. Linear mixed effects models were implemented to examine each association, adjusting for participant age, sex, and year of sample collection among other confounders. An interquartile (IQR) increase in serum perfluorononanoate (PFNA), per-
fluorohexane sulfonate (PFHxS), and perfluorodecanoate (PFDeA) was associated with a -1.66% (95% CI= -0.32, -0.23), -1.95% (95% CI= -3.41, -0.49), and -2.47% (95% CI= -4.48, -0.45) decrease in eGFR relative to the population mean, respectively. On the other hand, an IQR increase in serum 2-(N-methyl-perfluorooctane sulfonamido) acetate (Me-PFOSA-AcOH) and 2-(N-ethyl-perfluorooctane sulfonamido) acetate (Et-PFOSA-AcOH) was associated with a 1.66% (95% CI= 0.40, 2.93) and 1.27% (95% CI= 0.09, 2.46) increase in eGFR relative to the median. An IQR increase in serum perfluorooctanesulfonate (PFOS) was associated with a 10.21% increase in serum TSH (95% CI: 2.29, 18.74). In sex stratified models, an IQR increase in PFHxS was associated with a 24.1% (95% CI= 5.01, 46.66) increase in serum TSH in males; this association was not signifi-
cant in females however the interaction between PFHxS, sex, and TSH was significant (p<0.05). Our findings suggest an association between PFAS exposure and altered thyroid and kidney function in exposed communities.

1876 Systematic Review and Meta-Analysis of Diazepam and Labor Duration

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The therapeutic agents of the benzodiazepine (BZD) class, such as diaz-
epam (7-chloro-1,3-dihydro-1-methyl-5-phenyl-2H-1,4,benzodiazepin-
ium), exhibit the characteristic properties of the gamma-aminobutyric acid (GABA)-A receptors, a family of ligand-gated ion channels essential for the regulation of the central nervous system (CNS). In addition, GABA-A receptors are also located in the spinal cord and motor neurons, which mediate myorelaxant effects. Their potential role in labor is as yet unexplored. Therefore, we conducted a systematic review and meta-analysis to determine whether diazepam, a ligand of GABA-A receptors, impacts labor duration. We conducted literature searches in PubMed and Scopus and reviewed reference lists of review articles and original studies. We systematically and critically reviewed all the identified studies; for those with sufficient data, we used random-effects models to calculate summary mean differences between labor dura-
tion in women treated with diazepam and women in control groups. We conducted subgroup and sensitivity analyses when possible and assessed the presence of publication bias. We identified 12 studies for systematic review, none of which were of overall high quality, primarily due to co-administration of other analesic drugs during labor. Of the 12 studies, 9 had sufficient quantitative information to be included
a primary meta-analysis. We found that women treated with diazepam had a similar labor duration as placebo-treated controls (summary mean difference of labor duration: -0.41 hours; 95% CI: -0.97 to 0.16; I² = 63.2%; p-heterogeneity = 0.001), and this result was generally robust to several subgroup and sensitivity analyses. The funnel plot suggests that the presence of publication bias towards small studies favoring a shorter labor duration with diazepam treatment; however, Egger’s test for small study effects was not statistically significant (p = 0.07). Collectively, the clinical evidence indicates that diazepam does not prolong labor duration. This suggests that drugs currently being developed that target α2-containing GABA-A receptors are not likely to prolong labor via this mechanism.

1877 Endocrine-Disrupting Metals in Ambient Air May Be Associated with Increased Breast Cancer Incidence in the US

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Heavy metals are ubiquitous environmental pollutants. Recent studies have suggested that certain heavy metals could act as endocrine disruptors that mimic the actions of hormones, such as estrogens, and contribute to the development and progression of breast cancer. In this study, we examined the association between ambient air emissions of several endocrine-disrupting heavy metals and the incidence of female breast cancer in the United States by analyzing the county-level data from the Surveillance, Epidemiology, and End Results (SEER) Program and the National Emissions Inventory of the US Environmental Protection Agency. Linear regression analysis was conducted using SPSS 22.0 for Windows to examine the association in unadjusted and adjusted models. The adjusted annual age-adjusted incidence rates of female breast cancer were observed in the urban and more industrialized regions among the 9 SEER regions of the US over the time period of 1973-2014. Of the metals analyzed, air emissions of arsenic (β=5.21; p=0.004), cadmium (β=16.23; p=0.033), lead (β=375.66; p=0.001), and mercury (β=27.07; p<0.001), but not chromium VI (β=2.54; p=0.069), were found to be significantly associated with the incidence of female breast cancer after adjusting for race, poverty level, education, smoking prevalence, and obesity rate. Among these metals, emissions of lead showed the strongest association with breast cancer incidence. Our results demonstrated that exposure to heavy metals in ambient air may be associated with increased incidence of breast cancer. Further studies are needed to explore these interactions and to elucidate the mechanisms of action.

1878 Longitudinal Assessment of Liver and Kidney Functions among Adolescent Pesticide Applicators

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Egyptian adolescents work seasonally for the Ministry of Agriculture applying pesticides to cotton fields throughout the country. Organophosphorus (OP) pesticides are primarily applied to control cotton boll weevils. Liver and kidney impairments are major concerns among workers exposed to OP pesticides. The aim of the current study is to assess the effect of repeated exposure to OP pesticides on the liver and kidney functions among adolescent pesticide applicators. A total of 221 applicators were recruited from four field stations in Menoufia Governorate, Egypt, and they were followed for three consecutive years: 2014, 2015, and 2016. A non-applicator group was included during 2015 and 2016. Participants were examined three times each year: during the application season, immediately following the end of the season, and two months after the season ended. Blood samples and baseline medical information were collected at each test session and analyzed for cholinesterase activity, and liver and kidney functions. Functions included alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase, (ALP), gamma glutamyl transpeptidase (GGT), total bilirubin (TB), direct bilirubin (DB), total protein (TP), albumin, urea, and creatinine. While both acetyl cholinesterase (ACHE) and butyryl cholinesterase (BChE) activity showed changes over time, inhibition during exposure, and recovery after the end of exposure, only BChE exhibited differences between applicators and non-applicators over the three years of the study. The majority of serum measures of liver and kidney functions (ALT, AST, ALP, GGT, TB, urea, and creatinine) showed deterioration immediately after the end of the application season compared to during the application season, and then recovery two months after application had ended. Across the three years, serum GGT and TB did not return to baseline levels, suggesting a long-term impact on these measures of liver function. Exposure to OP pesticides significantly influenced several serum measures of liver and kidney functions, particularly immediately following application. Study outcomes demonstrate the sensitivity of liver and kidney function to exposure to OP pesticides and reflect that repeated exposure to pesticides may lead to progressive deterioration of these functions.

1879 Spatial Patterns of Childhood Leukemia Incidence and Air Toxics in Pennsylvania


Previous studies have examined childhood leukemia incidence with exposure to traffic- and industrial-related air toxics. While many of these studies used surrogates for these exposures, such as proximity to major roadways and traffic density, only a few have estimated rates of ambient concentrations of carcinogenic air toxics. Our goal was to examine potential associations for estimated concentrations of outdoor air toxics with childhood leukemia incidence. We obtained modeled, census tract-level concentrations of air toxics from the 2005 National-Scale Air Toxics Assessment (NATA). Incident leukemia cases (n = 1,245) in children and teens under 20 years of age in 2007-2014 were obtained from the Pennsylvania Cancer Registry. We calculated age-, sex-, and race-adjusted standardized incidence ratios (SIRs) for the study population at the census tract level using the expected number of leukemia cases based on the national average of leukemia incidence rates from the Surveillance, Epidemiology, and End Results Program. Exploratory spatial data analysis was used to assess spatial patterns of leukemia SIRs across Pennsylvania over the study period. We conducted spatial and temporal analyses to examine associations between air toxics and leukemia SIRs, adjusting for sociodemographic covariates derived from the US Census Bureau. The present analysis revealed that higher concentrations of formaldehyde and tetrachloroethylene were significantly associated with higher leukemia SIRs with coefficients of 0.22 (S.E.=0.11, p=0.042) and 0.83 (S.E.=0.42, p=0.048), respectively, after adjustment for sociodemographic factors. Benzene and 1,3-butadiene, which previous studies found were associated with increased childhood leukemia incidence, were positively but not significantly associated with leukemia SIRs in our analysis. In this analysis, air toxics such as formaldehyde and tetrachloroethylene are associated with higher incidence of childhood leukemia. Primary prevention strategies for childhood leukemia could incorporate the identification and elimination of sources of these air toxics.
Some investigators have suggested that personal cosmetic talc use is a risk factor for mesothelioma and ovarian cancer, while others maintain that the weight of evidence clearly establishes that cosmetic talc does not increase the risk of either disease. In this evaluation, we assessed the temporal association between U.S. cosmetic talc consumption and the incidence of mesothelioma and ovarian cancer in females. A similar investigation comparing peak asbestos consumption rates in the US demonstrated a corresponding peak in incidence of mesothelioma in males after an appropriate latency period. In this analysis, we evaluated cancer incidence in women because (1) many talc-containing products are marketed specifically for use by women, and (2) women are less likely to have experienced significant (confounding) workplace exposures to asbestos. Cosmetic talc usage rates by year (metric tons per year from 1975 to 2003), which were compiled by the United States Geological Survey (USGS), indicate that product use peaked in 1977 at approximately 70,000 metric tons per year and was never less than 16,000 metric tons per year during that time frame. Throughout the post-1975 time frame, several studies have reported that the rates of pleural and peritoneal mesothelioma in women has remained constant. Our evaluation of the Surveillance, Epidemiology, and End Results (SEER) 9 cancer registry database (1973-2014) is consistent with these findings and shows an age-adjusted incidence rate of mesothelioma for women in the range of approximately 2.5 to 4.9 per million. Similarly, age-adjusted incidence rates in the SEER registry for ovarian cancer remained stable over the last several decades, and have actually declined somewhat from approximately 16 annual cases per 100,000 in 1975 to 11 annual cases per 100,000 in 2014. We suggest that if cosmetic talc use was truly a significant risk factor for either disease, then given the decades of documented extensive use, an observable increase in these diseases would have been observed at some point in the last 10 to 20 years. These findings are consistent with the National Occupational Mortality Surveillance (NOMS) database, which has consistently reported no increased incidence of female pleural or peritoneal mesothelioma in occupations that are expected to routinely use cosmetic talc, such as hairdressers, barbers, and cosmetologists.
Textbooks of epidemiology on the use of the Bradford-Hill criteria (or considerations) have consistently devalued if not eliminated the analogy criterion for causal inference. As part of a broader inquiry into the practice of causal inference, I conducted a systematic review of the literature to examine whether the practice of causal inference reflects this methodological development. PubMed and TOXLINE were searched for English-language publications that refer to Bradford-Hill (i.e. methods papers) and/or employ the criteria (i.e. application papers) and were published between 1990 and the present. Of 247 publications identified, 46 met inclusion criteria. Of these 10 methods papers accurately reflect the idea promulgated in the textbooks that analogy can be achieved by simple similarity arguments (subject to the imagination of the authors), that it is a weak association, that it “doesn’t work”, and may be detrimental, that it is equivalent to coherence or biological plausibility, or that it should be abandoned altogether. Of the 36 practice papers where criteria were applied (with specific reference to Bradford-Hill), 7 authors ignored analogy and the remainder employed the simplistic notion that if A causes a disease B then “by analogy” a similar exposure A’ is likely to be a cause of B or any other disease. Analogy in theory and in practice has been effectively eliminated from causal inference in epidemiology. Even in situations in which the simplistic “similarity” argument is employed, the remaining criteria—e.g. strength, dose-response, consistency, and biological plausibility—reign.

Multiple epidemiological studies have consistently demonstrated that high levels (100-1,000 µg/L) of arsenic in the drinking water is associated with a marked increase in lung cancer risk. The same has not been consistently demonstrated at low levels. We have conducted an analysis of lung cancer incidence and arsenic levels in groundwater used as drinking water supply for US counties to examine the relationship at low (<50 µg/L) arsenic levels, the historical limit for both EPA and WHO. The exposure data are from the USGS ground water arsenic database which have been used in multiple studies and the outcome data (lung cancers, 2009-2013) are from the NCI. Co-variables are from the US Census Bureau and the CDC. The exposure metric was the median drinking water well arsenic level. Poisson analyses were conducted at low (<50 µg/L) arsenic levels, the historical limit for both EPA and WHO.

Chronic, low-dose exposure to inorganic arsenic (iAs) is connected to high incidences of cancer in the United States and worldwide. iAs has been attributed, in part, to changes in the epigenome, although studies of trans-generational effects on gene expression. Recent data has emerged on the adverse effects of MeHg and the nutritional intake (TWI) in 2012 for MeHg. However, new epidemiology data has emerged on the adverse effects of MeHg and the nutritional benefits from fish consumption. We reviewed the current literature and developed a graphical data compendium to visually present the data from epidemiology studies on neurodevelopmental effects of MeHg. We identified over 50 studies in the literature published since 2000 on prenatal exposure to MeHg and subsequent neurodevelopmental outcomes in children. Because these studies relied mostly on regression or correlation analyses, we compiled central tendency values to describe exposure. The reported values were graphically presented in a series of data-array figures and organized by biomarker, child age, cohort location, and neurodevelopmental outcome. By inspecting the visual array of data, a few key observations emerge. Adverse effects in childhood persisted into adulthood in some cohorts but not in others. While the majority of studies found detrimental effects of MeHg exposure on neurodevelopment, some studies did not find adverse effects of MeHg, despite adjusting for multiple confounders. Effects were observed at different levels of MeHg exposure, including estimated doses comparable to the EPA RfD, JECFA PTWI, and EFSA TWI. We conclude that the graphical analysis of this complex body of research is helpful in highlighting possible trends and uncertainties for further investigation.

Epigenetic (EG) changes induced by therapeutic agents and environmental xenobiotics have trans-generational effects on gene expression. EG alterations are inheritable and have significant potential to trigger pathological conditions. The addictive potential of opioids during fetal development can induce EG modifications, resulting in altered physiological states. To determine the mechanism whereby EG changes from opioids influence developmental progression, mouse embryonic stem (mES) cells were exposed to 1 to 1000 µm morphine sulfate (MS) for 24 hrs. Cell viability, global DNA methylation, and gene expression of DNA methyltransferases (i.e. DNMT1, DNMT3a, DNMT3b) were measured at the 24 hrs with RT-PCR and ELISA using corresponding anti-S-methylcytosine antibody. Cell viability of mES cells significantly decreased at 1000 µm MS only (P<0.001, N=3) but was unaffected at lower concentrations. Global DNA methylation levels were increased at 1 µm MS (apparent hormesis effect; P<0.001, N=3) yet decreased at 10 µm and 100 µm MS (P<0.01 and P<0.001, respectively, N=3). While no net changes were detected in DNA methylation levels following MS exposure up to 1000 µm, the cells exhibited concentration-dependent down regulation (1.0 to 2.5-fold) of all MT’s, with DNMT3a demonstrating the most sensitive response. The results suggest that opioids, at concentrations less than those needed to suspend viability, alter EG modifying enzymes during stem cell differentiation. DNMT1 is usually referred to the “maintenance methyltransferase” since it has a higher preference for hemi-methylated DNA. DNMT1 targets the DNA replication fork, by factors such as HHR1, that recognizes hemi-methylated DNA. HHR1 and UHR2 are members of the SRA family of MT’s (SET and B-box domains) that recognize (P<0.001) ubiquitous-like, domain-containing proteins that recognize and join with hemi-methylated DNA, and are essential for maintenance methylation. DNMT3a and -3b are the predominant de novo MT’s, while the latter is also involved with methylation of satellite DNA and pericentromeric regions. In particular, these MT’s are crucial for normal embryonic progression, expansion, and differentiation, thus supporting the understanding that opioids have high potential to cause a fetal addiction syndrome through the alteration of EG pathways.

Chronic, low-dose exposure to inorganic arsenic (iAs) is connected to high incidences of cancer in the United States and worldwide. iAs has been associated with the epithelial-to-mesenchymal transition (EMT) in which cancer cells lose their adhesion properties and increase in motility, allowing them to travel to other parts of the body and metastasize. The mechanism behind iAs-mediated carcinogenesis and EMT has been attributed, in part, to changes in the epigenome, although studies on this are limited. These changes can include histone post-translational modifications, incorporation of histone variants, or DNA methylation reprogramming. Our use of high resolution studies has identified...
changes in DNA methylation patterns and histone H2B variant regulation as key players in IA-mediated carcinogenesis and EMT. Using Methyl-seq, we show genome-wide and gene-specific changes in DNA methylation patterns that correlated to changes in gene expression in response to IA exposure. After removal of IA exposure, we observe moderate reversal of DNA methylation changes, gene expression patterns, and EMT. We also identified ten histone H2B variants that are dysregulated during IA-mediated carcinogenesis using top-down mass spectrometry. These variants are possibly modulating DNA compaction around the nucleosome, thereby altering gene expression patterns in chronically IA-treated cells. Our studies indicate that inorganic arsenic-mediated carcinogenesis is instigated, in part, by changes to the epigenome including DNA methylation and histone H2B variants.

**1890 Regulation of Chromatin Assembly and Cell Transformation by Exposure to a Physiologically-Relevant Concentration of Formaldehyde**

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Formaldehyde (FA) is an environmental and occupational chemical carcinogen. Most studies of FA-induced carcinogenicity have focused on DNA damage and mutagenesis induced by DNA adducts and DNA-protein crosslinks, however, little is known about the molecular mechanisms responsible for FA-induced epigenetic dysregulation. Previously we demonstrated that exposure to 0.5 mM of FA dramatically decreases lysine acetylation of the N-terminal tails of cytotoxic histones H3 and H4, thereby inhibiting chromatin assembly. Endogenous FA concentration in human blood ranges between 20 μM and 100 μM. Thus, to test whether similar outcomes can be seen at a more relevant FA dose, we treated human BEAS-2B cells with 100 μM FA and studied its effect on histone modification, chromatin assembly, transcription, and cell transformation. Continuous exposure of cells to the physiologically relevant concentration of FA significantly reduced the levels of cytotoxic H3K9&K14Ac and H4K12Ac. The reduction was likely due to the formation of FA-histone lysine adducts that give lysine formylation and Schiff base were detected by mass spectrometry in cells. Cellular fractionation and Western blot analysis show the amount of histone H3 in chromatin fraction decreases by about 20%; chromatin immunoprecipitation assays show the level of histone H3, an H3 variant, is significantly reduced at the majority of loci tested, suggesting that FA compromises chromatin assembly at a physiologically relevant concentration. Moreover, knockdown of the H3.3 gene, which mimics inhibition of chromatin assembly, altered expression of a number of cancer-related genes deregulated by FA and facilitated FA-mediated anchorage-independent cell growth. These results suggest that defective chromatin assembly may play important roles in FA-induced transcriptional deregulation and cell transformation. This work was supported by NIH grants R01ES026138-01, 3P30ES00260, R03ES024147, and R01GM099409 as well as National Natural Science Foundation of China Grant 31329003.

**1891 Characterization of Functional and Molecular Endpoints of Potential Adverse Health Effects Associated with Age, Diet, and Occupational Exposure in an Animal Model**


The exposome is the measure of all exposures of an individual in a lifetime from conception to death and how those exposures affect health. An individual’s exposome is highly variable and dynamic throughout their lifetime. The goal was to design an exposure paradigm that would address multiple exposome components, including lifestyle (e.g., diet), age, and occupational exposure (welding fume; WF) in a controlled animal model. Functional and molecular endpoints predictive of adverse health effects were identified by biological fluids of exposed animals that are translatable to human populations were examined. Male Fischer 344 rats were maintained on a high fat western (HF) or regular (REG) diet for 24 wk. At wk 7 during diet maintenance, groups of rats were exposed by inhalation of stainless steel WF (20 mg/m³ x 3 hr/d x 4 d/wk x 5 wk) or filtered air (control) until wk 12 at which time some animals were euthanized. A separate set of rats were allowed to recover from WF exposure until the end of the 24 wk period. Whole blood and bronchoalveolar lavage fluid were collected at wk 7 (baseline before WF exposure), 12, and 24 wk to assess blood cell differential and to recover serum, peripheral blood mononuclear cells (PBMCs), and lung phagocytes for epigenetic analysis and immune response. Significantly elevated % change in body weight and serum triglycerides were observed in groups maintained on the HF diet. At all time points, plasma triglycerides were higher by recovered phagocytes and PBMC telomere length were significantly decreased in the REG+WF, HF+air, and HF+WF groups compared to the REG+air group. A significant decrease also was observed in telomere length over the 24 wk regimen in all groups. In summary, age, diet, and occupational exposure (WF inhalation), important exposome components, altered immune response and epigenetic endpoints in rats. An animal model may be advantageous for studying the exposome because of the ability to control all external exposures and to measure potential adverse health outcomes of each animal over its entire lifespan and to link a specific internal biological response/endpoint with a specific exposure.

**1892 Combinatorial Effect of Butyl Benzyl Phthalate and a High-Fat Diet on Epigenetic Regulation of Adipogenesis and Obesity**

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Obesity has reached epidemic proportions worldwide. Exposure to endocrine disrupting chemicals (EDCs) such as those found in plastics may influence the development of obesity. Our studies investigate the effect of the EDC butyl benzyl phthalate (BBP) in combination with a high-fat environment on epigenetic regulation of obesity. We hypothesize that BBP and a high-fat diet (HFD) act synergetically to enhance obesity via epigenetic regulation of microRNA (miR) promoter methylation. C57Bl/6 male (8 weeks old) mice were fed chow diet (CD) (4% kcal from fat) or HFD (60% kcal from fat) with or without BBP (3 mg/kg/day) for 12 weeks. Mice were assessed for body weight and food intake and adipose tissue weights were measured. 3T3-L1 preadipocytes were treated with BBP, palmitic acid (PA), or a combination of both to induce adipogenesis. Adipogenic characteristics and epigenetic regulatory mechanisms were analyzed in mature adipocytes. Mice fed HFD+BBP had significantly increased body weights compared to HFD alone, and CD and CD+BBP fed mice (P<0.01 for HFD and P<0.001 for CD groups). Mice fed HFD+BBP also had significantly larger WAT weights compared to HFD (P<0.01) or CD groups (P<0.001). BBP (1 μM, 10 μM, 50 μM) exposure in 3T3-L1 cells induced adipogenesis in a dose-dependent manner, and BBP+PA increased adipogenesis over individual treatments with a significant upregulation of adipogenic gene expressions compared to PA alone (P<0.001). BBP significantly upregulated miRs-34a, 103, 107, and 125a expression in mature adipocytes (P<0.01). BBP+PA further significantly upregulated miR-34a, 103, 107, and 125a expressions compared to control or PA (P<0.001). DNA-methyltransferase (DANMT) expression in mature adipocytes were significantly decreased with BBP treatment (P<0.01) and further decreased in combination with PA (P<0.001). Concurrently, global DNA methylation was significantly reduced with BBP treatment (P<0.05) and further decreased with BBP+PA treatment (P<0.001 compared to control; P<0.05 compared to PA alone). Thus, BBP and a high-fat environment increased obesity in mice and increased adipogenesis in vitro. BBP and PA increased adipogenic miR expression while decreasing DNA methylation and DNMT expression. We are currently investigating miR promoter methylation in adipogenesis. This suggests that an HFD and EDC exposure can worsen an individual’s metabolic health by altering the epigenome program.

**1893 N-Ethyl-2-Pyridoline and DNA Methylation: Identification and Verification of Target Genes by Next-Generation Sequencing and Mass Spectrometry**

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N-ethyl-2-pyridoline (NEP) is frequently used as an industrial solvent and classified as a reproductive toxicant. However, no studies are available on the mode-of-action of NEP. Therefore, we orally exposed rats (n=5 per dose group) to 0, 5, 50, and 250 mg NEP/kg/d up to 28 days and studied the influence of NEP on DNA methylation, an early epigenetic event, in tissue samples of the liver, kidney, adrenal gland,
thymus, testis, epididymis and ovary. When directly comparing the 0 and 250 mg/kg/d dose groups, next generation sequencing (NGS) of enriched methylated DNA regions showed methylation changes predominantly in the liver, kidney, thymus and the adrenal gland of females but not in the ovaries, whereas the testes, kidney and liver was predominantly affected in males. In general, methylation differences were (up to 10-fold) more pronounced in the lab samples that were more affected by NEP rather than females. Strongly affected genes in both males and females were Map3k5, Vomr272, and Phka2. In addition, prominent methylation differences in Zbtb7a, Ehm2, and Man1a1 were observed in males and Golt1b and Mageb4 in females. All changes were found when studied both, the median and maximum observed differences of the enriched methylated DNA regions sequenced by NGS. By using MALDI-TOF MS as an independent technique we verified the NGS data. Wherever a full coverage by specific amplicons of the methylated DNA regions was possible in the MS-based approach, the NGS data could be confirmed by MS. In particular, Map3k5 showed prominent methylation differences (all hypermethylation) in almost all tissue samples, in both sexes, in all exposed dose groups, and both by NGS and MS. In addition, methylation differences of Phka2 and Vomr272 could be confirmed by MS in selected tissues of males and are also most likely due to sex NEP. Overall, our studies suggest that NEP might cause alterations in the MAPK and JNK-pathways and in carbohydrate and glycogen metabolism of rats. Ongoing studies investigate whether the observed effects on DNA methylation influence the downstream regulation of the respective genes.

1894 Exposure to Pesticides Alters the Sperm DNA Methylation in Sprayers from Nayarit, Mexico
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Pesticides are contaminants that represent a health problem worldwide. Their excessive use causes reproductive problems, including damage to sperm DNA and these alterations have been related to epigenetic modifications. DNA methylation is the most studied epigenetic mechanism, which involves the transfer of a methyl group to DNA in CpG sites catalyzed by DNA-methyltransferases. DNA methylation is crucial in spermatogenesis and it is known that methylation profiles in somatic cells. Our objective was to evaluate the DNA methylation in spermatoozoa of men exposed to pesticides and its relationship with the semen quality and DNA damage. A cross-sectional pilot study was conducted in urban sprayers and in a non-exposed group (n=60) from Nayarit, Mexico (Pacific coast). We evaluated the semen quality (WHO guidelines), the genetic damage by CSPA (Sperm chromatin structure assay, %DFI-DNA fragmentation index), and the global sperm DNA methylation (%5mC) using LINE1 by ELISA (Active motif methylation assay, %DFI-DNA fragmentation index), and the global sperm DNA methylation (%5mC) using LINE1 by ELISA (Active motif methylation assay). DNA methylation and gene expression were measured in CD14+ monocytes isolated from neonatal blood cell subtypes is uncharacterized. AHRR DNA methylation and gene expression were measured in CD14+ monocytes isolated from cord blood collected from nonsmoking (n=15) and smoking mothers (n=29). Cytotline levels were analyzed in cord blood serum. Bisulfite-treated DNA was pyrosequenced across 6 AHRR CpG sites. Gene expression of AHRR, and 3 additional candidate genes (CYP1B1, TMEM176A, and TMEM176B) was measured using RT-PCR. Across the 6 AHRR CpG sites we found a 9-15% methylation decrease in smoking mothers relative to nonsmoking mothers, with the most significant decrease at the CpG located at chr5:373490 (-15%, p=4.77E-06). In cord blood monocytes, no significant smoking-associated changes in gene expression of AHRR, CYP1B1, TMEM176A, or TMEM176B were observed. The CpG site at chr5:373378 (cg05575921) is widely accepted as a biomarker of smoking in neonatal and adult blood, consistent with our results in monocytes (-10.6%, p=2.60E-06). However, previous work in adults has revealed that several nearby CpGs in AHRR show similar or greater changes. This was less of an AHRR methylation decrease in cord blood monocytes relative to our previous findings in adult blood monocytes (-10% in cord blood vs -25% in adults). This effect size difference may be due to lower levels of tobacco use in pregnant women and, fetal blood may receive a lower dose of the active compounds in tobacco smoke due to maternal metabolism and the placental barrier. In adult monocytes, AHRR methylation level and gene expression are strongly correlated, however we found no significant relationship in cord blood monocytes. AHRR expression in adult monocytes is a transient response requiring recent smoking, thus time variation since the last cigarette smoked by mothers may introduce variation in gene expression. In this study, highly significant smoking-associated changes in AHRR methylation levels in cord blood monocytes were observed. However, relative to adults, we found more variability in cotinine, AHRR demethylation, and AHRR gene expression among subjects thus limiting statistical power to observe associations.

1895 Resveratrol Attenuates Allergic Asthma and Associated Inflammation in the Lungs through miRNA Regulation
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Asthma is an allergic condition characterized by airway hyper-responsiveness and increased bronchial spasm, inflammation and mucous secretion. More than 25 million Americans suffer from asthma, with children constituting around 7 million. The inflammation in asthma is mediated by Th2 cell activation with increased levels of IL-4, -5, and -13. Resveratrol (3,4,5-trihydroxystilbene)- a polyphenolic stilbene, has been shown to mediate anti-inflammatory properties. In the current study, we investigated if resveratrol could suppress allergic asthma. To that end, we induced asthma in BALB/c mice by injecting ovalbumin (OVA) with aluminum hydroxide intraperitoneally followed by 7 days treatment with resveratrol (100mg/kg) by oral gavage. Intranasal ovalbumin inhalation was given on day 0 and days 7 and 14. We found that resveratrol significantly attenuated the asthmatic lung (induced by ovalbumin) and caused reduction in CD3+CD4+ and CD3+CD8+ cell numbers in pulmonary tissue of sensitized mice when compared to vehicle controls. There was significant reduction in T-helper cells both in pulmonary tissue and BALF in resveratrol treated group with reduced levels of IL-5, IL-13, GM-CSF and TNF-a in BALF. Interestingly, miRNA-34a was downregulated in cells from the lungs of asthmatic mice treated with resveratrol. Ingenuity pathway analysis (IPA) demonstrated that miRNA-34a was targeting FOXP3, the transcription factor for Tregs that are highly immunosuppressive. Moreover, our PCR results showed that resveratrol-treated OVA mice expressed higher levels of FOXP3 in lung-infiltrating mononuclear cells. Immunofluorescent staining of pulmonary tissue also showed higher expression levels of FOXP3-expressing mononuclear cells in resveratrol treated mice compared to control group. Overall, our studies suggest that resveratrol treatment may be a potential therapeutic agent for asthma.

1896 Maternal Smoking, AhRR Methylation, and Gene Expression in Umbilical Cord Blood CD14+ Monocytes
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Sponsor: C. Keshava

Tobacco use during pregnancy is a major risk factor for adverse outcomes in children. While smoking is strongly associated with epigenetic modifications in whole blood DNA in both adults and neonates, the relationship between epigenetic modification and gene expression in cord cell subtypes is uncharacterized. AHRR DNA methylation and gene expression was measured in CD14+ monocytes isolated from neonatal cord blood collected from nonsmoking (n=15) and smoking mothers (n=29). Cotinine levels were analyzed in cord blood serum. Bisulfite-treated DNA was pyrosequenced across 6 AHRR CpG sites. Gene expression of AHRR, and 3 additional candidate genes (CYP1B1, TMEM176A, and TMEM176B) was measured using RT-PCR. Across the 6 AHRR CpG sites we found a 9-15% methylation decrease in smoking mothers relative to nonsmoking mothers, with the most significant decrease at the CpG located at chr5:373490 (-15%, p=4.77E-06). In cord blood monocytes, no significant smoking-associated changes in gene expression of AHRR, CYP1B1, TMEM176A, or TMEM176B were observed. The CpG site at chr5:373378 (cg05575921) is widely accepted as a biomarker of smoking in neonatal and adult blood, consistent with our results in monocytes (-10.6%, p=2.60E-06). However, previous work in adults has revealed that several nearby CpGs in AHRR show similar or greater changes. There was less of an AHRR methylation decrease in cord blood monocytes relative to our previous findings in adult blood monocytes (-10% in cord blood vs -25% in adults). This effect size difference may be due to lower levels of tobacco use in pregnant women and, fetal blood may receive a lower dose of the active compounds in tobacco smoke due to maternal metabolism and the placental barrier. In adult monocytes, AHRR methylation level and gene expression are strongly correlated, however we found no significant relationship in cord blood monocytes. AHRR expression in adult monocytes is a transient response requiring recent smoking, thus time variation since the last cigarette smoked by mothers may introduce variation in gene expression. In this study, highly significant smoking-associated changes in AHRR methylation levels in cord blood monocytes were observed. However, relative to adults, we found more variability in cotinine, AHRR demethylation, and AHRR gene expression among subjects thus limiting statistical power to observe associations.
DNA methylation status changes as a function of age in humans and animal models, a process that may play a role in chronic disease development. Recent reports have shown toxicant-mediated shifts away from the baseline rate of age-related DNA methylation, a concept termed “environmental deflection.” We tested the hypothesis that developmental exposure to bisphenol A (BPA) results in environmental deflection of the epigenome, as manifest via longitudinal epigenome-wide DNA modifications in matched mouse blood (2, 4, and 10 months of age). To characterize whether the aging epigenome is driven by changes in 5-methylcytosine (5-mC) or 5-hydroxymethylcytosine (5-hmC), DNA modifications were measured in longitudinal blood samples from isogenic mice developmentally exposed to Control (n=18) or Control+50 µg/kg diet BPA (n=18) diets. Genome-wide 5-mC and 5-hmC levels were measured using two genome-wide sequencing methods -- enhanced reduced representation bisulfite sequencing (eRRBS) and hydroxymethylated DNA immunoprecipitation sequencing (HMeDIP-seq). Examining the epigenome by age, we identified 38,300 uniquely differentially methylated CpGs (DMCs) and 8,613 differentially hydroxymethylated regions (DHMRs). Comparing age-related DMCs and DHMRs, 1,854 annotated genes showed both differential 5-mC and 5-hmC by developmentally exposed to BPA exposure. When testing for environmental deflection by BPA exposure using an age:exposure interaction term, we identified 54,693 significant DMCs, but zero significant DHMRs. Using ChIP-enrich, we tested DMCs and DHMRs for enriched gene ontology pathways. Combined, our genome-wide results show age- and BPA-related differential 5-mC and 5-hmC, including one gene - Nfic - at the exact same chromosomal region. At this shared region, 5-mC and 5-hmC levels both decreased with age. Reflecting these age-related epigenetic changes, Nf1c RNA expression from blood samples also decreased with age. In separate analyses by BPA exposure, we identified 64,284 unique DMCs and 9,950 DHMRs. One annotated gene - the imprinted locus Gnas - showed both significant age-related 5-mC and 5-hmC by developmentally exposed to BPA exposure. When testing for environmental deflection by BPA exposure using an age:exposure interaction term, we identified 54,693 significant DMCs, but zero significant DHMRs. Using ChIP-enrich, we tested DMCs and DHMRs for enriched gene ontology pathways. Combined, our genome-wide results show age- and BPA-related differential 5-mC and 5-hmC, but only minimal evidence for environmental deflection of age-related methylation by developmental exposure.

Phthalates have been linked to adverse pregnancy complications. Placental hypoxia plays an important role in the pathogenesis of the hypertensive disorder of pregnancy preeclampsia. MiR-210-3p (hypo-ami) has been shown to be upregulated in the placenta and plasma of patients with preeclampsia. A recent epidemiological study showed an association also resulted in differential methylation of genes in BAT. A subset of differentially methylated DNA loci in BAT were also detected in blood, demonstrating a treatment-specific consistency across tissues. These data suggest a role for the epigenome in mediating DDT-impaired thermogenesis.

Epigenetic regulation is considered as the mediator between gene and environment interaction. Previously, we have demonstrated that the methylation of tripartite motif containing 36 gene (TRIM36) enhanced in the course of cell malignant transformed induced by PAHs in a dose-dependent manner. The dynamic changes in methylation of tripartite motif containing 36 gene (TRIM36) CpG site 4, 5, 6, 8 was demonstrated that the methylation of TRIM36 hot spots methylated in the peripheral blood lymphocytes (PBLCs) of the subjects. The difference and correlation between the exposure, TRIM36 methylation, and DNA damage were analyzed to characterize the changes of TRIM36 hot spots methylation. We revealed that specific CpG site 4, 6, 8 of TRIM36 promoter is specifically hypermethylated in COEs-, and As-exposed population but hypomethylated in the DE- and benzene- exposure workers. The methylation of TRIM36 responds to different environmental exposure in a dose-dependent manner. Moreover, the status of TRIM36 methylation was negatively correlated with the gene expression in the COEs- and benzene-exposed workers, demonstrating that TRIM36 gene expression is regulated by DNA methylation. The mediation analysis indicated that genetic damage might be mediating the aberrant TRIM36 methylation. Combined analysis from COEs- and DE-exposed population demonstrated that the methylation of TRIM36 CpG site 4, 5, 6, 8 was correlated with levels of PAHs exposure and extent of genetic damage in a dose-dependent manner. The dynamic changes in methylation of TRIM36 suggest that CpG site-specific alterations might be attributable to mediate the adverse health effects and be the candidate biomarker for monitoring PAHs exposure.
1901 Placental Methylation at Metastable Epialleles Is Predictive of Childhood BMI at Ten Years of Age

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The placenta is a critical organ for understanding both fetal and later life disease. Metastable epialleles (MeS) represent time and tissue stable sites of methylation. Additionally, these sites can be influenced via cytosine methylation (CpG) by detrimental conditions surrounding conception. Thus, placental CpG methylation patterning at metastable epialleles could provide mechanistic understanding into the developmental origins of health and disease (DOHaD) hypothesis. Here we set out to assess placental CpG methylation at predicted metastable epialleles to understand their influence on later life body mass index (BMI). Placental methylation was measured using the Illumina 450k and 850k arrays in 202 placental samples. A total of 21 probes were identified across arrays representing candidate metastable epialleles. Infants in the study were subsequently followed up for height and weight measurements at 1, 2 and 10 years of age. Placental methylation at the metastable epialleles was tested for association to BMI at each of the follow up time points. A total of six sites, namely cg12004671, cg22757362, cg27225663, cg4878745, cg12949927, and cg13786083 displayed association with BMI at age, 2 or 10 years of age after adjustment for race, sex, socio-economic status, and gestational age. For these sites, cg048788745, cg12949927, and cg27225633 increasing methylation was associated with decreased BMI, while cg12004671, cg12949927, and cg22757362 increasing methylation was associated with increases in childhood BMI. These data represent sites in the epigenome associated with later life health outcomes, suggesting that placental methylation could provide predictive capacity for children’s health.

1902 Benzyl Butyl Phthalate (BBP) Regulates IncRNA H19 during Adipogenesis in C3H10T1/2 Stem Cell Line

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Over the last decade, there has been a dramatic and rapid deterioration in metabolic health primarily due to the emergence of the obesity epidemic and the rise in diabetes rates. Our studies show that BBP, a widespread endocrine disruptor, induces epigenetic stress during adipogenesis in C3H10T1/2 stem cell and 3T3-L1 preadipocyte, which may be one of the contributions to recent metabolic disorder crisis. In the present study, we hypothesized that BBP may deregulate long non-coding RNA (lncRNA) H19 to induce adipogenesis in mesenchymal stem cells (C3H10T1/2). After confluence, C3H10T1/2 cells were treated with or without BBP (0.01 µM, or 50 µM) for 8 days. Cells were then harvested and RNA was extracted on day 2, 4, 6, and 8. Our results showed that gene expression of H19 was significantly decreased from day 2 to day 4 under 50 µM BBP exposure (P<0.05). However, there was no significant change observed at day 6 and day 8. Consistently, RNA expression of miR-103/107, one target of H19 and implicated in adipogenesis, was also significantly increased from day 2 to day 4 (P<0.05 or P<0.01), except for let-7e. On day 6 though, only let-7b was significantly increased. Interestingly, insulin receptor substrate 1, one of the key insulin signal transduction regulators, was significantly downregulated from day 2 to day 8 under 50 µM BBP exposure (P<0.01). However, gene expression of insulin receptor and insulin receptor substrate 2 was not altered by BBP exposure. Our study suggests that IncRNA H19 may regulate adipogenesis at an early time point and BBP can lead to increased adipogenesis and metabolic dysregulation by impairing vital epigenetic regulators such as IncRNA H19 and its miRNA targets.

1903 MicroRNAs Influence Inter-Individual Variability in the Expression of Drug Metabolizing Enzymes and Transporters


Among humans, individuals exhibit a significant disparity in drug sensitivity, efficacy and toxicity which is largely due to inter-individual variability in the expression of genes encoding drug metabolizing enzymes and transporters (DMETs). MicroRNAs (miRNAs) belong to a group of non-coding RNAs that control gene regulation epigenetically. During the last decade there has been growing interest in the role of epigenetic factors, including the role of miRNAs, in the regulation of DMET expression. Many studies have reported correlations between the expression of specific miRNAs and the expression of DMETs; however, only a small number of studies have demonstrated the mechanisms underlying the correlations. To investigate the mechanisms by which miRNAs regulate DMET expression in human liver, we systematically analyzed expression files for 370 DMET genes and approximately 1200 miRNAs, selected candidate miRNAs that could target mRNA transcripts for key DMETs, and then applied a series of biochemical and biological assays to elucidate the molecular basis for miRNA-dependent DMET gene regulation. Reporter gene assays, electrophoretic mobility shift assays, enzymatic assays, proteomic assays, and bioinformatics analyses provided in vitro, in vivo, and in silico evidence establishing the specificity and potency of miRNA-dependent molecular mechanisms suppressing the expression of CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4, ALDH5A1, SULT1A1, SULT1A2, and SULT2A1 at an inhibition level of 15-35%. Furthermore, we applied integrative approaches to show that interactions between miRNAs and drugs can influence drug-drug interactions. Notably, these results provide molecular and biochemical insights into how miRNAs modulate DMET gene expression and contribute to variations in drug metabolism and toxicity in humans.

1904 Mechanism of Trans-Generational Inheritance of Reproductive Dysfunction Stemming from Environmental Bisphenol A Exposure in C. elegans


Our work aims to characterize the molecular mechanisms of memory of environmental exposures, using Bisphenol A (BPA) as a model chemical. Utilizing a transgenic repetitive array assay, carried out on the model organism C. elegans, we identified a germline desilencing response that lasted at least five generations, with and without initial ancestral exposure. The desilencing response was coupled with changes in gene expression and sterility at the F2 generation, with some F3 and later generations losing the original BPA response. The desilencing effect was coupled with several reproducible effects at the F3 generation, including a significant increase in both germline apoptosis and embryonic lethality. Importantly, the germline condition also showed a reduction in the repressive marks H3K9me3 and H3K27me3. The reduction of these repressive marks could be reversed with both chemical and genetic intervention targeting Junmonji demethylases jmjd-2 and jmjd-3/utx-1, required for reduction of H3K9me3 and H3K27me3 marks, respectively. The genetic and chemical intervention also reduced germline apoptosis and embryonic lethality at the F3 generations, rescuing the initial BPA response. Additional analyses showed us that the germline desilencing effect is not exclusive to the array as a profound alteration of the F3 germline transcriptome as well as a moderate upregulation of X-linked transcripts are also identified using RNAseq. Consistent with these transcriptional changes, we observe a 25% to 50% decrease of in H3K9me3 and H3K27me3 expression. These results suggest that environmental exposure to BPA can alter the transcriptome and protein expression of the germline, epigenome, and how to prevent transmission of these effects across generations.
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1905 PCB Exposure and Altered DNA Methylation in the Anniston Community Health Survey

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The Anniston Community Health Survey I and II (ACHS I, II) cross-sectional studies, were conducted from 2005-2007 and 2014 to explore the extent of PCB and other persistent organic pollutant (POP) exposures in Anniston’s population and to assess potential adverse health outcomes. DNA methylation profiles in blood cells have been associated with aging, environmental exposures and disease. We hypothesized that exposure to POPs may alter DNA methylation levels in the immune cells of Anniston residents. In ACHS-I, Anniston residents were selected using stratified random sampling, and ACHS-II participants were recruited from the ACHS-I cohort. 35 PCB congeners were measured in serum. Methylation was measured in whole blood DNA with Illumina arrays (ACHS-I, n=537, EPIC 850K; ACHS-II, n=332, 450K). Wet weight concentrations for the 35 PCBs were summed and log10-transformed. We modeled associations between methylation at each individual CpG and sum35PCB using multivariable linear regression adjusted for age, race, sex, current smoking (yes/no), total serum lipids, and estimated percentages of 6 white blood cell types. In ACHS-I we identified 16 CpGs sites at which methylation was associated with sum35PCB exposure (p<10^{-6}). Of these sites, 9 CpGs were unique to the 850K array. We observed 4 CpGs with greater than 2% differential methylation between the highest and lowest PCB exposure level (based on quartiles). Most statistically significant CpG (p = 6.38x10^{-6}) was a CpG in the serine phosphorylation enzyme AURKB was lowest at Days 3 and 7. Most other differentially regulated epigenetic modifiers (44 of 92 examined) gradually increased during differentiation. Modeling the basal expression of epigenetic modifiers has revealed critical time windows for epigenetic regulation of DPGs and laid the foundation for investigating the effect of xenobiotics that may influence the expression of DPGs through epigenetic mechanisms.

1906 Modeling the Expression of Epigenetic Enzymes and Drug-Processing Genes in Differentiating HepaRG Cells Revealed Critical Time Windows for Human Liver Development


During liver development drug-metabolizing enzymes (DME) and transporters (together called “drug-processing genes” (DPGs)) are regulated by epigenetic modifiers such as DNA methylation and histone modifications. Regulation of expression of DPGs may lead to adverse drug reactions by altering the expression of DPGs in newborns and children. In vivo regulation of human liver development is limited due to the scarcity of samples and ethical concerns. The goal of this study was to establish an in vitro model of human liver development for epigenetic regulation of DMEs; HepaRG cells are a line of human hepatocytes used to determine the regulation of DPGs at a fully differentiated stage. Modeling the basal expression of DPGs in mouse and human hepatocytes showed at most 2-fold higher expression of DPGs in mouse and human hepatocytes than in liver. In the present study, we examined changes in 63 genes that are DMEs and 35 genes that are enriched for histone modification marks H3K9me3 and H3K27me3 in the germline which can lead to the increased risk of liver disease. In a similar manner, we identified effects due to c-Jun and C/EBP delta levels in HepaRG cells. The results of this study demonstrated that regulation of DPGs in HepaRG cells is different from that in liver. The findings of this study will be useful for elucidating the mechanisms of human liver development.

1907 Examining the Effects of Ethanol on the Epigenome in C. elegans

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In sexually reproducing organisms, the development of germ cells is vital for the faithful transmission of the genome and its associated epigenetic marks across generations. Recent findings have suggested that DNA methylation alterations in mammalian germ cells in response to environmental exposures can be inherited across multiple generations. However, embryonic germ cells undergo two waves of epigenetic reprogramming that decreases DNA methylation and establishes a blanket slate. Thus, we believe that histone marks may be the mediators of epigenetic memory since germ cells rely heavily on repressive chromatin marks, H3K9me2/3 and H3K27me3 during reprogramming. In previous work using a Caenorhabditis elegans strain containing a highly repetitive GFP transgene that is silenced in the germline, we had discovered that BPA presents a desilencing effect in directly exposed worms as well as in their progeny in a transgenerational manner. In the current study, we performed RNAi to target the same known chromatin modifying enzymes in C. elegans in order to identify the genetic pathways that are involved in ethanol's transgenerational effects. This project therefore will identify potential mechanisms for the transgenerational effects of ethanol exposure and carry important implications for human reproductive health in the context of environmental exposures.

1908 Global Methylation and Hydroxymethylation Analysis in Livers of Mice Treated with Non-Genotoxic Carcinogens with and without Methyl Supplemented Diet


Many non-genotoxic carcinogens (NGCs) are known to cause perturbation in DNA methylation which can be an early event leading to changes in gene expression and onset of carcinogenicity. Phenobarbital (PB) has been shown to induce changes in liver DNA methylation (5-mC) and hydroxymethylation (5-hmC) patterns in mice in time dependent manner. The goal of this study was to assess if clofibrate, another well-studied rodent NGC, would produce epigenetic perturbations in mice and 2) if feeding mice Methyl Supplemented (MS) diet would alter/reverse changes induced by PB. CByB6F1 mice were treated with PB and MS diet for each time point were fed a methionine/choline enriched diet. Global liver 5-mC and 5-hmc levels were assessed by Liquid Chromatography- Ionization Mass Spectrometry (LC-MS). Gene expression analysis was conducted using microarrays (Affymetrix). Exposure to phenobarbital produced a significant decrease in global 5-hmc levels in mouse livers after both 7 and 28 days whereas clofibrate treatment resulted in 5-hmc level decrease after 28, but not after 7 days of dosing. A slight increase in 5-hmc levels was observed in MS diet subgroup compared to phenobarbital alone after 7, but not after 28 treatment days. None of the treatments produced a significant change in global 5-mC levels compared to controls. After 28 day exposure, the total number of expression changes in the liver was lower in MS diet group than in PB alone. Many PB induced genes essential to cell proliferation, growth and invasion showed at least 2 fold lower number of change in MS group compared to PB alone. These genes include S100A8, MCM5/6, Esco2, S0X10, BIRC5, RRM2, Gstm3, RAD51, Atrx as well as Cyp2b10; many of them are reported to be regulated by DNA methylation. Both increases in the global 5-hmc
level and decreased numbers of gene expression changes in MS diet fed mice after 7 days suggests possible early protective effect of methyl-rich diet. Although further work is needed to assess differential methylation in relation to gene expression and mechanisms of toxicity, LC/MS can be the first tier indicating global epigenetic changes induced by NGCs.

1909 Inter-Individual Variability in Epigenetic and Genotoxic Responses to 1,3-Butadiene in a Population-Based Collaborative Cross-Mouse Model

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1,3-Butadiene (BD) is a known human carcinogen that is both an occupational and environmental health hazard. Although it is well-established that genotoxicity is the key mechanism of BD carcinogenesis, epigenetic events have also been observed. Previous studies in a multi-strain mouse model revealed that inter-strain (e.g. inter-individual) differences exist in both BD-induced DNA damage and epigenetic effects. These studies indicated that variation in epigenetic alterations could drive the inter-individual susceptibility to BD genotoxicity. In the present study, we investigated whether or not there is population variability in epigenetic alterations and genotoxic effects in response to BD exposure by using the Collaborative Cross (CC) mouse model. We tested the hypothesis that there is variation in BD-induced epigenetic events and DNA damage. Male mice from 50 CC strains were exposed to 0 or 625 ppm of BD by inhalation (6 hr/day, 5 days/week) for 2 weeks. We evaluated genotoxic and epigenetic effects of BD in tissues that are a target (lung and liver) and non-target (kidney) of BD-induced carcinogenesis. Genotoxicity was assessed by measuring THB-Gua adduct levels. We observed that exposure to BD resulted in variable levels of THB-Gua adducts between strains and tissues. In order to investigate the epigenetic effects, we evaluated the levels of histones H4K20me3, H3K27me3, H3K9me3, H3K9ac, and H3K27ac in the livers. We observed variable response to BD as a strain-specific manner for all histone modifications as a result of BD exposure. Additionally, we analyzed the status of these histone modifications in the livers of unexposed mice and found that the strains with low levels of THB-Gua adducts after exposure to BD were characterized by a markedly high histone H3K27ac/H3K27me3 ratio, a mark of transcriptionally active chromatin. In contrast, this ratio was substantially lower in strains with high levels of THB-Gua adducts. This indicates that strain-dependent variability of BD-induced DNA damage and potentially greater tissue susceptibility to carcinogenesis may be predetermined by the pre-exposure epigenome status of a target organ.

1910Arsenic-Induced CPG Methylation Patterning in Placental JEG-3 Trophoblast Cells Via the Glucocorticoid Receptor: Support for the Transcription Factor Occupancy Theory

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Exposure to inorganic arsenic (iAs) is a major public health problem as millions of individuals worldwide are exposed above the WHO limit of 10 ppb in drinking water. Arsenic exposure is associated with altered epigenetic profiles such as DNA methylation that have been associated with detrimental health effects, including cancer, adverse cognitive development and pregnancy outcomes. One hypothesis explaining iAs’s ability to alter DNA methylation is the transcription factor occupancy (TFO) hypothesis. This posits that DNA methyltransferase cannot gain access to DNA when transcription factors have bound to and occupied response elements of selected genes, thereby influencing Cpg methylation patterning. Given that iAs can act on the Glucocorticoid Receptor (GR), we set out to examine whether iAs exposure affects GR and GR target gene Cpg methylation and expression. In support of the TFO, we hypothesized that sites of decreased methylation would be associated with increased expression and increased methylation would be associated with decreased expression. To test this, placental JEG-3 cells were exposed to 1 μM iAs for 24 hours. Following exposure, methylation levels were assessed with the EPIC Illumina platform and gene expression was assessed using the Qiagen GR PCR array. A total of 450 GR-associated Cpg sites representing 253 genes displayed significantly altered Cpg methylation levels in relation to iAs exposure. An integrated analysis of methylated GR genes and gene expression data revealed 130 Cpg probes representing 54 genes with overlap. Of these genes, a total of 25 displayed changes representative of the transcription factor occupancy theory, demonstrating either hypermethylation in association with decreased gene expression or hypomethylation in association with increased gene expression. These genes included FK506 Binding Protein 5 (FKBP5), AFA/FMR2 Family Member (AFF1), Protein Activator 3 (RAS3), and Damage Inducible Transcript 4 (DDIT4), which are all associated with the development of adverse cognitive outcomes or cancer endpoints. Taken together, these results demonstrate the ability of iAs to modulate GR gene methylation and subsequent gene expression, and the potential epigenetic reprogramming resultant from transcription factor occupancy.

1911 DNA Methylation Epigenetically Regulates NF-κB Pathway Genes during COPD Exacerbations

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Chronic Obstructive Pulmonary Disease (COPD) is a global health problem with ineffective current therapies and lack of knowledge about the pathobiology of the disease. Cigarette smoking is the leading cause of COPD, however, not all the smokers develop COPD. Exacerbations of COPD are caused by microbes and dust common to the environment with about 20-50% of exacerbations caused due to bacterial colonization in the lower airways of the patient. It is generally accepted that epigenetic mechanisms especially DNA methylation play important role during the disease prognosis in COPD. We thus hypothesized that DNA methylation patterns vary significantly in case of bacterial pathogen in COPD. To test this we used in vitro study model which mimics COPD exacerbations and performed methylation specific-PCR (MS-PCR) for NF-κB and STAT3 pathway genes. In brief, we challenged human alveolar type II epithelial cells (A549) with cigarette smoke extract (CSE) or DMSO (control) for 24 hr which included 3h challenge with bacterial lipopolysaccharide (LPS from Pseudomonas aeruginosa) before harvesting the cells. Samples were processed to determine the production of cytokines/chemokines, regulation of transcription factors and DNA methylation of specific genes. Our findings demonstrate significant increase in the transcriptional and translational profile of inflammatory mediators in CSE-exposed A549 cells challenged with LPS as compared to control group. We also observed significant decrease in the Cpg methylation at NF-κB and IKK promoter regions, however, the regulation at NIK and STAT3 promoter was insignificant. We also investigated the expression levels of Dnmts at protein level in various study groups. We observed increase in the expression of Dnmt2 and Dnmt3a in the CSE-treated cells challenged with LPS. Results from whole-exome bisulphite sequencing also shows downregulation of DNA methylation at CpG islands of NF-κB pathway genes. Overall our results indicate that activation of NF-κB mediated inflammatory pathway is regulated by alterations in DNA methylation at the CpG sites of these genes. Further studies are in progress to understand the mechanism of epigenetic regulation of NF-κB pathway in detail during COPD exacerbations which will help in the development of better and more effective therapeutic intervention strategies.

1912 Epigenetics and Genotoxicity: Potentially Interrelated Roles in Chemical Carcinogenesis

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The relationship between genotoxicity-induced mutagenesis and cancer has been extensively demonstrated and is well accepted, while the roles of epigenetic regulators that are involved in inter-individual differences in carcinogenic potential and carcinogenic processes is not as clear. We aimed to assess the state-of-the science on the interplay between epigenetic alterations, DNA damage and carcinogenesis, and to gain an understanding of the availability of epigenetics data for DNA-damaging agents that have been classified as carcinogens. We first investigated the molecular mechanisms by which epigenetic alterations can render cells more vulnerable to genetic damage, and contrasted these with epigenetic mechanisms necessary for proper damage response and repair. The following connections between epigenetics and DNA damage and repair were identified: (i) preferential binding of reactive chemicals or metabolites to methylated cytosines at mutational “hotspots”, (ii) histone modifications that affect chromatin structure, resulting in altered accessibility of DNA to damaging agents and/or DNA repair machinery and/or recruitment of DNA repair machinery to sites of damage, and (iii) changes in abundance and activity of miRNAs that regulate the expression of genes involved in DNA damage response. These relationships are supported by data for three chemicals: benzo[a]pyrene, 1,3-butanediol, and formaldehyde. For example, significant enrichment for the functional category ‘DNA replication, recombination, and repair’ was observed among the mRNA targets of two miRNAs altered by exposure to benzo[a]pyrene and 1,3-butanediol. Despite the importance of epigenetics in chemical carcinogenesis, there is a general lack of infor-
mation on epigenetic responses among DNA damage agents. Among the 48 chemicals/metals (not including pharmaceuticals) and related occupations classified as “carcinogenic to humans” by the International Agency for Research on Cancer, we identified through a review of literature and cancer monographs that genotoxicity was reported for 36, and published information on epigenetics exists for just over half of these substances. Understanding of genotoxic responses and chemical-induced cancer. This evaluation identified epigenetic mechanisms that are important in the carcinogenic potential of DNA-damaging chemicals, and may be considered as candidates for further research and inclusion in cancer hazard evaluation.

### 1913 Screening and Evaluation of Endocrine Disrupting Chemicals on Sirtuin Regulation and Inflammatory Response in Macrophages

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There has been an epidemiologic rise in the prevalence of metabolic disorders with emerging evidence linking low grade inflammation as an underlying mechanism. Macrophages have been implicated in promoting inflammation in diabetes and obesity. Our published evidence reported endocrine-disrupting chemicals (EDCs) induced sirtuin dysregulation in pre-adipocytes, placental cells, and liver cells. Sirtuins, a novel epigenetic histone deacetylase class regulator, have also been shown to play a role in inflammation. In this study, we screened six EDCs hypothesizing that EDC exposure leads to an inflammatory response in the macrophage via sirtuin regulation. RAW 264.7 cells were treated with 0.5, 5, and 50 μM of EDCs (Bisphenol A (BPA), Bisphenol F (BPF), Diethylene glycol diethyl ether (DEG), Mono (2-ethylhexyl) phthalate (MEHP), Perfluorooctane sulfonate (PFOS), BBP) and showed higher exposures than native communities fishing on tributaries that feed into the MDD River (Queros (median 0.9 ppm, range 0.05-44.8 ppm)). To evaluate the possible impacts of hazardous environmental stresses on epigenetic modifications mediated through virtual screening of 1154 pesticides on human DNMTs (DNMT1, PDB code: 35WR) and (DNMT3, PDB code: 2QVR). Molecular docking calculations for DNMTs-pesticide complexes were performed using AutoDock Vina. Binding-affinity values, contact patterns and molecular diversity were employed as selection criteria of consensus pesticides as virtual hits of DNMTs. The best DNMTs-pesticide complexes selected according to their high absolute affinity values (kcal/mol) for both DNMT1 and DNMT3 were Bromadiolone (11.0; 9.1), Flocoumafen (9.9; 9.0), Difenacoum (9.7; 9.1), and Difenacoum (11.4; 10.2). These chemicals were molecularly clustered and all belong to second generation rodenticides. The most frequent interacting residues for DNMT1-pesticide complexes were Trp1170A, Phe1170A, Met 696A, Glu698A; whereas for DNMT3 those were Glu303B, Pro739A, Lys740A and Glu303B. These results suggest that rodenticides used for pests control are potential DNMT ligands and therefore may modulate DNA methylations, finding with important environmental and clinical implications. This discovery may be useful to identify new epigenetic biochemical pathways activated by these xenobiotics, using this information as a starting point for future validations with in vitro and in vivo assays.

### 1914 Mitochondrial-Epigenetic Crosstalk Endpoints in PBMCs from Genetically Diverse Individuals with High Methyl Mercury Exposure Living Near ASGM in the Peruvian Amazon


The Madre de Dios (MDD) region of the Peruvian Amazon has experienced rapid artisanal small-scale gold mining (ASGM) expansion. Communities near ASGM have high methylmercury (MeHg) exposure via contaminated fish consumption. We evaluated MeHg exposure using total hair mercury (Hg) in n=2308 individuals across 23 MDD communities Diamante (median 5.2 ppm, range 0.4-21.4 ppm), Isla de los Valles (median 7.1 ppm, range 1.2-16.7 ppm), and Puerto Azul (median 5.2 ppm, range 2.2-14.1 ppm) fish directly on the MDD River, and showed higher exposures than native communities fishing on tributaries that feed into the MDD River (Queros (median 0.9 ppm, range 0.4-2.1 ppm), and Palotafo-Teparo (median 2.7 ppm, range 1.4-6.4 ppm)); or mining towns (Hupepetuca (median 0.9 ppm, range 0.003-20.3 ppm), and Quebrada Nueva (median 0.8 ppm, range 0.05-44.8 ppm)). To evaluate potential biological impacts of MeHg exposure in a genetically diverse study population, we measured mitochondrial and epigenetic endpoints in whole blood from a subset of baseline participants (n=32; n=16 “HIGH” >10 ppm total hair Hg, n=16 “LOW” <1ppm total hair Hg, matched on age and sex). Emerging evidence strongly supports cross-talk between these two systems. We measured mitochondrial DNA (mtDNA) damage and copy number (CN). We evaluated cell type proportions and DNA methylomes using Illumina 850K epic microarrays. Although neither mtDNA damage nor CN were associated with Hg, both DNA methylomes (β=0.65, p=0.009) and cell type proportions were significant predictors of mtDNA damage, but these relationships were attenuated on inclusion of Hg in the model. These results suggest that T cells are preferential targets for mtDNA damage. We are currently analyzing methylation data as potential mediators or consequences of mitochondrial responses. This work highlights the importance of a “systems biology” approach to biologically related toxicity endpoints.

### 1915 Alterations in Epigenetic Control: In Silico Evaluation of Pesticides as Potential Modulators of Human DNA Methyltransferases


DNA methylations are part of epigenetic changes that modulate gene expression, processes carried out by DNA methyltransferases (DNMTs). A wide range of chemicals can promote changes in the epigenome, including pesticides, making them attractive candidates of association with chronic diseases. In this study, human DNMTs were evaluated as potential targets for pesticides through virtual screening of 1154 pesticides on human DNMTs (DNMT1, PDB code: 35WR) and (DNMT3, PDB code: 2QVR). Molecular docking calculations for DNMTs-pesticide complexes were performed using AutoDock Vina. Binding-affinity values, contact patterns and molecular diversity were employed as selection criteria of consensus pesticides as virtual hits of DNMTs. The best DNMTs-pesticide complexes selected according to their high absolute affinity values (kcal/mol) for both DNMT1 and DNMT3 were Bromadiolone (11.0; 9.1), Flocoumafen (9.9; 9.0), Difenacoum (9.7; 9.1), and Difenacoum (11.4; 10.2). These chemicals were molecularly clustered and all belong to second generation rodenticides. The most frequent predicted interacting residues for DNMT1-pesticide complexes were Trp1170A, Phe1170A, Met 696A, Glu698A; whereas for DNMT3 those were Glu303B, Pro739A, Lys740A and Glu303B. These results suggest that rodenticides used for pests control are potential DNMT ligands and therefore may modulate DNA methylations, finding with important environmental and clinical implications. This discovery may be useful to identify new epigenetic biochemical pathways activated by these xenobiotics, using this information as a starting point for future validations with in vitro and in vivo assays.

### 1916 Identification of Smoking-Induced Changes in DNA Methylation in an Epigenome-Wide Scan

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Gene expression in eukaryotes is regulated in part by epigenetic modifications mediated through DNA methylation and stable histone modifications, an exquisite biological system that maintains cellular homeostasis. There is significant evidence that the dysregulation of epigenomic functional complexes disrupts information processing of the study of the possible impacts of hazardous environmental stresses on epigenetic modifications has attracted interest because it allows a better understanding of disease pathogenesis. To identify the genes involved in smoking-induced epigenetic alteration, we assessed the DNA methylation levels of 473,864 autosomal CpG sites in a total of 384 subjects from Japanese general population-based cohorts. Genomic DNA obtained from peripheral blood was bisulfite treated and genome-wide methylation profiles were obtained using the Illumina Human Methylation 450K BeadChip. After removing an outlier sample and low call rate sites, we detected 11 sites with 6 loci, including NFE2L2, ALPL2, DPR15, AHR9, IRAK3, and F2RL3, which had adjusted P values of less than 0.01 in comparisons between current and non-current smokers for the DNA methylation levels. To address whether the smoking-induced DNA demethylation is reversible, the difference in the DNA methylation levels among never, former, and current smokers was examined. In former smokers, the sites in ALPL2, AHR9, IRAK3 and F2RL3, which had P values of less than 0.01 in comparisons between current and non-current smokers, whereas the sites in DPR15 and NFE2L2 showed the
nearly the same DNA methylation levels observed in never smokers. The smoking-induced change in the DNA methylation levels is not dependent on smoking dose or years, but its restoration is dependent on the time since smoking cessation, suggesting that the epigenome adapts dynamically to smoking stress.

**1917 Anaemic Induction Disrupts Lipid Profile and Alters Lipoprotein Metabolism Gene Expression in a Rat Model**

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Iron metabolism in animals is altered by haemolytic anaemia induced by phycocyanin (Phyco) treatment. However, its effects on lipid metabolism remain elusive. The aim of this study was to examine the impact of anaemia on lipid profiles and lipoprotein metabolism gene expression in rats. Fourteen adult male Wistar rats were randomly classified into normal control and anaemia-induced groups (n = 7) respectively. Anaemia was induced by daily administration of Phyz at 10 mg/kg for eight consecutive days; after which blood was collected and liver excised. Lipid profiles of plasma and liver were determined spectrophotometrically while the expression of genes associated with lipid and lipoprotein metabolism was assayed by reverse transcriptase polymerase chain reaction (RTPCR). Anaemia resulted in a significant (p < 0.05) decrease in plasma HDL-C and triglycerides, and an increase in plasma VLDL-C, LDL-C and total cholesterol. We conclude that anaemia induces alterations in the expression of genes associated with lipid and lipoprotein metabolism. This is the first study to investigate the impact of anaemia on lipid profiles and lipoprotein metabolism gene expression in rats.

**1918 A Cellular Game of Telephone: Trans-Cellular Reprogramming in Responses to Toxic Stimuli**

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Exposure to air pollution is a leading cause of cardiopulmonary morbidity and mortality; however, while these effects outside the lungs have been associated with aberrant oxidative stress and inflammation, the underlying molecular mechanisms are poorly understood. We hypothesized that air pollutant exposure of human bronchial epithelial cells (HBEC) could alter the expression of pro-inflammatory and pro-oxidant stress genes in adjacent, but physically separated, human bronchial epithelial cells (HBECS) through alterations to the epigenome. To test this hypothesis, we exposed confluent monolayers of HBEC, grown on the apical surface of Transwell permeable membranes, to diesel exhaust particles (DEP) or polycyclic aromatic hydrocarbons (PAH). We analyzed the expression of two sets of genes, one associated with HBEC and the other with the basolateral compartment. A variety of pro-inflammatory and oxidative stress genes were induced in HBEC-1 cells cultured in the presence of DEP-exposed HBEC. Using IL-8 and HMOX1 as representative genes in the pro-inflammatory and oxidative stress responses, respectively, we then determined whether these changes in gene expression were associated with alterations to the epigenome. We assessed the abundance of four histone modifications with established roles as key regulators of gene expression, whether these changes in gene expression were associated with alterations to the epigenome. We assessed the abundance of four histone modifications with established roles as key regulators of gene expression, including di/trimethyl histone H3 lysine 4 (H3K4me3) and lysine 27 (H3K27me3). We observed that DEP-exposed HBECs resulted in a significant (p < 0.05) increase in the abundance of H3K4me3 and H3K27me2/3 and unmodified histone H3. The abundance of H3K27me3 increased within the promoters of both IL-8 and HMOX1 in HBEC-1 cells cultured in the presence of DEP-exposed HBEC. These indirectly exposed HBECs were associated with an increase in the expression of IL-8 and HMOX1 in HBEC-1 cells cultured in the presence of DEP-exposed HBEC. These indirectly exposed HBEC-1 cells also exhibited increased H4ac within IL-8 and HMOX1 enhancer regions. In addition, a reduction in H3 occupancy accompanied these histone modifications. This trans-cellular epigenetic reprogramming within the HBEC-1 cells suggests a more open chromatin conformation and was associated with an enhanced pro-inflammatory and oxidative stress response to a secondary challenge with lipopolysaccharide. Our findings demonstrate that pollutant-exposed epithelial cells reprogram the expression of pro-inflammatory and oxidative stress genes in adjacent cells. These findings ultimately provide a mechanistic mechanism by which exposure to air pollution can cause adverse health effects in tissues that are not directly exposed, and further heighten the importance of understanding the effects of air pollution on tissues beyond the airway.

**1919 Phthalates Contamination in Commercial Alcohol: Effects on Sirtuin Regulation**

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Phthalates have attracted public regulatory attention due to their possible adverse environmental and human health effects. Our group demonstrated the presence of endocrine disruptor phthalate in vegetable cans, baby bottles and microwaveable containers from the Mexican market. In a screening assay (GC/MS technique) of various samples of tequila and mezcal, we discovered that these beverages were contaminated with phthalates specifically DEHP and in many cases were above the permitted limits. Furthermore, computational molecular docking analysis showed that DEHP binds to sirtuin 3, a member of the Class III histone deacetylases family involved in regulation of metabolism. Therefore, we hypothesize that phthalate contamination in alcoholic beverages may synergistically dysregulate sirtuins during metabolic processes. HepG2 cells were exposed to different concentrations of DEHP (0, 100 nM, 1 μM, 5 μM, 10 μM, 25 μM, and 50 μM) with or without ethanol (0, 1 nM, 100 nM, 1 μM, 10 μM, and 25 μM) for 48 h. Sirtuin regulation and lysine acetylation were analyzed. Treatment with DEHP and ethanol alone or in combination for 48 h did not affect cell viability. DEHP alone significantly decreased gene expression of sirtuin 3 and 5 in a dose-dependent manner (P < 0.001) whereas sirtuin 1, 2, 6, and 7 showed nonmonotonic changes. Sirtuin 4 gene expression showed no changes in response to DEHP. Ethanol alone significantly decreased all sirtuins in a dose-dependent manner (P < 0.001). In comparison to ethanol or DEHP alone, the combination of DEHP and ethanol significantly downregulated the expression of sirtuin 7 while no significant effects were observed for any of the other sirtuins. Finally, after 72 h, 1 nM and 10 μM ethanol significantly increased protein acetylation while decreasing sirtuins 1 and 3 protein expression, with no effects detected on sirtuins 5, 6, and 7 protein expression. We are currently investigating the sirtuin regulation in human hepatocytes. In conclusion, a combination of DEHP and ethanol may change sirtuin regulation which may affect liver pathology.

**1920 N-Acetylcysteine Protects Endogenous High Glucose/Fatty Acid Toxicity and Streptozotocin-Induced Cytotoxicity in Pancreatic Beta Cells**

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Prevalence of diabetes is on the rise globally and causing extraordinary burden on the health systems, both in developed and developing countries. Pancreatic beta-cell mass destruction/neogenesis/proliferation (e.g. in type1) and/or malfunction (type2) have been implicated in diabetes etiology, pathology, progression as well as in responses towards disease managements. Oxidative stress and alterations in mitochondrial energy metabolism play important roles in diabetes-induced cellular complications. Several studies, including our own, have suggested that sirtuins are the basal-salient component. A variety of pro-inflammatory and oxidative stress genes were induced in THP-1 cells cultured in the presence of DEP-exposed HBEC. Using IL-8 and HMOX1 as representative genes in the pro-inflammatory and oxidative stress responses, respectively, we then determined whether these changes in gene expression were associated with alterations to the epigenome. We assessed the abundance of four histone modifications with established roles as key regulators of gene expression, including di/trimethyl histone H3 lysine 4 (H3K4me3), pan-acetyl histone H4 (H4ac), di/tri-methyl histone H3K27 (H3K27me2/3), and unmodified histone H3 (H3). The abundance of H3K27me3 is increased within the promoters of both IL-8 and HMOX1 in THP-1 cells cultured in the presence of DEP-exposed HBEC. These indirectly exposed THP-1 cells also exhibited increased H4ac within IL-8 and HMOX1 enhancer regions. In addition, a reduction in H3 occupancy accompanied these histone modifications. This trans-cellular epigenetic reprogramming within the THP-1 cells suggests a more open chromatin conformation and was associated with an enhanced pro-inflammatory and oxidative stress response to a secondary challenge with lipopolysaccharide. Our findings demonstrate that pollutant-exposed epithelial cells reprogram the expression of pro-inflammatory and oxidative stress genes in adjacent cells. These findings ultimately provide a mechanistic mechanism by which exposure to air pollution can cause adverse health effects in tissues that are not directly exposed, and further heighten the importance of understanding the effects of air pollution on tissues beyond the airway.
221 Comparative Effects of High Glucose and High Palmitic Acid Treatment on Cell Survival, Oxidative Stress, and Mitochondrial Bioenergetics in RIN-SF and HepG2 Cells

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According to WHO, over 400 million cases of diabetes were reported in 2014 and is estimated to be the 7th leading cause of death by 2030. As obesity is considered as one of the most important risk factors, this disease is also referred to as “diabesity”. Glucotoxicity and lipotoxicity, due to persistent hyperglycemia and dyslipidemia, and increased inflammation are the main causative factors affecting the progression and pathology of diabetes. Using both in vivo and in vitro models, we have previously reported increased oxidative stress and mitochondrial dysfunction in diabetes. Here, we have compared the effects of high glucose (up to 30 mM) and high saturated fatty acid, palmitic acid (0.3 mM), on cell survival, inflammatory responses, oxidative stress, mitochondrial function and apoptosis using rat pancreatic Rin-SF insulin secreting cells as well as human hepatoma derived HepG2 cells. Our results show that high glucose and high palmitic acid alone or in combination inhibits cell survival, as measured by MTT assay, and induces apoptosis by altering cell signaling and mitochondrial functions, in a dose-dependent manner. The gluco- and lipotoxicity in these cellular systems were also observed at the metabolic level as seen by alterations in energy metabolism in the mitochondrial and cytosolic compartments. Furthermore, we also observed that these cells were under severe oxidized and inflammatory stress as shown by increased reactive oxygen species (ROS) production and alterations in glutathione (GSH) dependent antioxidant homeostasis. Our results also show that treatment with N-acetylcysteine, a ROS scavenger, attenuated the effects of gluco-/lipotoxicity observed in these cells. These results may have implications in better understanding the etiology and pathophysiology of diabetes and associated complications.

222 Endoplasmic Reticulum Stress and MAPK Signaling Pathway Activation Underlie Leflunomide-Induced Toxicity in HepG2 Cells

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Leflunomide, used for the treatment of rheumatoid arthritis, has been reported to cause severe liver problems and liver failure; however, the underlying mechanisms are not clear. In this study, we used multiple approaches including genomic analysis to investigate and characterize the possible mechanisms of the cytotoxicity of leflunomide in hepatic cells. We found that leflunomide caused endoplasmic reticulum (ER) stress and activated an unfolded protein response, as evidenced by increased expression of related genes including CHOP and GADD34; and elevated protein levels of typical ER stress markers including CHOP, ATF-4, p-eIF2β, and spliced XBP1. The secretion of Gaussia luciferase showed increased cell survival against UVB-induced apoptosis in cell treated with leflunomide in an ER stress reporter assay. Inhibition of ER stress with an ER stress inhibitor 4-phenylbutyrate, and knockdown of ATF-4 and CHOP genes partially protected cells upon leflunomide exposure. In addition, both genomic and biochemical analyses revealed that JNK and ERK1/2 of MAPK signaling pathways were activated, and both contributed to the leflunomide-induced cytotoxicity. Inhibiting JNK activation using a JNK inhibitor attenuated the ER stress and cytotoxicity of leflunomide, whereas inhibiting ERK1/2 using an ERK1/2 inhibitor or ERK1/2 siRNA increased the adverse effect caused by leflunomide, suggesting opposite roles for the two pathways. In summary, our data indicate that both ER stress and the activation of JNK and ERK1/2 contribute to leflunomide-induced cytotoxicity.

223 Electronic Liquids Induce Osteotoxicity in Which Flavoring Is a Key Factor

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Electronic cigarettes (e-cigs) are a relatively new method of nicotine delivery advertised as a healthier alternative to traditional tobacco products. While early studies support this claim, the health effects of e-cig use are still widely unknown. As the popularity of e-cigs increases, especially among middle- and high-school age kids, so does need to determine the health consequences associated with e-cig use. Another significant health concern is that children have become seriously ill after ingesting the electronic liquids (e-liquids) used in e-cigs. Since smoking traditional tobacco products is a risk factor for osteoporosis, our lab hypothesizes that exposure to e-liquids can also impair bone function. For this research, the human osteoblast-like cell line MG-63 was used as a model. Cells were treated for 48 hours with dilutions of commercially available un-vaped e-liquids of various flavors, without or with (0.1 mg/mL) nicotine, for analysis of cell viability through MTT assays and immunofluorescence for collagen type I. Cell viability results showed a dose dependent decrease in all the e-liquids tested, but the effect was most pronounced in cinnamon flavored e-liquids and least pronounced in flavorless e-liquids consistently in all the brands tested. There were no consistent differences between treatments with or without nicotine. Immunofluorescence for collagen type I showed decreased protein expression after exposure to a non-cytotoxic dose of un-vaped e-liquid with or without nicotine. We conclude that exposure to e-liquids alters osteoblast function, and further investigation is warranted to determine the cellular mechanisms involved.

224 Insights into the Effect of Subtle Chemical Modification of Aryl Chloroethyurea (CEU) as Small Molecules Targeting Either β-Tubulin or Prohibitin and Thioredoxin-1

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Chloroethyureas (CEU) are protein alkylating agents displaying potent antineoplastic properties that covalently bind to β-tubulin and affect microtubule polymerization dynamics. A different CEU subset has been shown to induce cell growth inhibition without alkylating β-tubulin. Our research focus is to understand the mechanisms underlying the antiproliferative activity of that new class of compounds. From B16 and MDA-MB-231 cells incubated with [(14)C-urea]-CEU-25 and [(125)I]-CEU-98 were separated using 2D-electrophoresis followed by MALDI-TOF identification of modified proteins. Protein expression and distribution were investigated by Western blot analyses and immunocytochemistry. Cell cycle arrest was obtained by treating the cell line B16 with CEU-22 and its bioisosteric derivative CEU-98 are original CEU prototypes that covalently bind to β-tubulin via an ester linker on Glu198. The alkylation leads to microtubule depolymerization phenotype, cell cycle arrest in G2/M and inhibition of cell proliferation in vitro. A newly isolated subset of CEUs exemplified by the prototypical CEU-25, alkylates prohibitin (PHB) on Asp40 and thioredoxin isoform-1 (TRX1). CEU-25 arrests cells predominantly in G1 phase and inhibits Trx-1 and PHB nuclear translocation. The intracellular proteins alkylated by the new CEU subset were identified as the PHB and TRX1. Different protein targets profiles explain the G1 cell cycle arrest. Our research emphasizes that a subtle chemical modification might lead to drastic change of protein target. Our finding might help to design new potent anticancer drugs that will target specific and lethal biological pathway essential to tumor growth.

225 Epidermal-Specific Deletion of TC-PTP Promotes UVB-Induced Keratinocyte Cell Survival through the Regulation of VEGFR2/JNK Signaling

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UVB can contribute to the development of skin cancer by modulating PTK signaling. It has been suggested that UVB increases the ligand-dependent activation of PTKs and induces PTP inactivation. Our recent studies have shown that T cell protein tyrosine phosphatase (TC-PTP) deficiency predisposes mice to skin carcinogenesis induced by two-stage chemical regimens, which indicates its critical role in the prevention of chemically-induced skin cancer. In the current work, we report that activation of TC-PTP leads to increased keratinocyte susceptibility to UVB-induced apoptosis via the downregulation of VEGFR2/JNK signaling. TC-PTP-deficient (TC-PTP KO) mice showed significant resistance to UVB-induced apoptosis in epidermis. To assess the role of TC-PTP in UVB-induced apoptosis, we generated immortalized TC-PTP KO keratinocytes from the epidermis of TC-PTP KO mice. Immortalized TC-PTP KO keratinocytes showed increased cell survival against UVB-induced apop-
DNA Damage-Induced Apoptosis and MAPK Pathway Contribute to the Toxicity of Dronedarone in Hepatic Cells


Dronedarone, an antiarrhythmic drug, has been marketed as an alternative to amiodarone. The use of dronedarone has been associated with severe liver injury; however, the mechanisms remain unclear. In this study, the possible mechanisms of dronedarone-induced liver toxicity were characterized in HepG2 cells. Dronedarone decreased cells viability and induced apoptosis and DNA damage in a concentration- and time-dependent manner. Pretreatment of the HepG2 cells with apoptosis inhibitors (caspase-3, -8, or -9) or the necrosis inhibitor (Necrox-5), partially, but significantly, reduced the release of lactate dehydrogenase. Dronedarone caused the release of cytochrome c from mitochondria to cytosol, a prominent feature of apoptosis. In addition, the activation of caspase-2 involved in dronedarone induced DNA damage, and dronedarone exposure activated JNK and p38 signaling pathways. Inhibition of JNK and p38 by specific inhibitors attenuated dronedarone-induced cell death, apoptosis, and DNA damage. Additionally, suppression of caspase-2 decreased the activities of JNK and p38. Dronedarone triggered DNA damage was regulated by down-regulation of topoisomerase IIa at both transcriptional and post-transcriptional levels. Taken together, our data show that DNA damage, apoptosis, and the activation of JNK and p38 contribute to dronedarone-induced cytotoxicity.

Characterization of Whole Smoke and Smokeless Tobacco Exposure to a 3D Human Oral Tissue Model

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Oral disease is frequently associated with viral and environmental exposures as well as oral hygiene. The use of tobacco is an additional risk factor in the development of oral disease. The goals of this study were to evaluate cytotoxicity, inflammatory response and oxidative stress in response to extracts of CORESTA smokeless tobacco reference products (CRPs) exposed to a 3D human oral buccal model, EpiOral™, as well as to determine the potential cytotoxicity of whole smoke in this model. CRPs for snus (CRP1), moist snuff (CRP2) and dry snuff (CRP3) were each extracted in complete artificial saliva (CAS) with a ratio of 300 mg of CRP to 1 mL of CAS. CRP extracts were sterile-filtered and stored at 80°C until the time of exposure. CRP extracts (15 - 300 mg/ml) were added topically to the apical side of EpiOral™ tissues for 24 or 48 hours continuously. Cytotoxicity (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; MTT), oxidative stress (8-isoprostanate) and inflammatory response (cytokine release; IP10, IL-1α, IL-8) were measured after each time point. Whole smoke exposure were conducted by exposing the EpiOral™ tissues to whole smoke with depths of 8-isoprostane at 48 hours of exposure. Collectively, the data suggest that the EpiOral™ three-dimensional human cell culture model may be useful in differentiating smokeless tobacco products and evaluating between tobacco product categories.
Ethanol-induced apoptosis in neural crest cells (NCCs), a multipotent progenitor cell population, are implicated in the Fetal Alcohol Spectrum Disorders (FASD). Previous studies from our laboratory have demonstrated that sulforaphane (SFN), a vegetable-derived isothiocyanate, can prevent ethanol-induced apoptosis in NCCs. Epithelial-mesenchymal transition (EMT) is a process by which cells lose their epithelial phenotype and acquire mesenchymal phenotype, which is essential for NCC migration. The induction of EMT has also been shown to confer resistance to apoptosis in cancer cells. The objective of this study is to investigate whether ethanol exposure can induce apoptosis in NCCs by inhibiting EMT and whether SFN can prevent ethanol-induced apoptosis by epigenetically modulating the expression of EMT transcriptional factor Snail1. We found that exposure to ethanol resulted in a significant increase in apoptosis in NCCs. Co-treatment with SFN significantly reduced ethanol-induced apoptosis. Ethanol treatment also inhibited EMT, as indicated by an increased expression of E-cadherin, an EMT-suppressing marker, and a reduced expression of vimentin, an EMT-promoting maker. Treatment with SFN significantly reversed ethanol-induced changes in the expression of E-cadherin and vimentin, and restored EMT. We also found that ethanol exposure significantly decreased the mRNA expression of Snail1, which can be reversed by co-treatment with SFN. To determine whether ethanol and SFN regulate the expression of Snail1 by histone modification, the expression of kdm5a, a lysine-specific demethylase, and the levels of trimethylation of histone H3 lysine 4 (H3K4me3) at the promoters of Snail1 were determined. We found that ethanol exposure increased the expression of kdm5a which was reversed by SFN. The ChIP-qPCR analysis revealed that ethanol exposure significantly reduced the levels of H3K4me3 at the promoters of Snail1 and that SFN treatment reversed the ethanol-induced reduction of H3K4me3. Additionally, knock-down of kdm5a restored EMT and significantly decreased apoptosis. Taken together, these results demonstrate that ethanol exposure can induce apoptosis in NCCs by inhibiting EMT and that SFN can protect against ethanol-induced apoptosis by reverting ethanol-induced reduction in the levels of H3K4me3 at the promoters of Snail1, restoring the expression of Snail1 and EMT in ethanol-exposed NCCs.

1930 Sulforaphane Protects against Ethanol-Induced Apoptosis in Neural Crest Cells through Epigenetically Modulating the Expression of Snail1 and Restoring EMT

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Prostate cancer is one of the most frequently diagnosed malignancies and the second leading cause of cancer related death in men in the developed countries. The androgen deprivation therapy (ADT) is a classical treatment strategy, but development of resistance to ADT is a major obstacle for the management of advanced prostate cancer. Therefore, search for the novel therapeutic approaches are urgently needed. Fenofibrate, a fibrate, is now being used to treat hypercholesterolemia and hyperlipidemia. Recent reports have indicated that fenofibrate exerts anti-proliferative and pro-apoptotic properties in a few types of cancers. However, report on fenofibrate-induced effects on prostate cancer cells remains lacking. Here we aimed at testing whether fenofibrate can be repurposed for the suppression of human prostate cancer with its cell lines in a vitro model, and underlying mechanism. Methods: We used include cell viability, measured by MTT assays, apoptotic cell death, analyzed by flow cytometry, and Western blots and real-time PCR for the signaling pathway-related targets at both protein and mRNA expression levels, respectively. Results showed that fenofibrate inhibited the growth of androgen-independent prostate cancer cell lines DU145 and PC-3 in dose- and time-dependent manners. Then we chose 50 µM of fenofibrate as the optimal dose in the future studies and demonstrated that fenofibrate-induced cell death is predominately apoptotic death that is mediated by the activation of caspase-3- and AIF-dependent signaling pathways in both cell lines. Thereafter, the underlying mechanism was explored with DU145 cell line alone. We showed that fenofibrate increased the expression of Bad and decreased the expression of Bcl-xl and Survivin. Furthermore, fenofibrate induced a significant increase in apoptosis as measured by Annexin V/PI staining, with or without the addition of nicotine. Conclusions: These results suggest that fenofibrate indeed significantly inhibited human prostate cancer cell proliferation via apoptotic action, which will provide a great potential to be applied into clinics for the treatment of ADT resistance prostate cancer. Therefore, further study in mechanistic details by which fenofibrate suppress prostate cancer proliferation is mediated by its inhibition of the Jak2/Stat3-dependent cell survival pathway.

1932 Fenofibrate Induces Human Prostate Cancer Cell Apoptosis Likely Via Jak2-Stat3 Pathway

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Electronic cigarettes (e-cigs) continue to gain worldwide popularity as a healthier alternative to traditional tobacco products. The potential risks that e-cigs impose on human health are currently under investigation. The unidentified health risks associated with e-cigs use combined with newly implemented federal regulations on production standardization point to the need for e-cig research. Since tobacco use is a well-known risk factor for bone-related diseases, we are interested in the effect of e-cigs on bone-forming osteoblasts. We hypothesize that exposure to e-liquid can impair osteoblast function. Human osteoblast-like Saos-2 cells were exposed to different concentrations of unvaped commercially available e-liquids with either 0 mg/ml, 0.01 mg/ml, 0.1 mg/ml or 1 mg/ml of nicotine for 48 hours. Cell viability was assessed using an MTT assay. Alkaline phosphatase (ALP) activity, a widely used marker for osteoblast activity, was also measured. Unvaped e-liquids were found to significantly reduce cell viability in a dose-dependent manner. This reduction was exacerbated with flavorings agents and varied between different flavors, with fruity flavors having the most cytotoxic effects and no flavorings having the least effect. Furthermore, the cytotoxic effects of the e-liquids were similar in treatments with or without the presence of nicotine. There were no detectable changes in ALP activity in response to any of the e-liquid exposures with or without nicotine. These studies indicate that the degree of osteotoxicity depends on the flavorings. Ongoing studies are investigating mRNA expression of genes involved in osteoblast function including COL1A1, RUNX2, and ALP. This study provides insight into the potential impact of e-cig use on bone health. This research is supported by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under Grant #P20GM103408.

1933 The Effect of Electronic Cigarette Liquids (E-Liquids) on the Human Osteoblast-Like Cell Line Saos-2

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We evaluated the use of bioluminescent real-time viability and LDH release assays for toxicity studies of 3D cultures. The sensitivity (limit of detection <10 cells/well) and nondestructive nature of both assays address the need for monitoring drug toxicities in a time-dependent manner using a small number of cells. We used a novel bioluminescent LDH toxicity assay to monitor the time-dependent effect of panobinostat and doxorubicin on HCT116 spheroids formed in 384 ultra low adherence plates using 2,500 cells/well. Drug toxicity was measured at 24, 48 and 72 hours by removing 5μl of medium for each time point, diluting, freezing and analyzing the samples at the end of the experiment. Both drugs showed time and concentration dependent increases in toxicity with EC50 values decreasing from 1.8 μM to 3.8 μM for doxorubicin and from 17.1 μM to 2.3 μM for panobinostat between 24 and 72 hours of treatment. In both cases the toxicity was measured with >15 fold increase in signal at maximum drug concentration as compared to untreated control. The assay was further validated using 3D human liver microtissues. Treatment of microtissues with staurosporine and aflatoxin resulted in LDH release with calculated EC50 values of 2.9 μM and 3.5 μM, respectively. A bioluminescent real-time viability assay was also validated using induced pluripotent stem cell derived hepatocytes plated in 384-well ULA plates to form spheroids from 2,000 cells/well. The spheroids were dosed every 2 days with hepatotoxic compounds, amiodarone and tolcapone, and viability was determined after 2, 4, 7 and 11 days. For both compounds prolonged exposure increased toxicity and reduced EC50 between 2 days and 11 days of treatment (by up to 8 fold for amiodarone and more than 1,000 fold for tolcapone). These data indicate that both bioluminescent real-time viability and LDH toxicity assays provide convenient tools for measuring drug induced toxicity. The assays are compatible with small numbers of cells, allow monitoring of changes in toxicity over extended periods of time and preserve the viable cells for further analysis or use with other end-point assays.

Heavy Metals in C. elegans

A major route of exposure to various heavy metals is through contaminated soil and water. Research has shown that these substances play roles in the induction of various diseases such as cancer, neurodegeneration and birth defects. In the cell, proteins such as metallothioneins respond to heavy metal exposure and chelate the metal to prevent cellular damage. However, little is known about the cellular response in regards to DNA damage after heavy metal exposure. To provide a better understanding of this cellular response, the induction of both cell cycle arrest and apoptosis were investigated after exposure to copper, cadmium, iron, lead, nickel and silver in the nematode C. elegans. Growth assays were conducted to determine EC10 and EC50 concentrations, which were utilized to determine the DNA damage response pathway. Apoptosis and cell cycle arrest induction upon exposure to a given heavy metal was analyzed in the germline of adult animals after 24h exposure. Apoptosis in the germline was significantly induced in response to all the heavy metals in a dose dependent manner. Nickel, iron and cadmium had the greatest induction at their EC50 concentrations. Additionally, cell cycle arrest was induced in a dose dependent manner for all six metals. Nickel and silver resulted in the greatest induction of cell cycle arrest at their EC50 concentrations. Copper, cadmium, iron, lead, nickel and silver all induce apoptosis and cell cycle arrest in response to exposure suggesting cellular damage and possibly DNA damage from low dose exposures. Investigating the mechanism behind these inductions can help us to better understand the underlying causes to effects of exposure.
Development of a functional blood-brain barrier (BBB) is a complex process regulated by multiple cell types. BBB development consists of several key events during heterotypic cellularization of the embryonic neuroepithelium: vascular invasion and patterning from the perineural vascular plexus; neuroprogenitor proliferation, migration and differentiation in the subventricular zone; and cytokine/growth factor signaling co-opted from microglia, resident macrophages originating in the yolk sac. Disruption of these key events could lead to BBB developmental toxicity. For example, microglia disruption during development is hypothesized to disrupt BBB formation via decreased vessel branching and compromised vessel stabilization. To examine the effects of chemical exposure on the developing BBB, a cell agent-based model was built to recapitulate key events during development of the neurovascular unit. This computer model enables stochastic interactions between microglia and endothelial cells via a complex signaling network (Notch/dIl4, CSF1, VEGF, VEGFR). Molecular elements in these pathways were linked to available receptor targets through EPA’s ToxCast high throughput screening (HTS) dataset in order to translate bioactivity results from the HTS dataset into quantitative predictions of BBB dysmorphogenesis. A simulated dose-escalation study was conducted with Mancozeb, which showed a concentration-dependent response on ToxCast assays of three critical signals mediating microglial-endothelial interplay: CSF1 at low concentrations, VEGFC at an intermediate concentration range, and VEGFA at the upper bound. The predicted no-effect-level (pNEL) on the system transitioned into a predicted lowest effect level (pLEL) at 30% inhibition of CSF1R. The model outcome was decreased surface abundance and reduced branching of the invading vascular network. The pLEL (0.3 μm) from this computational NVU (cNVU) model agreed with the Mancozeb-specific tubulogenesis point-of-departure (0.6 μm) reported from in vitro culture system investigating tubulogenesis in human progenitor cells vs. embryonic (ECV304) from the VAL interface platform. Taken together, development and validation the cNVU model could help predict chemical perturbations to the complex biology of the developing BBB. This abstract may not reflect US EPA policy.

Quantitative Imaging-Based Phenotypic Screening of Adaptive Stress Response Pathways and Computational Methods to Predict Drug-Induced Liver Injury


Early phase drug safety assessment for hepatotoxicity remains a major concern. Improved understanding of mechanisms of action of toxicity is essential to guide the drug development process. Although prediction models can be trained using biological fingerprints of drugs, we present an innovative in vitro toxicity testing platform that addresses both these approaches. Based on transcriptomics data mining we developed a panel of reporter cell lines using BAC technology allowing endogenous regulation of the GFP tagged protein. With high-content live cell imaging and informatics tools we can quantify the temporal and concentration dependent dynamics of key proteins functioning as a hub in specific cellular signaling pathways. Here we focus on five key adaptive stress response signaling programs: 1) the oxidative stress pathway; 2) the UPR in the ER; 3) the heatshock response in the cytoplasm; 4) the DNA damage response; and 5) inflammatory NFκB-signaling. In addition, features of cytotoxicity were captured, including cell count, and stains for necrosis and apoptosis. Together these data allow a compound specific benchmarking of cellular adaptation versus progression to adverse cell outcome. Due to the temporal information relative transcription factor translocation rates and relative protein net production rates are obtained. This allows a physiologically relevant quantification of stress response activation as compared to setting a threshold which results in binary type of on/off switch data. Together these data allow us to define two important features of a tested compound: 1) a biological fingerprint representing all the identified signaling pathways from the pathways and 2) an in vitro therapeutic window which we define as the concentration space between adaptive stress pathway activation to onset of cell death. We present the application of above approach for the assessment of drug-induced liver injury liability using a stress response reporter screen with 118 DILI compounds with different levels of DILI severity. We identified significantly reduced concentrations for onset of adaptive stress response activation for severe DILI compounds and show how the biological fingerprints can be applied to obtain a 70% accuracy of DILI severity using support vector machines and deep neural networks.

1940 Systems Toxicology Assessment of Exposure to Different Snus Extracts and the Total Particulate Matter Fraction of Cigarette Smoke on Human Gingival Organotypic Cultures


Swedish snus, a type of moist snuff, is a smokeless tobacco product (STP) commercialized in the Nordic countries. Published studies indicate a possible association of Swedish snus use with oral pathological conditions, although many of them lack to account for the variability of product chemistry and use patterns. During the last years organotypic cultures have been successfully used as in vitro models for examining the short-term response to environmental exposures and thereby assessing the induced biological effects. The present study aimed at determining the biological impact of a repeated 72-h exposure of human gingival organotypic cultures to snus, extracted from a commercially available product, in comparison with extracts from a reference snus product (CRP1.1, Coresta) and the total particulate matter (TPM) smoke fraction from 3R4F cigarettes. The snus extracts and TPM were tested in two nicotine-matched dilutions - with the TPM dilutions selected to represent a toxic and a sub-toxic stimulus. In addition, a higher concentration of the snus extracts was included, reflecting nicotine levels observed in the saliva of snus users. To assess the exposure effects, we employed a Systems Toxicology approach that complements measurements of cytotoxicity, histopathology, and pro-inflammatory mediator secretion with computational network biology analysis of transcriptome changes. While TPM induced substantial morphological changes, cultures treated with both snus extracts, even at a twenty-five fold higher concentration than TPM, did not exert any obvious histopathological modification. Perturbations of toxicity-relevant networks, such as those related to cell stress and inflammation, and the secretion of pro-inflammatory mediators will be analyzed. This study is of importance to indicate whether Swedish snus products could potentially exert a reduced harm to the oral mucosa in comparison with cigarette smoke.

1941 Biological Test Procedure for Fresh Generated Smoke and Aerosols under Air Liquid Interface Exposure in 24 and 96 Multiwell Plates

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Smoke and aerosol contain particulate matter as well as volatile substances. Both fractions are of potential toxicological relevance and should be considered when assessing biological activity of smoke / aerosol mixtures. Rapid ageing of the mixtures lead to problems regarding trapping and effective delivery of both fractions to the cells. Therefore, dedicated exposure systems have been developed allowing the exposure of biological in vitro systems to freshly generated smoke / aerosols. Imperial Brands’ Smoke Aerosol Exposure In Vitro System (SAEIVS) is designed to expose cells in multiwell plates (MWP) at the Air Liquid Interface (ALI) conditions. The system allows direct contact of both aerosol particles and gas phase components with the apical humid cell surface, recreating exposure at the lung surface as it occurs in vivo. SAEIVS enables in vitro testing of aerosols generated from different product categories encompassing tobacco products and e-cigarette devices. Up to 5 tobacco products or e-cigarette devices can be puffed simultaneously and deliver the smoke/aerosol in undiluted or diluted form. Treated rows of wells are exposed to the generated smoke / aerosol whereby guaranteeing stable exposure conditions over an extended time period sufficient for testing of several hundred puffs. The genotoxicity testing is performed with hamster lung cells V79 exposed grown on inserts and placed in 24 MWPs. Mutagenicity testing is realised by bubbling background suspension directly with freshly generated smoke / aerosol thereby guaranteeing stable exposure conditions and close contact of bacteria cells with the test atmosphere. Salmonella typhimurium Strain TA100 is used in this case because it is highly sensitive to mutagenic substances in both the gas phase and in the particulate matter of smoke / aerosol.
impact neurogenesis through BBB disruption. Taken together, this model integrates new in vitro cell-based tests with existing ToxCast data to translate cell-cell interactions within the NVU into a predictive toxicology framework of BBB disruption. This abstract does not reflect US EPA policy.

1944 Systems Toxicology Assessment of Flavors Compounds Present in E-Vapor Products Using Human Primary Bronchial Epithelial Cells


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Assessing the toxicity of e-cigarettes is challenging considering the large variety of flavored e-liquid mixtures and devices commercially available and the lack of standardized testing methods. While most flavors used in e-liquids are usually considered as safe when ingested or applied topically, there is limited data about their toxicity when they are inhaled. We recently developed a high-throughput approach to assess the biological impact of e-liquid ingredients on primary human lung epithelial cells in vitro. Submerged cells were exposed to serial dilutions of various single flavor compounds in a solution composed of propylene glycol (41%), vegetable glycerin (38%), and nicotine (0.6%). First, real-time cell viability was assessed over a 24h exposure period by impedance-based diagnostic, prognostic, or exposure surveillance utilities. Disclaimers: Research was conducted in compliance with the Animal Welfare Act, and all other Federal requirements. The views expressed are those of the authors and do not constitute endorsement by the US Army.

1943 An Integrative Systems Toxicology Model for Neurovascular Developmental Toxicity

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The blood-brain barrier (BBB) limits passage of toxicants into brain tissue and may be an important tissue interface for developmental neurotoxicity, although the extent to which drugs and chemicals interact with BBB anatomical development is not well known. We utilized a computational systems model of the neurovascular unit (cNVU) in concert with in vitro ToxCast chemical effects data on neurogenesis and angiogenesis assays to build a predictive model of BBB developmental disruption. A subset of 38 ToxCast chemicals was tested for effects on endothelial cell (HUVEC) and neuroprogenitor cell (hNPC, hNC, hNN) behaviors at a range of concentrations. ToxPi potency scores were derived for each chemical across a range of 8 neurogenesis and 9 angiogenesis cellular behaviors. We identified 3 major clusters of cellular effects (neurogenic, neuro-endothelial, and negligible). There were 311 ToxCast assays associated with the positive effects groups, 32 of which could be mapped to one of 21 critical nodes in a nascent systems model of NVU development. ToxPi scoring was used to predict putative BBB disruptors from among the compounds tested, in addition to the complete ToxCast chemical library. PFOs for example, a perfluorinated compound of human health concern, had one of the highest NVU-relevant ToxPi scores suggesting a potential effect on BBB development. PFOs also affected network formation in the neurogenic, but not angiogenic systems. It also tested positive in several inflammatory assays in ToxCast (e.g., TGFβ1, VCAM1, MCF5, IL8, CSF1R, TIE2) suggesting an integrative model would reveal an impact on cytokine signaling. Moreover, at least one study found that PFOs exposure increased BBB permeability. Both PFOs and the synthetic thalidomide analogues, SNPR-33, tested positive for angiogenic effects as detected by ToxCast hits on VEGF signaling (e.g., VEGFR1, VEGFR2, VEGFR3). Both of these compounds also impacted neurogenic endpoints in vitro and suppressed TGFβ1, one of three ToxCast assays directly implicated in progenitor development in the cNVU model. These results highlight potential molecular initiating events that may lead to BBB disruption.

1942 Gene Expression Profiling in Multiple Brain Regions and Peripheral Tissues of Soman Exposed Rats


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Soman (GD) is a potent acetylcholinesterase inhibitor that results in increased levels of acetylcholine leading to a cholinergic crisis upon exposure. This crisis induces convulsions and causes seizures and death if left untreated. Understanding the effects of GD is vital to developing a method of detection and treatment for exposure. We studied gene expression changes associated with GD exposure and resultant seizure activity in rats. Adult male rats were exposed to GD with or without medical countermeasures. Seven to eight hours after exposure, samples were collected from heart, kidney, liver, lungs, spleen, and brain regions, including amygdala, hippocampus, hypothalamus, piriform, medial prefrontal cortex, parietal cortex, and thalamus. Expression patterns demonstrated significant differences between seized and nonseized animals. The magnitude of gene expression changes were tissue types mapped closely to previously characterized patterns of histopathological damage, with the most significant changes occurring in the piriform cortex. Those dysregulated genes associated with seizure activity exhibited enrichment in several cellular functions including protein binding, cell division, phosphorylation, and the inflammatory response. These patterns were well conserved across tissues; amygdala, hippocampus, and piriform that exhibited histopathologic evidence of seizure activity. In the liver, seizing animals showed significant down-regulation of genes involved in oxygen reduction process. Ongoing work will fully characterize the genes and pathways altered by GD exposure and those associated with GD-induced seizure responses across different organs to identify genes and pathways which might have diagnostic, prognostic, or exposure surveillance utilities. Disclaimers: Research was conducted in compliance with the Animal Welfare Act, and all other Federal requirements. The views expressed are those of the authors and do not constitute endorsement by the US Army.
senting chromatin organization, cell cycle regulation, lipid metabolism, and other pathways. Molecular events downstream from the canonical pathways were discovered by heuristic and network inference methods. In conclusion, our systems biology transcriptomics, proteomics, metabolomics, modeling and inference methods identified the MoA of unknown agents within 30 days. Sponsored by the US Army Research Office and the Defense Advanced Research Projects Agency; accomplished under Cooperative Agreement W911NF-14-2-0020.

1946  **Elucidating Systems-Level Mechanism in Toxigenic Profiling of Hepatitis and Cholestasis Using Human Primary Hepatocytes**


Drug-induced liver injury (DILI), such as hepatitis and cholestasis, remains an elusive problem in the drug development process. Lack of connection between the clinical hepatotoxic phenotypes and associated complex molecular-level events further hinders in detecting the intrinsic toxigenic biological properties of drugs and chemicals. The ability to comprehensively relate transcriptomics data to (hepatotoxigenic) profiling could lay a foundation for future toxicity evaluation of active molecules. Thus, this study aims to elucidate the system-level mechanistic in hepatotoxic phenotypes that could be used to predict the compound’s human organ-level toxicity. Based on the drug-induced hepatotoxicity study published by the Japanese Ministry of Health, microarray data for 30 compounds (generated from human primary hepatocytes treated for 24 hours) were downloaded from Open TG-GATEs and processed using standard microarray analytics pipelines. To investigate specific gene sets associated with the respective hepatotoxic phenotypes, weighted gene coexpression network analysis (WGCNA) was performed. WGCNA clusters genes with similar expression patterns into modules and correlates each module to a biological phenotypic trait (e.g. hepatitis or cholestasis). Based on the expression patterns across 30 compounds, ~15000 genes were clustered in 65 distinct coexpression modules. Interestingly, GO enrichment and Reactome pathway analysis of statistically significant (p-value < 0.05) hepatitis- and cholestasis- correlated modules showed major functional differences between the two phenotypes. Hepatitis-specific modules were mainly associated with protein metabolism, cell cycle arrest, and apoptosis. In contrast, pathways such as DNA repair, cell cycle, Notch, Wnt, JNK pathway and other pathways. Molecular events downstream from the canonical pathways were discovered by heuristic and network inference methods. In conclusion, our systems biology transcriptomics, proteomics, metabolomics, modeling and inference methods identified the MoA of unknown agents within 30 days. Sponsored by the US Army Research Office and the Defense Advanced Research Projects Agency; accomplished under Cooperative Agreement W911NF-14-2-0020.

1947  **Evaluation of 2-Methoxy-4-Nitroaniline (MNA) in 14-Day Toxicity, Hypersensitivity, and Genotoxicity Studies**


2-Methoxy-4-nitroaniline (MNA), an intermediate in the synthesis of azo dyes used in textiles and paints, is structurally similar to carcinogenic anilines. Human exposure occurs predominantly through handling of dye dust, and the use and disposal of MNA-containing products. MNA is genotoxic; the pathology findings are suggestive of possible erythrocyte damage and reactive phagocytosis by macrophages in the liver and spleen, and regenerative erythropoiesis.

1948  **MimEX GI Is A Novel and Robust 3D Gastrointestinal Tissue Model for Toxicology and Disease Modeling**


Three dimensional cell culture models of the gastrointestinal epithelium are quickly being adopted for toxicology, drug discovery, and disease modeling. These more complex models provide a tremendous benefit over cell-line and primary cell-based methods, including the mimicking of native intestinal cytoarchitecture and importantly, the recapitulation of physiological attributes of the tissue. Researchers using current 3D models, such as gastrointestinal organoids, experience difficulties with model viability, and tissue adherence. Overcoming these obstacles is paramount for the logistical incorporation of 3D tissues into high throughput toxicity and disease modeling workflows. Here, we introduce MimEX™ GI, an innovative human tissue model system that utilizes the unique characteristics of adult “ground state” stem cells to generate 3D gastrointestinal organ tissue on a 2D surface. In this poster, we demonstrate that the MimEX™ GI platform can be used to clonally isolate and expand “ground state” stem cells from specific regions of the adult gastrointestinal tract. Using MimEX™ Differentiation Media, we show that a high-density monolayer of region-specific gastrointestinal stem cells will differentiate back into its respective tissue of origin, ex vivo. This differentiation is uniform and is oriented such that the apical surface of the mucosa is accessible within the well. Finally, using a high throughput permeability assay, we demonstrate the barrier function of MimEX™ GI tissue, and its response to multiple compounds, as evidence for the flexibility of this tissue in high throughput drug screening.

1949  **'Omics-Based Analysis of 6-Month Inhalation Study for Assessing the Effect of Cigarette Smoke and Cessation in Mouse Lung**


Smoking cessation is thought to be an effective approach to reduce the risk of cigarette smoke (CS)-related diseases. Although several studies reported that smoking cessation improved CS-induced adverse events, such as inflammatory responses, the impact of smoking cessation on global physiological changes is not fully understood. In this study, we performed a 6-month inhalation study using female C57BL/6J mice exposed to filtered air, shams, and 6% CS by volume for 6 months. The effect of smoking cessation was assessed by transcriptome analysis of lung tissue samples. The results indicated that the disruption of central carbohydrate metabolism was observed in CS-inhalation groups, and was also improved by smoking cessation. To quantitatively evaluate the impact of smoking cessation, the amplitude of both gene expression and metabolite profile were calculated as a score, based on the fold change in differential measurements. These scores increased period-dependently in CS-inhalation groups. In contrast, the scores of cessation groups decreased depending on the duration of smoking cessation, and
reached almost the same level as the sham group after 5 months of cessation. These results suggest that smoking cessation could reduce CS-induced adverse events, particularly NF-κB related inflammatory responses and central carbohydrate metabolism, in mouse lung at both the transcriptome and metabolome level.

### 1950 Assessing Genetic Risk from Chemical Exposures through Unbiased High-Content Phenotyping


Humans experience varying biological responses to chemical/environmental stimuli. Predicting differential responses involves understanding how an individual’s genetic profile can influence diverse responses to exposure/treatment. Understanding how genetics influences response to chemical exposure at the cellular level is critical for advancing personalized medicine initiatives and next generation prognosis and/or treatment methods. Genetically characterized human B-lymphoblast cell lines (LCLs) were treated for 48 hours with a panel of 9 compounds with diverse mechanisms of action over a concentration range (based upon the average LCL response). Each plate contained vehicle (0.1% DMSO) and positive controls (10% DMSO) for cell death using Jurkat control cells. The LCLs tested were stained with viability dyes and imaged. To analyze the images and extract phenotypic information, we developed an automated high content analysis pipeline (Clarity Bioanalytics) to measure 11,000+ morphological cell features. Redundant features were eliminated, and the remaining informative features were scored, ranked, and analyzed. Clarity Bioanalytics was used to manage, analyze, and score the high content analysis data. Algorithms were used to define the phenotypic signature for each chemical toxicant. SNP data for each LCL were downloaded from the dbSNP repository and processed for genome-wide association. Pharmacologic quantitative trait loci were mapped based on the genetic profiles of LCLs displaying “normal” (i.e., outliers) phenotypic signatures. Preliminary results from the feature evaluation and selection phase of the project indicate we can identify unbiased (sets of) features that are specific for one or more of the individual compounds. We have screened over 200 LCLs with the compound panel, identified phenotypic signatures that signify exposure to a specific compound and correlated differential phenotypic responses to particular genetic polymorphisms (i.e., SNPs). The identification of genetic correlations to differential response patterns is crucial for personalized assessment of biological effects of chemical exposure.

### 1951 Use of Human Derived Cell Lines to Increase the Biological Relevance of Cigarette Condensate, Non-Tobacco Materials, and E-Liquids Testing


The in vitro testing strategy of tobacco products is mainly based on the testing of extracts from cigarette smoke condensates. An accepted industry wide assay battery has been developed over recent years which delivers reliable results for the toxicological assessment of tobacco products. The testing battery comprises the Neutral Red Uptake (NRU) assay, the in vitro Micronuclei (IVM), and the Ames test. This combination of tests evaluates relevant biological endpoints and is also used for the toxicological evaluation of non-tobacco materials (NTM) like ingredients and e-liquids. All methods are based on the corresponding OECD guidelines which leave some choices regarding bacteria strains used in Ames test and cell lines utilized in NRU and IVM assays. With increasing experience the tests have been improved especially with regards to relevance. To build on this approach further, we use human cell lines to measure cytotoxicity and genotoxicity of smoke extracts, non-tobacco materials and e-liquids. Human liver HepG2 and bronchial epithelial (BEAS-2B) are used in the NRU assay where both show high sensitivity against cigarette condensate. The optimum exposure times were evaluated using a live-cell imaging system. The IVM assay is performed with the human lymphoblastoid cell line TK6. Due to the p53-competency of TK6, false positive effects are significantly reduced in comparison to widely used rodent cell lines. The assay battery is completed by the mutagenicity testing in the Ames test.

### 1952 Effects of Sub-Chronic Exposure to Arsenic on Energy Metabolism in the Liver and Brain Tissues


Arsenic is an element that is abundant in nature and has been associated with various diseases. This research work was conducted to investigate the sub-chronic effects of arsenic on energy metabolism in the cytosolic and mitochondrial fractions of the liver and brain of adult male albino rats. These rats were exposed to 25ppm, 50ppm and 100ppm arsenic as sodium-arsenate in their drinking water for eight weeks. Control animals received distilled water for the same duration after which animals were sacrificed. Activities of Hexokinase (HK), Pyruvate kinase (PK) and Lactate dehydrogenase (LDH) were determined in the cytosol while Sulfuric dehydrogenase (SDH), Malate dehydrogenase (MDH), NADH dehydrogenase (complex I), combined complexes I+III and Cytochrome C oxidase (complex IV) were determined in the mitochondrial fraction using spectrophotometric method. Arsenic significantly (p<0.05) decreased HK, LDH, MDH and complex I activities in the hepatocytes. However, the inhibition was dose-dependent for complex I. In the brain tissue, there was an up-regulation of the activities of HK and PK. Similarly, activities of complex I and combined complexes I+III had over two fold increase in animals exposed to only 100ppm arsenic. A positive dose-dependent relationship was observed in the effects of arsenic on complex IV in both tissues. These findings indicate that arsenic affects energy metabolism but its effects may differ in the tissues.

### 1953 Delineation of the In Vivo Transcriptional Regulatory Network in Livers of Rats Dosed with a Compound That Produces Electrophilic Stress


The liver is the primary site of drug metabolism. Hepatocytes express high concentrations of metabolic enzymes and transporters that enable oxidation, reduction, hydrolysis, conjugation and excretion of endogenous and exogenous molecules. Homeostasis is maintained by numerous signaling pathways which sense the metabolic needs and stressors in the cell and mediate appropriate responses. Frequently these responses are orchestrated via transcription factors (TFs) which bind target DNA and alter expression of specific gene networks. One example is nuclear factor (erythroid-derived 2)-like 2 (aka NRF2 or NFE2L2) which binds AREs to promote gene expression in response to electrophilic stress. ARE containing genes are induced in hepatocytes by the withdrawn hepatobiliary drug ticlopidine which is oxidized by CYP2C19 to form an electrophilic reactive metabolite (Takakusa et al. 2008). A multi-omics based approach was utilized to better understand the in vivo changes induced by ticlopidine at the mRNA, TF and pathway levels in rat liver. Male Sprague-Dawley rats were treated with 600 mpk ticlopidine for 4 days by oral gavage. RNA-sequencing (RNA-seq) was performed on liver samples to identify differentially expressed genes (DEGs) upon compound treatment. Unbiased pathway analyses of DEGs identify up- and down-regulated pathways as well as candidate upstream TF regulators including included oxidative stress pathways regulated by NRF2. Chromatin immunoprecipitation sequencing (ChiP-seq) was performed using NRF2 antibodies to identify genes bound directly by NRF2 in control and ticlopidine treated animals. Additional studies were performed using both direct and indirect NRF2 activating compounds in WT and NRF2 knockout rats. The integrated datasets delineate the genes and pathways directly activated by NRF2 in response to metabolic activation from indirect or orthogonal biological responses. Furthermore, we are applying this multi-omics approach of combining RNA-seq and ChiP-seq to additional pharmaceutical compounds and xenobiotic responsive TFs to elucidate the biological mechanisms of therapeutic response and/or toxicity liabilities.

### 1954 A Quantitative Systems Toxicology Modeling Framework for the Gastrointestinal Immune System


Drug toxicity in the gastrointestinal (GI) tract involves complex and dynamic combination of mucosal damage, immune responses, and the gut microbiota. A variety of gut cells and cytokines interact at multiple spatio-temporal scales, making drug-induced GI toxicity difficult to predict experimentally and complex to understand mechanistically.
To improve quantitative mechanistic understanding and prediction of drug-induced GI toxicity, a framework of quantitative systems toxicology (QST) modeling for the GI-immune system was developed. Our ordinary differential equation (ODE)-based model, adapted from previously published computational models, describes dynamic interactions among diverse cell types in the gut, including commensal and pathogenic bacteria, dendritic cells, T cells, macrophages, neutrophils, and intestinal epithelial cells. The model is used to simulate the effect of drugs with a different mechanism of action on GI mucosal integrity and inflammation. The drugs tested in silico have one of the following modes of action: intestinal epithelial cell lineage damage, T cell suppression, or neuroprotection. Third, increased post-infection recovery time and prolonged bacterial burden are observed in the presence of immunosuppressive agents. Fourth, drug-induced neutropenia leads to a higher bacterial burden post-infection. Overall, our QST framework can serve as a sustainable and expandable platform to better understand and predict the complicated dynamic behavior of the drug-challenged GI-immune system, and to facilitate more rational preclinical study design and safety risk assessment.

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1955 Unravelling Essential Key Events in Mitochondrial Adverse Outcome Pathways


The study focuses on key events relating compound-induced mitochondrial perturbation to hepatotoxicity. The obtained data serve as input for the establishment of quantitative adverse outcome pathways (qAOPs) specific for mitochondrial toxicity. Ultimately, these qAOPs could contribute to the improvement of toxicity prediction and risk assessment of new and existing chemicals. Analytical tools for assessment of mitochondrial functioning in HepG2 cells, including membrane potential, ROS formation, GSH expression and ATP production, were optimised for use in an automated real time confocal microscopy platform. The parameters were monitored along with measurements of cell functioning, including viability. In addition, gene and protein expression are studied using TempSeq for transcriptomics and using BAC-GFP reporter technology for single cell real time analysis of candidate protein expression. Time and concentration-resolved high content analysis of mitochondrial functionality and cell fate outcome were applied in a single exposure setting for mitochondrial toxicants including complex I, II and III inhibitors. Cluster analysis based on all mitochondrial functioning parameters demonstrated a clear separation of the different complex inhibitors. CI and CII inhibitors reduced membrane potential with different potencies. CI inhibitors induced ROS formation and diminished ATP production at lower concentrations compared to CII inhibitors. No effect was observed after CII inhibition in the applied concentration range. Cell viability after CI and CII inhibition was reduced only when cells were switched from glucose- to galactose-containing medium, indicating that a switch to glucose consumption leads to a CI-dependent and adaptive response to CI inhibition. Besides compound characterisation, the data was used to develop a qAOP. Based on the relationship between parameters and the AOP paradigm: for concentrations above the threshold value a reduction in membrane potential was observed along with a subsequent induction of an unfolded protein response and thereof a loss of cell viability. Ongoing experiments address the impact of repeat dose exposures and generate information on global transcriptional changes. The obtained data provides further insight into the chain of events triggered by mitochondrial toxicants. Altogether, this will support optimisation of the testing platform for assessment of mitochondrial toxicity.

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1956 Collaborative Cross Mouse Population-Based Dose-Response Analysis of Liver Transcriptomic Responses with Trichloroethylene at the Levels of Genes and Pathways

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Studies of gene expression are common in toxicology and provide important clues to mechanistic understanding of adverse effects of chemicals. At the same time, gene expression is heavily influenced by the genetic background of an individual, and the genotype-expression relationships may mediate inter-individual variability in toxic effects of chemicals. To date, few studies have dissected the dose- and genetic background-dependent effects of chemicals on the transcriptome. In this study, we tested the hypothesis that the genetically-diverse CC mouse population will enable characterization of genetic background-dose-expression relationships in response to the model liver toxicant TCE. Mice from 50 CC strains were exposed to single oral dose of TCE and tissues were collected. Livers were evaluated for both the level of trichloroacetic acid (TCA), a major TCE metabolite, and gene expression using RNA sequencing. Peroxisome- and fatty acid metabolism-related pathways were among the most dose-dependent enriched gene sets across the population. However, regression modeling revealed that nearly half of the TCE-induced liver transcriptomic effects were strain-dependent, with clinical relevance.

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1957 Application of Systems Pharmacology to Identify Exposure Response Markers in Peripheral Blood after Switching to a Candidate Modified Risk Tobacco Product: The Tobacco Heating System 2.2


As part of current harm reduction strategies, candidate modified tobacco products (MRTPs) are developed to offer adult smokers who want to continue using tobacco products alternative products as potential smoking cessation aids. Typically, studies assess effects on biomarker levels comparing MRTPs to either cigarettes, while potentially reducing individual risk and population harm compared to smoking cigarettes. One of these candidate MRTPs is the Tobacco Heating System (THS) 2.2 which does not burn tobacco, but instead heats it, thus producing significantly reduced levels of harmful and potentially harmful constituents (HPHCs) compared to combustible cigarettes (CC). The assessment of MRTPs against combustible cigarettes requires the establishment of exposure-response markers. Biomarkers derived from blood offer for the general population a less invasive alternative than sampling the primary site, such as the airways. Various diseases and exposures, including cigarette smoke, have been shown to alter the molecular profile of the blood. Leveraging this fact, a whole blood derived gene signature that can distinguish current smokers from either non-smokers or former smokers with high specificity and sensitivity was previously reported. Four controlled, parallel group, open-label clinical studies were conducted with subjects randomized to three monitored groups: (1) switching from CCs to THS2.2 (or its mentholated version, respectively); (2) continuous use of CC brand; or (3) smoking a signature consisting of only 11 genes on the blood transcriptome. Of these effects on the transcriptome were similar whether TCE dose, or level of trichloroacetic acid (TCA), a major TCE metabolite, and gene expression sets were analyzed.

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1958 Identification of Human Lung Epithelial Metabolic Responses to Acute Benzo(a)pyrene Exposure

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Polycyclic aromatic hydrocarbons (PAH) are a family of compounds commonly produced in the burning of wood, fuels, or grilled meats. Several PAH members, such as benzo(a)pyrene (BaP) have been classified as carcinogenic; however, other risk factors include acute inflammation. The concentration of BaP in plasma has been found to be in nanomolar to micromolar range based on exposure duration. Previous studies by our laboratory found alterations in gene expression, amino acid and drug metabolic pathways in the sera of individuals with elevated benzo(a)pyrene as well as several other metabolic pathways. The purpose of this study was to determine the mechanism of BaP on
metabolism and cellular function using A549 lung epithelial cells. The A549 cells were grown until 90% confluence in complete culture media (10% FBS) and then incubated in low serum media (0.5% FBS) overnight before treatment with vehicle or 3 µM BaP for 24h. Treatment at these concentrations was calculated to be approximately 3 times higher than the highest concentration observed in human plasma. The cells were then lysed in 1X RIPA buffer containing 14 internal standards and extracted samples were analyzed by a Q-Exactive mass spectrometer by following the workflow for high-resolution metabolomics as previously reported. The results show that 8532 m/z features that were detected, 621 metabolic features were significantly different after BaP treatment compared to control. Using pathway enrichment analyses, 22 metabolic pathways were significantly affected by BaP, including amino acid, urea cycle, and fatty acid metabolisms, several of which were observed in the human sera samples. Subsequent time course studies were performed as described above (0-24h) using the median physiological concentration of BaP (14.2 nm) determined from our human study. Significant alterations in amino acid metabolism, such as tyrosine, glutamine, and methionine were found using high-resolution metabolomics analysis in a time-dependent manner. Taken together, these results suggest a novel mechanism of acute BaP exposure to A549 cells by altering amino acid pathways which are critical for proper protein function as well as lung function. Future studies using an increased number of participants as well as multiple cell culture models are currently underway and will be significant to determining the effects of BaP exposure on individuals who have undergone chronic BaP exposure and the development of lung cancer.

59 Interferon Signaling Chemical, Pentachlorophenol, Identified by Percellome Toxigenomics Project

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Among about 140 chemicals tested on mouse liver in the Percellome Toxigenomics database, pentachlorophenol (PCP) was identified as a unique chemical that induces interferon signaling. Adult mouse liver transcriptome responses 2, 4, 8 and 24 hours after a single oral administration of PCP (0, 10, 30 and 100mg/kg) was monitored by Affymetrix GeneChip MOE430 2.0. The expression data were analyzed by the Percellome method and expressed as 3D surface with axes of time, dose and copy numbers of mRNA per cell. In the first 8 hours, approximately 100 probe sets (PPS) related to PXR/SXR and Cytp2A4 and other metabolic enzymes were induced, whereas c-Fos and c-Junb were suppressed. At 24 hours, about 1,200 PPSs were strongly induced. Homemade softwares Rsort and PercellomeExplorer were used for comprehensive cross-referencing against the database. Among those 1,200 PPSs, about a half belonged to metabolic pathways including Nrf2-mediated oxidative stress response networks that are shared among many of 140 chemicals. The remainder half belonged to the interferon signaling networking gene sets (ISG), and was unique to PCP. Toll like receptors and other pattern recognition receptors, interferon regulatory factors and interferon alpha itself were included. On the other hand, inflammatory cytokines were not induced. In summary, acute symptoms of PCP, such as hyperthermia and profuse sweating, might be mediated by the ISG (other than the ISG), which has been shown to modify mitochondrial uncoupling mechanism (U Toxicol Sci. 2013: 38:643-54). Recently we added a few chemicals to the Percellome database that modify the expression of ISG genes. Common features with PCP will be discussed.

60 Transcriptional Profiling in Cultured Cells to Determine Chemical Biological Activity: Genistin Case Study

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We have used transcriptional profiling and gene set enrichment analysis, particularly the 50 Hallmark gene sets (MSigDB v6.2), as well as other annotation sources, to better define the biological activity associated with specific chemicals. Gene sets were considered active when they were populated with at least 3 genes from the transcriptional profile being evaluated (Fisher’s exact test, p<0.05). Genistin was selected as an example since it has been established that this isoflavone has multiple biological activities, including the inhibition of protein tyrosine kinases, DNA topoisomerase II and other enzymes, as well as estrogenic activity. Genistin’s transcriptional profile was determined in MCF7, HepG2, HepaRG and Ishikawa cells, after exposure (0.1 mM) for 6h, using a comprehensive (U219; Affymetrix) or a custom made (L1000; Genometry) microarray. For each cell type evaluated, the genes included in the transcriptional profile were selected by their statistical significance (false discovery rate, FDR <0.05) and fold change as compared with the appropriate control (+/- 1.2), cells exposed to vehicle (DMSO). As can be expected, the transcriptional profiles determined in the comprehensive array, in any of the cell types evaluated, include a large number of up- and down-regulated genes, and allows for a more robust gene set enrichment. However, the custom array provides data that defines biologically active gene sets various of which agree with the ones defined by the larger set of genes obtained from the comprehensive array. Overall, MCF7 and Ishikawa cells are more responsive to genistin than HepG2 and HepaRG cells, and this response is different from each cell type. There is a strong concordance of the gene sets upregulated by genistin that are part of the early and late response to estrogen, the p53 pathway and networks, mTORC1 signaling and the TNFα signaling via N-FKB, and apoptosis, among others; as well as the down regulated gene sets including cell cycle control, PI3K/AKT/ mTOR pathway and apoptosis among others.

1961 Mechanistic Modeling of Mitochondrial Biogenesis within DILIsym Could Explain Clinically Observed Adaptation of Serum Alanine Aminotransferase (ALT) Elevations


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Resolution of serum ALT elevations despite continued drug dosing, termed “adaptation”, is commonly observed in clinical trials, but the underlying mechanisms behind this phenomenon remain unclear. Mitochondrial dysfunction is one of the major mechanisms underlying drug-induced liver injury (DILI). When mitochondrial function is insufficient for energy demand, mitochondrial biogenesis is often activated and we speculated this may be a mechanism underlying adaptation. Solithromycin, a 4th generation macrolide developed for the treatment of community acquired pneumonia, caused serum ALT elevations in a minority of patients in clinical studies, with improvement often observed during continued dosing (or with rapid recovery thereafter). Quantitative toxicology modeling of solithromycin using DILIsym showed that ALT elevations were mediated predominantly by mitochondrial electron transport chain (ETC) inhibition, suggesting that mitochondrial biogenesis might have contributed to the adaptation observed. In the current study, biogenesis was mechanistically represented within DILIsym, and its impact on time dependent ALT elevations resulting from solithromycin treatment was assessed. In DILIsym, mitochondrial ETC enzyme content and activity are modeled based on synthesis and degradation rates. Modeled decrements in liver ATP levels were employed as a stimulus for increase in the synthesis rate of these enzymes. Kinetic parameters for biogenesis were optimized based on clinical ALT data from patients administered solithromycin (Km = 0.055 μM; Hill = 4.14-14 mmol/l; Hill = 1.5; ATP decrement delay constant = 96 hr). Solithromycin effects on serum ALT were simulated in the absence and presence of biogenesis in a human simulated population (SimPops) that includes variability in mechanistic pathways underlying DILI. Addition of mitochondrial biogenesis to the model predicted the observed behavior of ALT at continued exposure. Overall, DILIsym modeling suggests that mitochondrial biogenesis contributes to hepatic adaptation to xenobiotic stress.

1962 A Distinct Class of Antioxidant Response Elements Is Consistently Activated in Tumors with Nrf2 Hyperactivating Mutations

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NRF2, a key regulator of the transcriptional response to oxidative stress, regulates expression of cytoprotective genes via its interaction with DNA sequences known as antioxidant response elements (AREs). Mutations that disrupt the interaction; caused by NRF2 and KEAP1, an important inhibitor of NRF2, lead to NRF2 hyperactivation and promote tumorigenesis. The exact mechanisms underlying NRF2’s oncogenic...
properties remain unclear, but likely involve aberrant expression of select NRF2 target genes. We tested this possibility using an integrative genomics approach, combining our own NRF2-centric functional genomics data with thousands of tumor genome and transcriptome profiles, to get a precise view of the direct NRF2 target genes dysregulated in tumors with NRF2 hyperactivating mutations. This approach revealed a cancer-specific NRF2 target gene set that is consistently upregulated in NRF2 hyperactivated tumors. This set of NRF2 “cancer target genes” includes canonical redox-related NRF2 targets (NQO1, etc.), as well as several target genes that have not been previously linked to NRF2 activation; NRF2-driven upregulation of this gene set is largely independent of the organ system where the tumor developed. This critical subset of NRF2 targets is significantly enriched for genes functionally linked to oncogenesis, and several features differentiate these genes from other NRF2 targets. One of the key distinguishing features is that the NRF2’s cancer targets are regulated by high affinity, switch-like AREs that fall within genomic regions that possess a distinct, ubiquitously permissive chromatin signature. This finding implies that the NRF2 cancer target genes are highly responsive to oncogenic NRF2 in most tissues because they lack the epigenetic restraints that restrict expression of most other NRF2 target genes. Notably, the NRF2 cancer target gene set also serves as a reliable proxy for NRF2 activity, independent of mutational status, and high inferred NRF2 activity is associated with significant decreases in survival in at least 10 different cancer types, including hepatocellular carcinoma, papillary kidney carcinoma, and squamous cell carcinoma. Together, these results suggest that this prognostically valuable gene set is uniquely responsive to NRF2 activity in many cell types, and will be central to dissecting the functional implications of NRF2 activation in several cancer contexts.

1965 Mass Spectrometry Based Untargeted Metabolomics for Dose-Response Analysis of Liver Injury Compounds: Utility and Comparisons to Transcriptomic Analysis

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This work is part of our initial efforts to determine how metabolomics data can aid in analysis of compounds that are potentially toxic and may have negative effects on human health. Understanding the role that xenobiotics have on endogenous metabolism can help determine which compounds are potentially hazardous. Untargeted metabolomics of in vitro cell systems were studied in a traditional dose-response analysis to investigate the effects of cells at different concentrations of toxic compound exposure. A set of compounds at ten different concentrations were incubated with 2D confluent HepaRG cells over a period of 96 hours. After every 24 hours, a sample of media was removed from each well. A Thermo Vanquish liquid chromatography/LC system was used in conjunction with a Thermo Q Exactive Plus for untargeted analysis. LC conditions consisted of a 20-minute gradient run using a Thermo Hypersil Gold aq column. MS and MS/MS data were collected in both positive and negative mode. Thermo’s Compound Discoverer 2.0 and XCMS online were used for data analysis. One compound investigated in this work is Tamoxifen. Transcriptional analysis of HepaRG cells exposed to Tamoxifen showed changes in lipid metabolism pathways. Additionally, cell imaging showed evidence of hepatic steatosis. Untargeted metabolomics data of media from in vitro incubations of HepaRG cells with different concentrations of Tamoxifen compared favorably to the transcriptional changes observed, in that transcriptional changes were observed in lipid metabolism pathways that were coincident with changes in lipid concentrations in the spent media. Selected metabolites were used to perform dose-response analysis to determine at what concentrations tamoxifen could cause clear metabolic level changes. In the metabolomics experiment 507 metabolite features were identified with a median benchmark dose of 31.7 μM and a benchmark dose of 16 μM for the first quartile. In comparison, the median benchmark dose for the transcriptomics experiment was 57.6 μM with a benchmark dose of 33.7 μM for the first quartile. Thus, the metabolomics experiment found benchmark doses at approximately 2x lower concentrations than the transcriptomics experiments. These initial results suggest that metabolomic changes may be more sensitive than transcriptomics.
Gene Expression Profiling and Machine Learning to Characterize the Molecular Mechanisms of P38K Inhibitor-Induced Gastrointestinal Side Effects

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Iatrogenic gastrointestinal disorders are among the most debilitating side effects of first line chemotherapy. In many cases, colitis, diarrhea and other drug-induced conditions limit tolerability of cancer treatment, such as taxanes where a high percentage of patients experience diarrhea. Some targeted therapies, such as P38K inhibitors, also cause gastrointestinal side effects. Thus, understanding the molecular mechanisms of GI toxicity caused by chemotherapeutic and targeted agents may provide insights into key genes and pathways, highlighting potential targets suitable for co-therapies that can mitigate them. To this end, we have used computational methods to explore the connections between the endpoints and assignment of cellular dysfunction (IBD). GI effects caused by bacteria and viruses, and GI toxicities caused by drugs or compounds. In total, we have investigated over 60 publicly available datasets that included compounds such as irinotecan, wolmanimm, methotrexate and multiple P38K inhibitors. We also performed whole-transcriptome profiling of the human Epithelial Gastrointestinal in vitro model treated acutely with diverse P38K inhibitors at their respective plasma steady-state concentrations. We show that gene expression profiles following treatment with irinotecan, a compound that causes severe GI toxicity, look most similar to GI diseases such as CD and IBD. In contrast, molecular signatures of P38K inhibitors were distinct from that of GI diseases. Surprisingly, gene expression profiles of P38K inhibitors shared similarities to the molecular signatures of a specific enteropeptic bacterial toxin with the cell cycle and autophagy pathways being most transcriptionally disrupted. This suggests that using irinotecan as a GI toxicity model may not be suitable to study molecular mechanisms for P38K-induced GI toxicity. We also found that inhibitors targeting different P38K isoforms have different levels of similarity to the bacterial toxin signature. We conclude that P38K-induced GI toxicity has a unique molecular signature that is different from GI diseases or irinotecan-induced GI toxicity. Instead, it has a novel association to the molecular mechanism of toxicity caused by an enteropeptic bacterial toxin, with some degree of isoform differences.

Mend the Gap: Organizing Toxicity Endpoints under the Hallmarks of Aging’s Nine Categories of Cellular Dysfunction

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Toxicologists and medical researchers have traditionally approached the mechanistic understanding of biological dysfunction from opposite ends of the causal chain. Toxicologists develop adverse outcome pathways (AOPs) beginning with a stressor-induced molecular initiating event. In contrast, molecular signatures of P38K inhibitors were distinct from that of GI diseases. Surprisingly, gene expression profiles of P38K inhibitors shared similarities to the molecular signatures of a specific enteropeptic bacterial toxin with the cell cycle and autophagy pathways being most transcriptionally disrupted. This suggests that using irinotecan as a GI toxicity model may not be suitable to study molecular mechanisms for P38K-induced GI toxicity. We also found that inhibitors targeting different P38K isoforms have different levels of similarity to the bacterial toxin signature. We conclude that P38K-induced GI toxicity has a unique molecular signature that is different from GI diseases or irinotecan-induced GI toxicity. Instead, it has a novel association to the molecular mechanism of toxicity caused by an enteropeptic bacterial toxin, with some degree of isoform differences.

Utilization of Scrap Tires Versus Firewood and Liquefied Petroleum Gas as Fuels for Singeing Meat in Ghana: Chemical Emissions Via Smoke

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Utilization of scrap automobile tires for singeing food animal carcasses at meat processing facilities in Ghana (and other countries) has been ongoing for over 50 yr. This practice can release chemical pollutants such as carbon monoxide (CO), benzene, sulfur dioxide (SO2), particulate matter (PM), etc. into the air via smoke. Exposure to these chemicals has been associated with adverse health effects such as respiratory impairment, mutagenesis, and cancer. This study aimed at determining the levels of CO, benzene, SO2, PM10, and PM2.5 emitted via smoke produced at three slaughter slabs (using tires and firewood) and Kumasi Abattoir (which uses liquefied petroleum gas (LPG) as a fuel). A Chip Measurement System and an Aerosol Monitor were used to quantify the chemical levels in real time. Results indicate that the average levels of both PM10 and PM2.5 from LPG-based smoke (0.7±0.2 mg/m3; 95% CI 0.3-1.1) and tire smoke were significantly lower than that of firewood (24.2±5.2 mg/m3; 95% CI 13.1-35.3) and PM10 (95% CI 13.1-35.3, respectively) and tire smoke was lower than that of tire smoke (24.2±5.2 mg/m3; 95% CI 13.1-35.3, respectively) and tire smoke was lower than that of firewood (24.2±5.2 mg/m3; 95% CI 13.1-35.3, respectively). Tire smoke was lower than that of tire smoke (24.2±5.2 mg/m3; 95% CI 13.1-35.3, respectively) and tire smoke was lower than that of firewood (24.2±5.2 mg/m3; 95% CI 13.1-35.3, respectively). Tire smoke was lower than that of tire smoke (24.2±5.2 mg/m3; 95% CI 13.1-35.3, respectively) and tire smoke was lower than that of firewood (24.2±5.2 mg/m3; 95% CI 13.1-35.3, respectively). Tire smoke was lower than that of tire smoke (24.2±5.2 mg/m3; 95% CI 13.1-35.3, respectively).
nificantly higher compared to those of LPG. These data suggest that slaughter slab operators and residents nearby these meat processing facilities may be at high risk for adverse health effects linked to these pollutants. A more comprehensive study is warranted to sufficiently characterize this tire burning-health effect link.

### 1970 Concordance of Pathophysiologic Responses in Mice Exposed to Different Biomass Smoke Conditions Via Aspiration and Inhalation


We have previously reported that aspiration of an equal mass (100 µg) of particle matter (PM) in flaming biomass smoke condensate caused greater lung toxicity in mice than samples from smoldering smoke. In this study, we conducted inhalation exposures on a subset of the biomass smoke fuels and conditions, and compared with the previous results before and after dosimetric adjustment for inhaled PM. Biomass smoke from peat, eucalyptus and oak fuels was generated under smoldering and flaming phases with PM levels precisely maintained by an automated smoke emission controlling system. Mice were exposed for 1 hour/day for 2 days and then assessed for lung toxicity at 4 and 24 h after the second exposure. PM levels were ~40 and ~4 mg/m3 from the smoldering phases, and ~3 mg/m3 from the flaming phases, respectively. BALF neutrophils (PMN; <0.05), eosinophils (EOS; not significant) in comparison to air exposure in HDM-allergic mice. Biochemical markers of lung injury (e.g., protein, LDH, albumin, cytokines, enzymes) were measured at 4 h although effects were equivalent for both conditions by 24 h. A significant increase in ventilator timing (as measured by Penh, potentially indicating airflow obstruction), was observed in mice exposed to flaming peat and for both flaming and smoldering eucalyptus immediately after each day of exposure, in agreement with the inflammation results. No pathophysiological responses were seen following exposure to either combustion condition of oak, which mirrored the responses following aspiration exposure. Overall the results show good concordance in responses between aspiration and inhalation studies depending on type of fuel and combustion conditions and confirm that PM from flaming condition is, on a mass basis, more toxic than that from smoldering smoke. This abstract does not represent US EPA policy.

### 1971 Differential Effects of Wildfire Biomass Smoke Inhalation on Allergic Inflammation in Mice


Wildfires emit high concentrations of airborne particulate matter (PM) and volatile organic compounds which can impact sensitive populations such as asthmatics. Health effects of these biomass emissions may vary significantly depending on fuel type and burn conditions. We assessed the relative acute toxicity of biomass smoke produced from controlled burns of peat, oak, or eucalyptus under smoldering (SM; 510 °C; ~40 mg/m3 PM; PAH levels) and flaming (FL; 640 °C; ~3.5-4 mg/m3 PM; PAH levels) conditions via transdermal inhalation of airborne smoke with equivalent carbon monoxide levels in house dust mite (Der p1) allergen (HDM) Balb/CJ mice. Control and HDM-sensitized mice were challenged with HDM 1 day before nose-only exposure to air, SM, or FL smoke (1 h/d x 2 d). Lung inflammation and histopathology were characterized with respect to the control group. Similar finding were found for genes related to xenobiotic metabolism, Cyp1a1; DNA damage, Ddih3; metal exposure, Mt1; and aromatic hydrocarbon receptor, AhR. Histological evaluation of treated animals revealed hepatic steatosis and inflammation. This study evidenced exposure to inhalable coal dust promotes changes at molecular, cellular and histopathological levels in mice. This information should serve as a basis for government entities to implement solid environmental standards that favor sustainable mining development in the country, with minimal impact on human or environmental health. Colciencias-UNIcartagena 589-2013 and 567-2012.

### 1972 Toxic Effects of Inhalable Coal Dust in BALB/c Mice

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Coal mining is a strategic sector for Colombia’s development and is one of the most widely used energy sources globally. Coal dust generated from mining operations has been associated with the increase of chronic diseases in inhabitants and in different organisms near mines. The objective of this study was to evaluate the toxic effects of coal dust with a particle size of less than 74 µm on Mus musculus BALB/c mice. For that purpose, mice were exposed during 10 weeks to sand spiked with different concentrations of coal dust (0, 0.25, 0.5, 1, 2 and 4% w/w) obtained from a sample of bituminous coal collected from a mine in Cesar, Colombia. After exposure, mice were euthanized for the extraction of blood and tissue samples for different assays. Peripheral blood smears revealed that the average values for polychromatim erythrocytes, micronuclei and microcytes were statistically greater than those in the control group. In the case of normoblasts, a significant increase was observed only at the two greatest tested concentrations. TBARS assay did not show significant differences in oxidative damage between exposed groups, although the relative mRNA expression of genes relate to oxidative stress, Nf2 and Sod1, displayed a sub-expression profile with respect to the control group. Similar finding were found for genes related to xenobiotic metabolism, Cyp1a1; DNA damage, Ddih3; metal exposure, Mt1; and aromatic hydrocarbon receptor, AhR. Histological evaluation of treated animals revealed hepatic steatosis and inflammation. This study evidenced exposure to inhalable coal dust promotes changes at molecular, cellular and histopathological levels in mice. This information should serve as a basis for government entities to implement solid environmental standards that favor sustainable mining development in the country, with minimal impact on human or environmental health. Colciencias-UNIcartagena 589-2013 and 567-2012.

### 1973 Transgenerational Effects of Coal Dust on Tribolium castaneum, Herbst

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Coal dust is a primary air contaminant from coal mining operations, where it has been recognized to produce harmful health effects. However, it is unclear to what extent its detrimental properties would impact future generations, and whether alterations in the progenies might be dose-dependent. The objective of this study was to determine the transgenerational effects of chronic exposure to coal dust on the red flour beetle, Tribolium castaneum (Herbst) at three life stages. Fifty insects in the adult instar were exposed during 10 weeks at different concentrations (0.0, 0.25, 0.5, 1.0 and 2.0%) of coal dust, particle size <38 µm, mixed with ground oats as food substrates. The average LC50 for F0 insects was 1.06%, whereas for larval, pupa and adults values were 0.18, 0.85 and 0.63%, respectively. Morphological damage assessed at F1 revealed a concentration-dependent frequency of several abnormalities, including larvae without antenna or extremities, and lack of T1, T2, T3 legs. On the other hand, it was found that F1 larvae derived from parental beetles did not achieve a complete conversion into the next growth stage, displaying a lack of femur, tibia, tarsus and elytron. In the same way, pupae without genital lobes were recurrently observed, and at high concentrations, those exhibited protuberances between segments IV, V and VI of the abdomen and undeveloped eyes. In short, sub-inhalable coal dust particles induced transgenerational effects on T. castaneum, highlighting the need to further study the impact of this airborne pollutant on wildlife and human populations. Colciencias-UNIcartagena, Grants: 589-2013, 567-2012 and 757-2016.
Coal is a key energy source in the world, therefore, its mining has steadily been increasing, simultaneously promoting environmental contamination. The aim of this research was to determine the morphological, physiological and behavior changes of *Eisenia fetida* exposed to bituminous coal dust from a coal open mine located in the department of Guajira, Colombia. The coal sample was obtained from the Cerrejon coal mine, and subsequently pulverized up to coal dust ≤ 38 µm. The worm cultivation was standardized under laboratory controlled conditions to evaluate lethality and physiologically-based assays using concentrations of 1, 2, 3 and 4% coal dust in artificial soil. The weight, morphological and behavior changes were visualized at 7, 14 and 28 days of exposition; an avoidance assay carried out after 48 hrs of exposition, and histopathological analysis were carried out at the end of the experiment. Mortality reached 5% at 4% coal dust in soil. The avoidance test followed a trend directly proportional to the coal concentration in soil. Morphological and behavioral alterations of the specimens were evident within 14 days of treatment at concentrations of 3 and 4%, presenting significant changes after 28 days of exposition, consisting of excessive production of mucus, lethargic and violent movements, excessive time to bury in the treated substrate and loss of weight. Histopathological findings revealed significant changes in the size of the ectodermic cells, as well as the expansion of the intercellular spaces in muscle tissues in organisms exposed to 3 and 4% coal dust in soil. In conclusion, *E. fetida* exposed to coal dust experienced morphological, behavioral and histopathological changes, suggesting this pollutant may induce population problems in macro invertebrates present in mining operation areas. *Colciencias-UniCartagena, Grants 589-2013, 567-2012 and 647-2014.*

### 1975 A High Fat Meal after Peat Smoke Inhalation Unmasks Latent Cardiometabolic Responses in Rats


Stress tests are used clinically to uncover underlying disease and predict future cardiovascular risk. Previously, we used treadmill exercise stress in rats to reveal latent effects of air pollution inhalation. Other daily stressors, when modeled experimentally, may have similar utility. For example, consumption of a high fat (HF) meal causes transient vascular changes. To evaluate the effects of air pollution exposure on cardiovascular responses to a HF challenge. Healthy male Wistar Kyoto rats were exposed once (1 hr) to filtered air (FA), or low (LP; 0.36 mg/m³) or high (HP; 3.76 mg/m³) concentrations. Rats were monitored for heart rate (HR), blood pressure (BP), electrocardiogram (ECG), and metabolic markers, and pulmonary and systemic inflammatory markers, and flow cytometry to assess circulating monocyte phenotype were carried out in sensitized mice (n= 8/group) were challenged with HDM 1 day before coming). By contrast, only exposure to LP increased sensitivity to aconitine-induced cardiac arrhythmia relative to exposure to FA (p<0.01). Few changes in systemic markers were evident. Taken together, HP caused overt responses not present with LP that were potentially mediated by autonomic responses. Perhaps of greater concern is the finding that exposure to lower levels that better approximate most human ambient exposures to smoke plumes only caused latent effects, indicating that the effects of exposure may be insidious. This abstract does not reflect US EPA policy.

### 1977 Smoldering Eucalyptus and Red Oak Smoke Inhibit Respiration in an Allergic Asthma Mouse Model

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Short- and long-term exposures to biomass wildfire smoke pose significant health risks to firefighters and impacted communities. Susceptible populations including asthmatics may be particularly sensitive to the effects of biomass smoke emissions. We examined pulmonary responses to biomass smoke generated from eucalyptus, Irish peat, or red oak burns under low temperature smoldering or high temperature flaming conditions in female control and house dust mite (HDM)-allergic Balb/c mice. For each fuel type, particulate matter levels were maintained at ~40 (smoldering) or ~3.5-4 (flaming) mg/m³ while CO levels were comparable (~60-120 ppm) under both conditions. Control and HDM sensitized mice (n= 8/group) were challenged with HDM 1 day before exposure to air or biomass smoke for 1 hour/day for 2 consecutive days. Pulmonary responses were assessed using head-out plethysmography chambers to measure thoracic flow and ventilatory parameters prior to (20 min) and during (1 hr) biomass smoke exposure. Both smoldering eucalyptus and red oak reduced breathing frequency in control and HDM-allergic mice. These fuel types also significantly elevated inspiratory time under smoldering conditions compared to the flaming treatment groups. Under flaming conditions, eucalyptus and red oak significantly increased minute volume and peak inspiratory flow. These changes were observed during both days of exposure. However, relaxation time was significantly prolonged during day 1 exposure to smoldering eucalyptus only. In contrast, peak expiratory did not alter these ventilatory parameters under either condition. We conclude that exposure to eucalyptus or red oak smoke inhibits respiration to a greater degree than peat smoke under smoldering conditions. This abstract does not represent US EPA policy.
Potential exposures from burn pit emissions have raised concerns related to the significant numbers of deployed military personnel with respiratory problems and diminished exercise tolerance. As a result, there is a need to understand potential molecular changes seen upon inhalation of airborne hazards as well as development of accurate biomarkers indicating accurate exposure and individuals monitoring. A joint NAMRU-D/AFRL collaborative in vivo study using whole body exposures of male Sprague Dawley rat to air borne hazards was conducted in response to these needs. Using an ambient breeze tunnel (ABT) at Battelle, West Jefferson OH, whole body exposures of rats were conducted for five days (6 h/day) to emissions generated from a surrogate burn pit composed of mixed waste representative of materials burned in-theater. Blood samples were collected at pre-exposure and five day post-exposure. Six sets of plasma samples from each time point were pooled and processed together. Rat plasma proteins were depleted utilizing IgY immune-depletion spin columns and the depleted fractions were labeled with TMT6, reduced, alkylated, and digested with trypsin. The digested protein solutions were desalted via C18 spin columns and fractionated into seven samples using a strong cation exchange (SCX) spin column. All seven fractions were run on a reverse phase nanoAcquity UPLC-LTQ Orbitrap Velos mass spectrometer (MS) system for analysis, and acquired MS and MS/MS data were searched against the FASTA database for protein identification and quantitatively analyzed using the SEQUEST algorithm in the Proteome Discoverer software suite. About 2800 proteins and 157 protein groups were identified and found to be > 2 fold differentially expressed in pre/post exposure set comparisons. The differentially expressed protein biomarkers were exported and further analyzed using Ingenuity Pathway Analysis (IPA) software. Interestingly, proteins identified have been also identified in published inhalation exposure studies of volatile organic compounds and particulate matter with a subset shown to be involved in inflammatory lung diseases. In compliance with DODI 3216.01.

Far from being sterile, the normal lung contains an individualized micro-biome that may influence if and/or how the lung responds to inhaled airborne hazards. Military personnel exposures to sand and/or burn pit emissions and concerns on potential development of respiratory issues led to a collaborative effort between NAMRU-D and AFRL to identify molecular alterations, including changes in the lung microbiome, which are initiated by inhaled toxicants. Sprague-Dawley rats were exposed to air (control), Burn Pit emissions (BPE), sand, or sand + BPE using whole body exposure chambers. BPE exposures from mixed solid waste were conducted 6 h/d for 5 days, whereas sand exposures (PM2.5) were conducted a total of 20 h/day, 5 days/wk, for 4 weeks, with ventilatory function tests conducted on all groups. Bronchial alveolar lavage fluid removal and lung tissue collection was accomplished in euthanized animals at post-exposure time points (4, 32, 90 days), with histopathology completed on lung tissue. Operational Taxonomy Units (OTUs) of bacterial populations were identified in lavage and lung tissue using bacterial genomic DNA extraction and 16S rRNA sequencing. Compared to controls, sand exposures did not alter the inhibitory effects of pine PM on scratch wound repair. Further investigation of the significance of homocysteine and HERPUD1, as well as other elements of the ER stress response is needed to better understand how TRPV3 signaling may influence the acute and sub-acute pneumotoxic effects of wood smoke PM. Supported by ES017431, and ES027015.

**1979 The Mechanisms Underlying TRPV3s Role in Lung Pathology following Chronic Exposure to Wood Smoke Particulate Matter**

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Transient receptor potential vanilloid-3 (TRPV3) is a Ca2+ channel expressed in the skin where it regulates cell growth, differentiation, and wound repair. Recently, our laboratory demonstrated TRPV3 expression in human lung epithelial cells, and a role for TRPV3 in regulating cellular responses to inhaled wood smoke particles (PM). We showed that PM-stimulated PM activates TRPV3, and that PM-induced histone deacetylation is dependent on TRPV3 activity. Additionally, mice treated sub-acutely with pine PM via oropharyngeal delivery displayed increased scratch wound repair rates, it did not alter the inhibitory effects of pine PM on scratch wound repair. Further investigation of the significance of homocysteine and HERPUD1, as well as other elements of the ER stress response is needed to better understand how TRPV3 signaling may influence the acute and sub-acute pneumotoxic effects of wood smoke PM. Supported by ES017431, and ES027015.


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Combustion of solid fuels in small boilers is a widely used form of heating family houses. These combustion processes emit large quantities of harmful emissions. Epidemiological studies show that PM created in heating appliances contains carcinogens and mutagens and thus may have health impacts. Previous studies have noted that the quality of combustion is affected by the combustion technology, user operation, and fuel, all of which affect the formation of emissions. In this study, various solid fuels (hard coal, lignite, dry wood, wet wood, lignite briquettes, wood pellets) were burnt in 4 types of boilers representing both old structural designs (over-fire and under-fire boilers) and also up-to-date combustion devices (gasification and automatic boilers). Nominal and reduced performance outputs of boilers were tested to compare the concentration of organic PM components and their genotoxicity. For this purpose, the organic components of collected total particulate matter (formed by 93-100% by PM2.5) were extracted, 16 priority PAHs were quantified and the analysis of the genotoxic potential of extracts was assessed by acellular assay of DNA adducts in calf thymus DNA. We found, depending on the boiler’s technology, fuel quality and output (reduced or nominal) the mass of emitted PM2.5 varied from 0.2 to 64 kg/ton of fuel. The concentrations of the representative carcinogenic PAH - benzo[a]pyrene varied from 5 to 18,000 mg/ton of fuel. Such huge differences in PAH content are reflected by results of the genotoxic potential in acellular assay: DNA adducts in calf thymus DNA (50 μg of extract for 24 h) after metabolic activation of PAHs varied from 6 to 140 adducts/10^9 nucleotides. The results of the study suggest that: (1) The highest genotoxicity was observed for over-fire boilers; (2) Reduced output exhibited more emissions and higher genotoxicity than nominal output; (3) Modern boilers produced less emissions and exhibited lower genotoxicity. In summary, huge differences in mass, composition and genotoxic potential of complex mixtures of organic compounds forming small PM emissions from various boilers were found. Supported by Czech Science Foundation (P-503-12-G147), Ministry of Education (LO1403), and Research Infrastructure NanoEnviCz (LM2015073).

**1978 Blood Proteomic Biomarker Discovery Using an In Vivo Inhalation Model of Burn Pit Emission Exposures**

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Potential exposures from burn pit emissions have raised concerns related to the significant numbers of deployed military personnel with respiratory problems and diminished exercise tolerance. As a result, there is a need to understand potential molecular changes seen upon inhalation of airborne hazards as well as development of accurate biomarkers indicating accurate exposure and individuals monitoring. A joint NAMRU-D/AFRL collaborative in vivo study using whole body exposures of male Sprague Dawley rat to airborne hazards was conducted in response to these needs. Using an ambient breeze tunnel (ABT) at Battelle, West Jefferson OH, whole body exposures of rats were conducted for five days (6 h/day) to emissions generated from a surrogate burn pit composed of mixed waste representative of materials burned in-theater. Blood samples were collected at pre-exposure and five day post-exposure. Six sets of plasma samples from each time point were pooled and processed together. Rat plasma proteins were depleted utilizing IgY immune-depletion spin columns and the depleted fractions were labeled with TMT6, reduced, alkylated, and digested with trypsin. The digested protein solutions were desalted via C18 spin columns and fractionated into seven samples using a strong cation exchange (SCX) spin column. All seven fractions were run on a reverse phase nanoAcquity UPLC-LTQ Orbitrap Velos mass spectrometer (MS) system for analysis, and acquired MS and MS/MS data were searched against the FASTA database for protein identification and quantitatively analyzed using the SEQUEST algorithm in the Proteome Discoverer software suite. About 2800 proteins and 157 protein groups were identified and found to be > 2 fold differentially expressed in pre/post exposure set comparisons. The differentially expressed protein biomarkers were exported and further analyzed using Ingenuity Pathway Analysis (IPA) software. Interestingly, proteins identified have been also identified in published inhalation exposure studies of volatile organic compounds and particulate matter with a subset shown to be involved in inflammatory lung diseases. In compliance with DODI 3216.01.

Far from being sterile, the normal lung contains an individualized microbiome that may influence if and/or how the lung responds to inhaled airborne hazards. Military personnel exposures to sand and/or burn pit emissions and concerns on potential development of respiratory issues led to a collaborative effort between NAMRU-D and AFRL to identify molecular alterations, including changes in the lung microbiome, which are initiated by inhaled toxicants. Sprague-Dawley rats were exposed to air (control), Burn Pit emissions (BPE), sand, or sand + BPE using whole body exposure chambers. BPE exposures from mixed solid waste were conducted 6 h/d for 5 days, whereas sand exposures (PM2.5) were conducted a total of 20 h/day, 5 days/wk, for 4 weeks, with ventilatory function tests conducted on all groups. Bronchial alveolar lavage fluid removal and lung tissue collection was accomplished in euthanized animals at post-exposure time points (4, 32, 90 days), with histopathology completed on lung tissue. Operational Taxonomy Units (OTUs) of bacterial populations were identified in lavage and lung tissue using bacterial genomic DNA extraction and 16S rRNA sequencing. Compared to controls, sand exposures did not alter the inhibitory effects of pine PM on scratch wound repair. Further investigation of the significance of homocysteine and HERPUD1, as well as other elements of the ER stress response is needed to better understand how TRPV3 signaling may influence the acute and sub-acute pneumotoxic effects of wood smoke PM. Supported by ES017431, and ES027015.
The global public health impact from household fine particulate matter (PM$_{2.5}$) resulting from heating and cooking sources is extremely large. However, there is a limited understanding of health effects associations with specific PM$_{2.5}$ chemical constituents as well as the underlying mechanisms of these adverse health effects. Use of a high-throughput screening platform enables biological response data to be collected to address these research gaps. To establish the utility of this experimental design, a subset of homes in Kheri, India that participated in the Prospective Urban and Rural Epidemiological (PURE)-AIR pilot study were selected to identify differences in chemical and biological measurements of household PM$_{2.5}$. Personal air monitors collecting PM$_{2.5}$ were worn by female participants and paired with stationary monitors, resulting in personal and home PM$_{2.5}$ filters for six households. PM$_{2.5}$ was removed from filters via sonication in methanol. Aliquots of individual filter samples were removed for chemical analysis (polycyclic aromatic hydrocarbons (n=61), elements (n=20)) and oxidative potential assessment. Remaining PM$_{2.5}$ of the same collection method was then pooled (n=6/group) and the soluble fraction of PM$_{2.5}$ from DMSO extraction was prepared for developmental toxicity testing in zebrafish (n=32/treatment) starting at 6 hours post fertilization (hpf). Pooled sample aliquots were used for chemical analysis and oxidative potential assessment with identical methods to individual filter analyses. Significant differences were observed in oxidative potential between personal and home PM$_{2.5}$ for individual and pooled samples. Significant mortality in zebrafish was observed starting at 24 hpf in personal PM$_{2.5}$ samples and by 120 hpf in home PM$_{2.5}$ compared to blank filter controls. Chemical analysis is underway to identify correlations between these biological responses and chemical constituents. This research is the first study to use paired home and personal PM$_{2.5}$ samples with chemical, oxidative potential, and developmental toxicity data, identifying differences in these measurements between household and personal PM$_{2.5}$. Importantly, it outlines procedures for large-scale analysis of the PURE-AIR study which includes planned PM$_{2.5}$ measurements in 4,000 homes and will ultimately allow for correlation of human health effects with chemical and biological data to improve identified health metrics for PM$_{2.5}$ exposures.

Mice inhaling fine air borne particulate matter (PM$_{2.5}$) exhibit defects in bone marrow stem cells. These include a depletion of hematopoietic stem cells (HSCs), as identified in a colony-forming assay, and functional impairment of cultured endothelial progenitor cells (EPCs), including decreased tube formation in vitro and attenuated vascular repair potential in vivo. One potential mechanism of PM$_{2.5}$-induced pathology is the induction of oxidative stress, leading to the oxidation of biomaterials (e.g. lipids) which are then distributed systemically through the circulation to impact the functionality of distal tissues, including the bone marrow. To test this mechanism and limit oxidative stress, we supplemented murine drinking water with 1mg/ml carnosine. Carnosine is a naturally occurring di-peptide (β-alanine-histidine) which quenches reactive oxygen species and removes toxic products of lipid peroxidation. We then exposed these mice and control mice drinking normal water to concentrated ambient particles (CAPs) or filtered air and examined effects on bone marrow stem cells. We observed that mice drinking normal water and exposed to CAPs had a 40% reduction in HSC colonies compared to control mice inhaling filtered air. In contrast, mice drinking carnosine-containing water and exposed to CAPs demonstrated no HSC depletion. Similarly, while EPCs cultured from mice drinking normal water and exposed to CAPs were deficient in forming tubes and vascular repair, comparable cells from mice drinking carnosine-containing water and exposed to CAPs had no such defects. Thus, supplementation with carnosine mitigated the adverse outcomes of PM$_{2.5}$ exposure on bone marrow stem cells and represents a practical and feasible interventional strategy for limiting the cardiovascular toxicity of PM$_{2.5}$.
1986 Increasing the Pulmonary Antioxidant Defense Prevents PM₂.⁵-Induced Changes in EPC Function and Homeostasis

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Fine particulate matter (PM₂.⁵) exposure is associated with decreased endothelial function, suggesting that PM₂.⁵ exposure induces endothelial injury. We showed in humans and mice that PM₂.⁵ exposure decreases circulating endothelial progenitor cells (EPCs) that are considered to contribute to vascular maintenance and repair. However, it is unclear whether PM₂.⁵ exposure induces EPC dysfunction and how PM₂.⁵ inhalation affects EPC function and homeostasis. As an increase in pulmonary oxidative stress is suggested to mediate cardiovascular PM₂.⁵-toxicity, we examined whether increasing the pulmonary antioxidant capacity in transgenic mice overexpressing extracellular superoxide dismutase in the lungs (ecSOD-Tg) could prevent the PM₂.⁵-induced effects on EPCs. We exposed ecSOD-Tg and wildtype (WT) mice for 9 days (6h/day) to concentrated ambient PM₂.⁵ (CAP). Mice breathing HEPA filtered air were used as controls. In WT mice, CAP exposure decreased reduced/oxidized glutathione ratios (GSGH/GSSG) in the lungs and GSH levels in circulating and bone marrow-resident EPCs (Flk-1⁺/Sca-1⁻ cells). In contrast, no CAP-induced redox changes were found in the lungs or EPCs of ecSOD-Tg mice. Pulmonary ecSOD overexpression also restored EPC trafficking, anti-VEGF-signaling and plasma NO levels that were impaired in CAP-exposed WT mice. While bone marrow-derived EPCs from CAP-exposed WT mice formed lesser tubers and failed to migrate into injured hind limb ischemia, ECAP exposure did not impair EPC-function in vitro or in vivo in ecSOD-Tg mice. Gene array data, confirmed by qRT-PCR, showed that in bone marrow-derived EPCs of WT mice CAP-exposure increased the expression of anti-apoptotic (Bcl2, bcl-xl) genes and decreased pro-apoptotic (Bax, Bim) gene expression. In contrast, no changes were observed in ecSOD-Tg mice. In summary, PM₂.⁵ inhalation induces profound defects in EPC function, implicating a role for PM₂.⁵-exposed EPCs in vascular health. These findings suggest that the pulmonary antioxidant capacity in transgenic mice overexpressing extracellular superoxide dismutase in the lungs (ecSOD-Tg) can prevent the PM₂.⁵-induced effects on EPCs.

1988 Chemical Composition Influences the Toxicological Responses Elicted by Size-Segregated Urban Air Particulate Matter from Nanjing, China

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Air quality events and high urban particulate matter (PM) levels are a major concern in China. PM exposure is associated with increased cardiorespiratory morbidity and mortality. PM sources include industry, traffic, construction and biomass burning. In this study, we assess how the atmospheric processes and local emission sources cause variations in the chemical composition of inhalable PM from Nanjing, China, and how this affects the toxicological responses of exposed human alveolar epithelial cells. Day- and nighttime size-segregated urban air PM was collected in Nanjing, China during August and October 2013. The PM samples underwent extensive chemical characterization and toxicological profiling using adenocarcinomic human alveolar epithelial cell line A549. The cells were exposed to four particle size ranges (PM₁₀-₂.⁵, PM₁.⁰-₂.⁵, PM₁.₀-₀.₂ and PM₀.₂) at five doses (25, 75, 150, 200, 300 µg/mL) for 24 hours, whereafter cellular metabolic activity (CMA), cell membrane integrity, oxidative stress, genotoxicity, inflammatory response and cell cycle state were measured. PM₀.₂₅ elicited very high oxidative stress, genotoxicity and inflammatory responses. For PM₁.₀₋₂.⁵, the changes in CMA, cell cycle, and inflammatory response were high. PM₁.₀₋₀.₂ caused elevated genotoxicity and inflammatory responses, and moderate changes to CMA and cell cycle. PM₂.⁵ elicited great changes to CMA, cell cycle and inflammatory responses. Metals, especially Ca and Al, were the dominant constituents of PM₁.₀₋₂.⁵, whereas in smaller size-ranges, sulfate and nitrate were the major constituents. Polyatomic hydrocarbon (PAHs) were most abundant in PM₁.₀₋₁.₀ and PM₁.₀₋₀.₂. Instead, PM₂.⁵ contained very low amounts of oxygenated PAHs, which were prominent in the other size ranges. We observed significant variations in PM chemical composition and toxicological responses between the daytime and nighttime campaigns as well as between day and night. Thus, atmospheric processes and emission sources affect the chemical composition, which in turn, affects the elicited toxicological effects.

1987 Air Pollution Exposure Activates NLRP3 Inflammasome and Exacerbates Obesity-Induced Insulin Resistance

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We have previously shown that air pollution exposure enhanced insulin resistance and increased inflammatory response in humans. However, the underlying mechanisms are not fully understood. In this study, we investigated the role of NLRP3 inflammasome in mediating the adverse effect of air pollution on glucose intolerance. Wild-type and NLRP3 knockout mice on a normal chow diet or high fat diet were exposed to filtered air or concentrated ambient PM₂.⁵, 6 hours/day, 5 days/week, for 12 weeks, using a versatile aerosol inhalation system. The cells were exposed to four particle size ranges (PM₁₀-₂.⁵, PM₂.⁵-₁.₀, PM₁.₀-₀.₂ and PM₀.₂) at five doses (25, 75, 150, 200, 300 µg/mL) for 24 hours, whereafter cellular metabolic activity (CMA), cell membrane integrity, oxidative stress, genotoxicity, inflammatory response and cell cycle state were measured. PM₀.₂₅ elicited very high oxidative stress, genotoxicity and inflammatory responses. For PM₁.₀₋₂.⁵, the changes in CMA, cell cycle, and inflammatory response were high. PM₁.₀₋₀.₂ caused elevated genotoxicity and inflammatory responses, and moderate changes to CMA and cell cycle. PM₂.⁵ elicited great changes to CMA, cell cycle and inflammatory responses. Metals, especially Ca and Al, were the dominant constituents of PM₁.₀₋₂.⁵, whereas in smaller size-ranges, sulfate and nitrate were the major constituents. Polyatomic hydrocarbon (PAHs) were most abundant in PM₁.₀₋₁.₀ and PM₁.₀₋₀.₂. Instead, PM₂.⁵ contained very low amounts of oxygenated PAHs, which were prominent in the other size ranges. We observed significant variations in PM chemical composition and toxicological responses between the daytime and nighttime campaigns as well as between day and night. Thus, atmospheric processes and emission sources affect the chemical composition, which in turn, affects the elicited toxicological effects.

1989 Respiratory Dose Analysis for Components of Ambient Particulate Matter

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Particulate matter (PM) in the atmosphere is a complex mixture of particles with different sizes and chemical compositions. Although PM is known to cause health hazard, specific attributes of PM that may cause health effects are somewhat ambiguous. Dose of each specific component of PM together with relevant dose metrics may shed insights into understanding of PM related health effects. We have attempted to analyze lung deposition dose of typical bimodal ambient aerosols composed of two distinct aerosols with mass median diameter of 0.3 um (A1) and 5.0 um (A2) with geometric standard deviation of 1.8-2.0. Mass fractions, MF = A1/(A1+A2), were varied in order to reflect aerosol characteristics of different regions. Lung deposition including tracheobronchial (TB) and alveolar (AL) regions was calculated by using a mathematical model built upon Weibel's lung morphology for mass, surface area and number of each of three size fractions, ultrafine (UF, PM₀.₁), fine (F, PM₀.₁₋₂.₅) and coarse (C, PM₂.₅₋₁₀) at inhalation patterns mimicking resting and mild to moderate exercise. Overall, mass deposition of C decreases whereas deposition of F increases in both TB and AL as MF increases during normal breathing at rest. Combined deposition of C and F shows a decrease in TB but an increase in AL resulting in a slight increase in TB+AL. Surface area deposition is resulted mainly by F with a minor contribution from UF at MF=0.2 whereas number deposition comes from both UF and F at a ratio of 1:5.1. During exercise, mass deposition increases greatly (~140%) in TB but decreases (15%) in AL, resulting in a moderate increase of 40% in TB+AL. In C, whereas F decreases by 15% ~ 40%. This results in variable changes (+/-20%) of F+C as compared with resting condition. Surface area and number
deposition shows a decrease of ~20% in a wide range of MF. In conclusion, PM size fractions of ambient aerosols contribute differently to lung deposition dose in terms of mass, surface area and number. Significance of roles of each size fraction and their combined effects need to be considered in toxicological and health risk assessment.

1990 Metabolic Impact Induced by Total, Water Soluble, and Insoluble Components of PM2.5 Acute Exposure in Mice

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The systemic biological effects on metabolic responses induced by PM2.5 and its components were poorly understood. This study was aimed to evaluate the toxicity of different components of PM2.5 via metabolomics approach. In the present study, we adopted 1HNMR-based metabolomics approach to evaluate metabolic profiles in mice after acute exposure to Total-PM2.5, water soluble components of PM2.5 (WS-PM2.5) and water insoluble components of PM2.5 (WIS-PM2.5). First, we characterized the morphological features and chemical composition of PM2.5. Then, the metabolites changes of serum and urine in mice were systematically analyzed using 800 MHz 1HNMR techniques in combination with multivariate statistical analysis. Total-PM2.5 exposure affected metabolites mainly involved in amino acid metabolism, protein biosynthesis, energy metabolism and metabolism of cofactors and vitamins. WS-PM2.5 exposure influenced lipid metabolism and carbohydrate metabolism. WIS-PM2.5 exposure mainly perturbed amino acid metabolism and energy metabolism. The results suggested that acute exposure to the Total-PM2.5, WS-PM2.5 and WIS-PM2.5 in mice exhibited marked systemic metabolic changes. In addition, the insoluble fraction of PM2.5 contributed greatly to the toxicity of PM2.5.

1991 Not All Particulate Matter Is Equal: Examining Toxicity by Source

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Exposure to particulate matter (PM) causes cardiopulmonary morbidity and mortality. PM exposures are generally classified by particle size because smaller particles can penetrate deeper into the lung, causing greater toxicity. But in addition to varying in size, PM from different sources also varies in composition. In order to screen for differential toxicity of PM from various sources, we conducted exposures of primary bronchial epithelial cells cultured at air-liquid interface from a panel of healthy human donors to identical particle masses of six different pollutants: diesel exhaust particles from two different sources (A-DEP and C-DEP), wood smoke particles (WSP), coarse (PM10), and fine (PM2.5), and ultrafine (PM0.11) concentrated ambient particulates (CAPS) collected in Chapel Hill, North Carolina. We compared the relative ability of these materials to induce the expression of oxidative stress and antioxidant defense genes and assessed the inter-individual variability in exposure outcomes. Exposure to A-DEP and C-DEP caused changes in the expression of different genes, with more genes upregulated following exposure to A-DEP. This indicates that the engine from which diesel exhaust particles (DEP) are generated and how they are processed affects their toxicity. Second, the A-DEP and WSP caused strong upregulation in the same set of genes across all donors: NQO1, HMOX1, TRBN1D, GCLC, GCLM, and SOW1. This suggests that similar mechanisms drive the toxicity of these two types of PM. Finally, coarse, fine, and ultrafine CAPS elicited different responses, with smaller CAPS causing more drastic changes in gene expression. Since the exposure setup used for these experiments does not model lung deposition, this is further evidence that differential toxicity between these particles is attributable not only to their size but also to their composition. Additionally, if a donor was less responsive to one exposure, then they tended to be less responsive to the others. This indicates that each donor may contain an overall response phenotype that influences their sensitivity to an array of air pollutants. Overall, these data highlight how the degree of PM toxicity is influenced both by PM source and by inter-individual variability. Exploring these differences in PM exposure outcomes will improve our understanding of how these toxins cause adverse health effects and help identify and protect susceptible populations. This abstract does not reflect US EPA policy.

1993 Effect of Sex and Fine Particulate Matter Exposure on Heart Rate Variability of ApoE Knockout Mice


Exposure to air pollution has been associated with increased risk of developing and exacerbating cardiovascular disease. We have previously shown that after an 8-week exposure to fine particulate matter less than 2.5 μm (PM2.5), male ApoE−/− mice, which are susceptible to developing atherosclerosis, develop atherosclerotic plaques and show changes in heart rate variability (HRV), a measure of cardiac autonomic control. In this study, the effect of sex and a long-term exposure were assessed. ApoE−/− male and female mice were implanted with electrocardiogram (ECG) telemetry devices and exposed 4 days per week for 11 weeks to either PM2.5 concentrated ambient particles (CAPs), or filtered air (n=5). Ambient particles were concentrated approximately 10 times and vented levels were maintained with the Choctaw Enrichment System. Ambient polycyclic aromatic hydrocarbons (PAH), a class of semi-volatile organic compounds, were collected and analyzed from polystyrene foam and XAD-2 polystyrene resin. Particle phase PAHs were also collected on a Teflon filters and subsequently extracted for analysis using gas chromatography-mass spectrometry. PAHs are known contributors to the toxicity of PM. Several measures of HRV were calculated from ECG data collected during the 11 weeks and averaged daily over 4 hours in the evening during the animals’ dark cycle on both exposure and non-exposure days. With this long-term exposure study, the cumulative effect of PM over time on HRV was monitored. HRV was analyzed in the time and frequency domains to assess autonomic control of the heart, and particularly parasympathetic, input to the heart. Immediate changes to HRV seen during week one were mostly dependent on if animals were subjected to exposure that day or not. As the study continued, PM elicited more chronic effects on the standard deviation of normal to normal intervals (SDNN) and low frequency (LF) root mean square of successive differences in RR intervals (RMSSD). Sex differences between the male and female heart rate variability changes were also seen. Specifically, for measures of parasympathetic nervous system input in both time and frequency domains, males show a greater response to PM2.5. These changes are thought to be related to parasympathetic control of the heart and represent a defense response to the toxic insult in the lungs. Toxicity of particulate matter may be in part due to sex and not only dependent on PM size and chemical composition as previously shown.
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Air pollution has been associated with increased incidences of cardiovascular disease leading to increased daily mortality and hospital admissions. Fine PM (Dp < 2.5 µm) contains a mixture of metallic elements, road dust, and includes ultrafine PM (Dp < 0.1 µm) encompassing vehicle emissions which, due to their large surface area/unit mass, allows for greater adsorption of reactive organic molecules and increased availability for interaction with potential cellular targets. The objective of this study was to provide experimental evidence to elucidate the epidemiological finding that older women may be more susceptible than men to the cardiovascular effects associated with ambient particulate matter exposure. Groups of genetically altered ApoE-/- mice (preventing developing atherosclerosis) were exposed to either filtered air (FA) or to concentrated ambient PM2.5 (CAPs). Mice were exposed 5 hours/day, 4 days/week for 8 weeks at the University of California, Irvine, about 1600m southwest of a major freeway. Blood pressure was measured weekly while implanted cardiac transducers continuously monitored ECGs from the mice. ECGs were analyzed at specific post-exposure times to detect changes from the baseline measurements at various waveforms. Female mice exposed to PM2.5 CAPs experienced a decrease in both systolic and diastolic blood pressure for most of the exposure period compared to FA controls; no difference was seen between exposure groups for male mice. No loss of any sex exposed to CAPs for 1 month exhibited a decrease in RR, PR, and QT-interval compared to filtered air (FA) controls. Furthermore, the T-wave amplitude in exposed male mice increased with increased exposure time compared to controls while the ST-segment elevation of exposed female mice remained depressed for the duration of the exposure. Mice of both sexes exhibited J-point waveform depression when exposed to PM2.5 CAPs. Hypotension can result from conditions such as heart disease and bradycardia while ST-segment changes are common measures of myocardial dysfunction in humans that may lead to life-threatening cardiac events. Further investigation into the observed changes need to be performed to explicate the sex-specific mechanisms corresponding to changes resulting from CAPs exposures.

Intestinal Polycyclic Aromatic Hydrocarbon (PAH)-DNA Adducts in Beluga Whales with PAH Exposures and High Rates of Gastrointestinal Cancer

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For a half century, beginning in 1926, carcinogenic polycyclic aromatic hydrocarbons (PAHs) were disposed into the Saguenay River of the St. Lawrence Estuary (SLE) by local aluminum smelters (Quebec, Canada). PAHs present in the river were hypothesized to be etiologically linked to gastrointestinal cancers observed in 6.2% of 178 adult beluga found dead along the shorelines. Because DNA adduct formation provides a critical link between exposure and cancer induction, we investigated PAH-DNA adducts in paraffin blocks of SLE beluga intestine. Using an antiserum specific for DNA modified with several carcinogenic PAHs, we stained consecutive sections of paraffin-embedded intestine from 51 SLE beluga (0-63 yr), and 20 beluga (0-46 yr) living in areas with low PAH contamination (Eastern Beaufort Sea, Canada; Barrow AK, and several aquariums). Stained sections showed nuclear light-to-dark pink color indicating the presence of PAH-DNA adducts concentrated in intestinal crypt epithelial lining cells. A simple color-based scoring system of whole tissue sections revealed higher scoring values for the 51 SLE beluga compared with the 20 non-SLE controls (p = 0.003). In addition, the H-scoring system, applied to coded individual photomicrographs, confirmed that the SLE beluga had levels of intestinal PAH-DNA adducts significantly higher than the non-SLE controls (p = 0.003). The presence of high levels of PAH-DNA adducts in SLE beluga intestine strongly connoted carcinogenic PAH exposures with intestinal cancer induction in these whales.
An increasing number of pharmaceutical and industrial chemicals are being classified as endocrine disrupting compounds (EDCs). These chemicals can interfere with hormonal homeostasis and lead to developmental disorders, cancer, and other pathologies. One such EDC is 17α-ethynylestradiol (EE2) used in oral contraceptives. EE2 can inadvertently be introduced into aquatic environments through municipal wastewater treatment facilities. Exposure of male fish to EE2 is known to increase the expression of the egg yolk precursor protein vitellogenin (VTG). This induction has been predominantly used as a molecular marker of exposure to estrogenic EDCs and resulting feminization. However, the mechanisms behind VTG induction are not fully known and we hypothesize that it is regulated via DNA methylation. To test this hypothesis, we used a modified sequencing approach called targeted genome bisulfite sequencing (TGBS) to determine the percent methylation at the vtg1 promoter. DNA methylation was assessed in the livers of adult male zebrafish exposed to 20 ng/L EE2 for 0.25, 0.5, 4, 7 and 14 days. A significant increase in mRNA was observed in the EE2-exposed fish as early as 5 days. However, the increase in DNA methylation at the CpG sites, however, was not observed until after 4 days of EE2 exposure. This decrease brought the overall methylation of vtg1 promoter in male zebrafish at the same level as that of female controls suggesting that it may lead to feminization. The persistence of these changes was assessed by discontinuing EE2 exposure. We observed that mRNA levels returned to basal levels after 7 days post EE2 removal. In contrast, DNA methylation levels remained significantly decreased. These data confirm the ability of EE2 to induce a genotypic change in VTG mRNA expression that can lead to feminization. Collectively, these data suggest a role for DNA methylation in VTG induction and subsequent feminization of male zebrafish after exposure to EE2. Our work identifies a novel epigenetic marker of exposure to estrogenic EDCs and resulting feminization that may serve as an indicator of previous exposure to EE2 which will aid in ecological risk assessment of EDCs.

**1999** Toxic Effects of Coal Dust Extract on Embryonic Development of Zebrafish

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Coal mining has been linked to several impacts on environmental health. The aim of this study was to evaluate the phenotypic alterations and gene expression changes generated in zebrafish embryos exposed to methanolic coal extracts. Samples of coal dust obtained from a coal mine in La Loma, Colombia, were extracted with methanol in a Soxhlet apparatus, and the solid extract dissolved in dimethyl sulfoxide for biological assays. Lethal and phenotypical alterations were monitored using an Olympus SZX12 stereomicroscope and analyzed by in situ hybridization. RNA sequencing and RT-PCR were employed to determine the alterations in gene expression associated with the observed phenotypes. The coal extract showed a 48 hpf (hours post-fertilization)-LC50 of 223.68 mg L-1 (SE: 29.48) and a 48 hpf-LD50 of 161.55 mg L-1 (SE: 17.16). Using the induction of acute toxicity as read-out, morphological and physiological embryo-toxic effects were registered in a concentration-dependent manner. Three phenotypes (P1, P2 and P3) were determined in embryos exposed to methanolic coal extract and all of them showed shortening of brain regions; defects in the formation of the pineal and somites; as well as defects in heart tube in in situ hybridization. Gene expression profile analysis by RNA sequencing for the phenotype groups identified alterations of several genes related to the composition of intermediate filaments, oxidation-reduction processes, calcium ion binding, focal adhesion, and ECM-receptor interaction pathway. These findings allowed to postulate changes in gene expression of several genes as biomarkers of exposure to pollutants derived from coal mining. Besides, coal dust extract causes severe alterations in embryonic development of vertebrate aquatic organisms, which could lead to poor health of biota and humans exposed to such chemicals.

**1998** Changes in CpG-Methylation of Vitellogenin 1 in Adult Male Zebrafish after Exposure to 17α-Ethynylestradiol (EE2)

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The aim of this study was to evaluate the phenotypic alterations and gene expression changes generated in zebrafish embryos exposed to EE2 which will aid in ecological risk assessment of EDCs. Biologically active xenoestrogens cause severe alterations in embryonic development of vertebrates. The adhesion, and ECM-receptor interaction pathway. These findings allow for the development of new therapeutic strategies to prevent adverse developmental outcomes.

The toxicity of 17α-ethynylestradiol (EE2) on zebrafish was assessed using an estrogenic assay. Lethal and phenotypical alterations were monitored using an EthovisionTM. Tissue analysis indicated successful maternal transfer of Hg from were fed either a high or low MeHg diet to assess the effect on adult fish. Future research will characterize potential effects on early developmental and reproductive processes not captured here.

**2000** Bioaccumulation and Effects of Dietary Exposure to an Alternative Brominated Flame Retardant ( Bis(2-ethylhexyl) Tetraphosphomethylphthalate (TPBH), in Atlantic Killifish, Fundulus heteroclitus

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Bis-(2-ethylhexyl) tetraphosphomethylphthalate (TPBH), is a high production volume chemical classified as an alternative flame retardant (FR), which replaces legacy FRs withdrawn from US markets due to health and environmental concerns. This study provides experimental data on the bioaccumulation and biological effects of dietary exposure to TBPH from an estuarine fish, Atlantic killifish, Fundulus heteroclitus. Briefly, individually tagged male and female adult fish were fed a gel-based diet amended with carrier or contaminant for 28 days, followed by 14 days consuming uncontaminated food. Dietary amendments were designed to resemble prior studies of fish dietary exposures to TBPH. Tissue concentrations achieved were ~2-TPBH_Lo to 20-TPBH_HI fold higher than those measured in a limited number of reports of TBPH in field-collected biota. Modeled estimates suggest that about 50% of the measured bioaccumulation occurred over about 12 days. Growth of males exposed to TBPH for about 22 days; there was no effect of sex or size on the rates of incorporation or loss of TBPH from fish tissue. Over the course of the experiment (42 d), male fish grew (~0.5 % body dw/d) but female fish did not; unexpectedly, reproductive condition (gonad:body weight ratios) declined in both sexes, independent of treatment. Treatment of zebrafish with extremely high dietary concentrations of TBPH can contaminate fish tissues above the highest levels observed in the environment to date, although these exposures produced few adverse biological effects on adult fish. Future research will characterize potential effects and biological outcomes in offspring.
to eggs. Overall, this contributes to the ongoing development of an ecological modeling framework in a fish with an extensive toxicological and genomic background. Ultimately, we are working to connect molecular mechanistic, physiological, reproductive, and behavioral responses to population level fitness.

2002 Effect of Cadmium Exposure on Larvae and Sperm of Prochilodus magdalenae
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Cadmium is a heavy metal found naturally in the earth’s crust associated with other minerals. Exposure to this metal may damage animal reproduction, decreasing the rate of fertilization of organisms, such as fish. The Prochilodus magdalenae is the most important fish species in Colombia, and it is widely used by riparian communities of many Colombian rivers. The aim of this work was to determine the toxic effects of cadmium chloride on 0, 1, 6, 7 and 8 days post-hatching larvae and sperm of P. magdalenae. The results indicated that cadmium-induced a dose-dependent effect on the lethality of the exposed larvae that depends on its development stage. The heavy metal also altered sperm quality by decreasing total motility and speed of rapid and medium swimming spermatozoas. The heavy metal also impaired sperm curvilinear and straight-line velocities in a concentration-response dose. The results evidenced that the exposure to cadmium causes physiological changes in the initial stages of development of P. magdalenae, probably increasing the risk of reducing the fertility rate of this vulnerable fish species. UniCartagena (2016-2017), Colciencias, 647-2014.

2003 Development of Wildlife Toxicity Reference Values (TRV) for Perfluorooctane Sulfonate (PFOS)

Perfluorooctane sulfonate (PFOS) is an anthropogenic compound that resists thermal, chemical and biological degradation, and vertebrae or microbial metabolism. Because of widespread use, PFOS remains a persistent environmental contaminant commonly found in wildlife and human organs, tissues and fluids. We reviewed the acute, sub-chronic and chronic toxicity of PFOS in mammalian, avian, reptilian and amphibian species with the purpose of developing toxicity reference values for mammals, birds, reptiles, and amphibians. Despite limited wildlife exposure data, available laboratory animal studies enabled identification of target organs and derivation of NOAEL and LOAEL dose-response endpoints. US Army Public Health Center Technical Guide 254 - Standard Practice for Wildlife Toxicity Reference Values (TRVs), guided our development of class specific TRVs. Although considerable data were available for mammals and birds, amphibian data were limited, and data relevant to reptiles were absent. Tentatively derived oral TRVs (mg/kg-day) based on the most sensitive species for mammals was 0.004 (NOAEL-based) and 0.04 (LOAEL-based) in male rats based on reproductive dietary studies. For the most sensitive avian species, the derived oral TRVs (mg/kg-day) were 0.015 (NOAEL-based) and 0.064 (LOAEL-based) from reproductive studies of adult mallards exposed to dietary PFOS. For amphibian species (frogs), derived TRVs (mg/L) were 0.0003 (NOAEC-based) and 0.00075 (LOAEC-based) from sub-chronic exposures. These tentative TRVs were based on the most sensitive species available, and might be protective of the entire class of wildlife species and useful in ecological risk assessments of PFOS.

2004 Evaluation of the Estrogen Receptor and the Dopamine Signaling Pathway as a Possible Target of Bifenthrin Toxicity in Zebrafish Embryos

Bifenthrin (BF) is a pyrethroid insecticide widely used in urban and agricultural applications. Previous studies in juvenile fish have shown that environmentally relevant (ng/L) concentrations of BF can affect the endocrine system causing the over production of 17β-estradiol (E2) and altering the expression of dopaminergic pathway components in the central nervous system. We have also noted that BF acts as an anti-estrogenic compound in embryos. To examine the role of the estrogen receptor (ER) embryos were exposed for 96 hours to a mixture of 0.15 and 1.5 µg/L BF and an ER agonist (Ethinyl Estradiol - EE2). Additionally, ER morpholinos (ERα, ERβ1, ERβ2) were injected in embryos and exposed to 0.15 and 1.5 µg/L BF and EE2. The relative levels of transcripts related to the dopaminergic and the hypothalamic-pituitary-gonad axis (DR1, and vitellogenin - VTG) were investigated by qRT-PCR, and dopamine and its metabolites (HVA and 3,4-dihydroxyphenylacetic acid) and E2 concentrations were evaluated by LC-MS/MS. Preliminary results show that BF decreased the estrogenic effects of EE2 in the embryonic stage. These results show that the ER might be one of the targets of BF sub-lethal effects. Further analysis of differentially expressed genes coupled with endocrine responses will help assess other potential sub-lethal targets of BF toxic and sub-lethal effects using zebrasfish as an animal model.

2005 Environmental Impact of Microbes: Its Effect on Awba Dam

Recreational use of water is often given inadequate consideration and care. In Nigeria, many of these are increasingly contaminated by domestic sewage and industrial effluents. This study is therefore relevant in assessing the environmental impact of microbes on ecotourism in Awba dam. A total of nine water (n=9) and soil (n=9) samples were collected at entry, middle and the end of the Awba dam for heavy metal analysis and microbial assay. Samples were assessed for heavy metals using an official procedure and atomic absorption spectrophotometry. Total aerobic plate count, Isolation and characterization of strains was done using standard methods. For enumeration of E. coli O157:H7, colonies were characterized using standard methods. The disc diffusion agglutination technique was utilized for serology. The presumptive E. coli isolates were subjected to agglutination tests with specific E. coli O157:H7. For the antibiotic sensitivity test, the Bauer-Kirby disc diffusion method was used to test the sensitivity of the isolates. ANOVA and Duncan multiple range test was used for data analysis. All the values obtained for the total aerobic count and total coliform count for soil and water were higher than EPA recommended value for recreational waters. For the Antibiotic Sensitivity Profile, isolates from Awba dam showed the highest sensitivity (16.17mm) to ciprofloxacin while lowest was with Augmentin (8.25mm). The isolates at the control point showed highest sensitivity to CPR and NIT (14mm) and least for AUG (5mm). Generally, E.coli O157:H7 isolates were highly sensitive to Oftoxacin and Ciprofloxacin(93.3%) while the isolate was completely resistant to Ampicilin and Cefuroxime. The presence of E.coli O157:H7 in the dam can make the dam unfit for recreational activities and also for the community household chores, if not well treated. The University management should device means of controlling waste water that enters into the dam by providing alternate channels of discharge. This will reduce the growth and spread of the microbes in the soil and water of the dam.

2006 The Effects of Pristine and Carboxylated Multi-Walled Carbon Nanotubes on Phagocytosis by Macrophages
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The global production and applications of multi-walled carbon nanotubes (MWN Ts) have increased in recent years despite evidence that MWN Ts cause pulmonary fibrosis in lab animals that may lead to mesothelioma. Studies to understand the pathological mechanisms of MWN Ts focus on macrophages as they are first responders to invaders in the body. Recent work in our lab shows that both human and mouse macrophages preferentially accumulate pristine MWN Ts (C-MWN Ts) than pristine MWN Ts (P-MWN Ts). Preliminary results suggest that multiple types of receptors expressed in macrophages may be involved in the selective accumulation of C-MWN Ts compared to P-MWN Ts. To investigate potential impacts of accumulated C-MWN Ts and P-MWN Ts on endocytic function of macrophages, this study focused on two approaches: 1. Mouse macrophage RAW 264.7 cells were pre-exposed to C-MWN Ts or P-MWN Ts at 37 °C, washed and challenged with Alexa Flou 488 conjugated dextran, a fluorescent marker used to monitor the phagocytic activity of the cells, using fluorescence microscopy. The fluorescent intensities of the cells treated with C-MWN Ts or P-MWN Ts were comparable to the control cells. The results suggested that the MWN Ts were quenching Alexa Flou 488 fluorescence, interfering with this approach. 2. To avoid fluorescence quenching by MWN Ts, the second approach employed polystyrene beads as a phagocytic marker. Cells treated with C-MWN Ts or P-MWN Ts were washed and then challenged with non-functionalized 0.5-1.0 µm diameter blue polystyrene beads. The phagocytosed
beads were readily visible using bright field light microscopy. Moreover, a method recently developed in our lab allows the detection of MWNTs in 3D cell imaging using Confocal Raman Laser Scanning Microscopy (CRLSM). Since the polystyrene beads have a strong aromatic ring vibration Raman shift at 1000 cm-1, easily distinguished from the MWNT Raman shift at 1350-1600 cm-1, the polystyrene beads will also be detectable using the CRLSM in this approach. The evidence suggests that blue polystyrene beads avoid the quenching of fluorophores by MWNTs and that the beads are a better tool to investigate the effect of selective C-MWNT accumulation by cells.

### 2007 Bioaccumulation and Biopersistence of Model Chemical Mechanical Planarization Slurries in Daphnia magna

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The purpose of this study is to determine the bioaccumulation and biopersistence in *Daphnia magna* (D. magna) of model nanoparticle (NP) slurries containing either ceria (CeO₂) or alumina (Al₂O₃). The semiconductor manufacturing industry uses these NP slurries in the chemical mechanical planarization (CMP) process to polish wafer surfaces. Semiconductor manufacturers generate high volumes of the slurries and the slurries are interesting in the impact the slurry might have on aquatic organisms. In previous studies, we reported the acute and chronic toxicities associated with model CMP slurries in *D. magna*. The key findings were that the long-term exposure of CeO₂ and Al₂O₃ CMP slurries at concentrations that imposed no life-threatening danger to *D. magna* nevertheless cause serious reduction in reproduction which may endanger the *D. magna* population in an ecosystem. In the present study, bioaccumulation (BA) and biopersistence (BP) of NPs accumulated by MPs in D. magna when exposed to CeO₂ or Al₂O₃ slurries. Adult *D. magna* (8 to 10 days old) used in the bioaccumulation studies were exposed to various concentrations of CeO₂ or Al₂O₃ NPs for 4, 12, 24, and 48 h and the amounts of NPs accumulated by MPs in D. magna were determined by digesting the organism in acid using a microwave followed by ICP-MS analysis. Preliminary results of bioaccumulation with the Al₂O₃ slurry showed that exposure time increases, the amount of Al₂O₃ NP accumulated by *D. magna* also increased. Moreover, preliminary results with the CeO₂ slurry showed that bioaccumulation increased with increasing exposure time and concentration. Biopersistence studies were performed with NPs that will determine the length of time and the NP amount that remains in *D. magna* following exposure to the slurries.

### 2008 In Vitro Bioactivity and Chemical Composition of Recipient Waters Impacted by Onsite, Small Scale, and Large Scale Waste Water Treatment Facilities


Contamination of surface water by micropollutants (MPs) is an environmental problem of concern. MPs can be contaminated by MPs from multiple sources, including waste water treatment facilities. In rural areas of Sweden, on-site waste water treatment facilities are common, and the MP removal efficiency of these facilities has been questioned. In this study, we collected seasonal water samples from seven sites along a river system. The sampling sites are differentially impacted by on-site, small scale, and large scale waste water treatment facilities, respectively. Water was sampled in a time-integrated manner, using passive diffusion samplers. Samples were analyzed for bioactivity using in vitro assays for specific toxicity mechanisms including activation of aryl hydrocarbon receptor (AhR), nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB), nuclear factor erythroid-2-related factor 2 (Nrf2), estrogen receptor (ER), and androgen receptor (AR) agonistic and antagonistic effects. Furthermore, occurrence of 80 MPs, including pharmaceuticals, personal care products, pesticides, and perfluoroalkyl substances were analyzed using a target approach based on liquid chromatography coupled to tandem mass spectrometry. The highest levels of MPs were detected at the sampling sites heavily affected by a large scale waste water treatment facility. The levels of MPs showed a seasonal trend with highest levels of MPs in September. Likewise, the AhR activity showed a clear seasonal trend where the samples from most sampling sites collected in June and September activated AhR whereas samples collected in November or March did not show AhR activity. With the exception of one single water sample, we did not detect any activity of NFκB, Nrf2 or AR. One water sample showed biological activity in multiple toxicity biosassays, but the observed bioactivity could not be explained by the occurrence of target chemicals. None of the water samples activated the ER. In conclusion, the toxicological and chemical characterization performed in this study revealed a seasonal variation in both bioactivity and chemical composition of the water samples. The chemical analysis could, however, not explain the observed bioactivity.

### 2009 Role of Directly and Indirectly Acting Chemicals on Development and Oxidative Stress in Various Early Life Stages

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Recent studies in our laboratory comparatively explored toxicity responses associated with oxidative stress (OS) pathways in fathead minnow (*Pimephales promelas*) and zebrafish (*Danio rerio*) at early life stages. However, discrepancies between responses of these two common fish models have not been fully investigated across developmental ages. Here we aimed: 1. to explore how oxidative stress responses vary throughout development and evaluate if these responses were conserved between these two model organisms; 2. to investigate how these responses varied between directly acting chemicals versus those that are metabolized throughout development. Fathead minnow and zebrafish were exposed to R-carvone (a directly acting compound with a Michael Addition mechanistic domain) or bisphenol-A (BPA, a metabolized, indirectly acting compound with a receptor-mediated mechanistic domain) following standardized toxicity guidelines from the Organization for Economic Cooperation and Development (OECD no. 236) for embryonic exposures and the US Environmental Protection Agency (EPA) for larval exposures. Eight studies were conducted to determine 96-h LC₅₀ values, which showed that zebrafish were more sensitive than fathead minnow embryos. R-carvone exposed to control and 40% of the 96-h LC₅₀ value. At 24, 48, 72, and 96 hours post exposure, observations were made and tissues collected for gene expression. These observations included mortality, hatching rate (embryos), and developmental deformity assessment. Expression of the measured OS associated genes using qPCR included gclc, gsp1, nrf2a, and sod1, using actb1 as a reference gene. R-Carvone, a directly acting compound, was more potent to embryos than larvae (e.g., fathead minnow embryos 4 times more sensitive than larvae). Conversely, BPA, a metabolized compound, was more potent to larvae than to embryos (i.e., zebrafish embryos 2 times more sensitive and fathead minnow almost 9 times). Hatching rate was delayed, though not statistically, following exposure to R-carvone. Both species exhibited developmental deformities following exposure to both compounds with a slightly higher prevalence following exposure to R-carvone. Results show that responses to study chemicals varied throughout development. Moreover, differences in toxicity of directly and indirectly acting compounds appear to be unique to age. Additional comparative studies are needed to better understand the uptake, metabolism and biological toxicity relationships among fish models and ages.

### 2010 Developmental Effects of Ortho-Substituted PCB-153: Evidence for Altered Glucose Homeostasis in PCB-Sensitive Killifish and in Zebrafish, but Not in PCB-Tolerant Killifish from a Superfund Site

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Atlantic killifish (*Fundulus heteroclitus*) inhabiting the New Bedford Harbor (NBH) Superfund Site have evolved resistance to toxic effects of ortho- (dioxin-like) polychlorinated biphenyls (DL-PCBs) and other compounds that act via the aryl hydrocarbon receptor (AhR). However, the majority of PCBs in NBH are ortho-substituted (non-DL, o-PCBs); their impacts on fish populations are not well understood. To determine whether the NBH killifish population has adapted to o-PCBs, we exposed embryos from NBH and a reference site (Scorton Creek; SC) to 2,3,4,4′,5,5′-hexachlorobiphenyl (PCB-153), a model o-PCB. Water-borne exposure of SC embryos produced embryo concentrations of PCB-153 equal to or greater than those in eggs of fish from NBH. PCB-153 was not acute cytotoxicity to developing F2 killfish (SC or NBH) at concentrations up to 28 μM. RNA-seq showed that SC embryos exposed to PCB-153 (28 μM for 6 h at 10 days post fertilization) had reduced expression of genes involved in glucose metabolism and homeostasis, which was not seen in the NBH embryos. In additional experiments, 10 dpf embryos from SC exposed to DMSO, 2.8 μM PCB-153, or 28 μM PCB-153 for 6 or 24 hours showed reduced expression of glucose metabolism genes. The low
concentration (2.8 µM) caused reduced expression following 6-hr exposures, while the high concentration (28 µM) increased expression after 24 hr. Parallel studies in 48-hpf zebrafish embryos exposed to PCB-153 (28 µM) for 6 or 24 hr revealed several genes that were down-regulated in common with SC killifish embryos, including genes involved in glucone metabolism and homeostasis, coagulation, and erythropoiesis, and several beta crystallin genes. The results suggest that PCB-153 may disrupt glucone homeostasis in developing embryos and that NBH killifish have reduced sensitivity to PCBs, suggesting complex adaptation to chemical mixtures at this Superfund site. Supported by P42ES007381 (Boston University Superfund Research Program) and US EPA.

2011 The Fate of Atmospheric Mercury (Hg) in Ephemeral Wetlands

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Bioaccumulation of mercury in aquatic food chains represents a risk both to human and wildlife that feed on fish or other aquatic organisms. Once in an environment, methylmercury (MeHg) can be formed from inorganic Hg by microbial action. MeHg is the most harmful and toxic form of Hg because it is the easiest for biota to accumulate. Mercury enters the environment from a multitude of ways including: industrial activities, mining, and atmospheric deposition. Water level fluctuation can increase levels of Hg in semi-permanent and permanent wetlands. Previous studies have focused on Hg accumulation in fish and thus did not examine wetlands that dry frequently. Such wetlands have hydroperiods ranging from 3-12 months and support tremendous biodiversity. What may be at risk of high levels of Hg exposure. Our goals were to determine if Hg levels in sediments and biofilms of ephemeral wetlands relate to hydroperiod and if Hg levels in sediments of wetlands are higher at the edge, due to increased water fluctuation. We sampled and sediments and biofilms in twelve southeastern ephemeral wetlands with hydroperiods ranging from long (9-12 month) to short (3-9 months). We sampled from three different locations within each wetland: edge, midway from center and the center. Samples were lyophilized, homogenized, sorted and weighed, and then put on a DMA 80 for mercury analysis. We found significant variation in Hg concentrations among the wetlands, but that variation was not explained by hydroperiod or location sampled for sediment or biofilm. There was a trend for shorter hydroperiod wetlands to have more Hg, but it was not a significant relationship. Contrary to our predictions, there was a trend of higher Hg in the center of the wetland, but again this was not significant. Additional studies are needed to investigate what is driving the wetland variation in Hg concentrations.

2012 Toxic Effects of Textile Waste Water Discharged on the Atoyac River: Zebrafish as Study Model

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70 percent of mexican’s rivers are polluted, mainly due to discharges of industrial waste water. In the states of Puebla and Tlaxcala, the textile industry has contributed to the pollution of Atoyac river due to extremely high water consumption and the complex combination of danger substances used and deposited in the river without previous treatment. The region of river located in Villa Alta (between the limits of Puebla and Tlaxcala) is very polluted, consequently its population has serious health problems. The aim of this work is to evaluate the toxicity of the textile residual water waste, using zebrafish (Danio rerio) as study model. We calculate the average volume of textile waste water discharged into the river, as 1.12 L/s, equivalent to 2,903,040 L/month. Water samples from textile waste water discharges were obtained in dry season, we measured 18 physicochemical parameters, some of which exceed the maximum permissible limits established by the Mexican Official Standard for wastewater discharges (NOM 001-SEMARNAT-1996); among them: total nitrogen 62.43 mg/L, sedimentary solids 400 mg/L, fat and oils 38.28 mg/L, Biochemical Oxygen Demand (BOD5) 969.33 mg/L, Chemical Oxygen Demand (COD) 6272 mg/L, Total Suspended Solids (TSS) 5250 mg/L. The average concentration of dyes in textile waste water was 0.0104 ppm, temperature 28 °C and pH = 7.33. Toxicity was determined through lethal dilution 50 (LD50) test, 15.2/40 L was the LD50 of textile waste water for zebrafish. Our preliminary results show that water discharged into the Atoyac river is toxic and lethal. This water affects liver and gills morphology of zebrafish, demonstrating a potential risk along the whole zone, where the river provides for agriculture and other human activities.

2013 Toxicity Effects of Leachate of Cigarette Butts on Caenorhabditis elegans and Its Use as Adsorbent of Methylene Blue


Cigarette butt (CB) waste is a public health problem because they contain chemical compounds that are toxic to living beings, moreover when they are released to water, they can adsorb other pollutants, being a risk to aquatic life; however, this risk has been understudied. In this research, cigarette butts were collected in two beaches of Cartagena (Colombia), Playa Blanca (PB) and Punta Arena (PA), and their ethanolic leachates were obtained by Soxhlet extraction. Caenorhabditis elegans was exposed to these leachates and the number of dead and alive worms after 24 hr was counted. New (NCB) and recently smoked cigarette butts (SCB) were used as control. In addition, SCB was used as adsorbent of methylene blue in an aqueous solution (0.25%) in order to evaluate their potential use in wastewater treatment. The leachate of CB decreased survival in a concentration dependent manner. The effect was major in nematodes exposed to PA and PB than those to SCB and NCB, indicating the presence of more pollutants probably adsorbed from the water. SCB leachate was more toxic than NCB, suggesting that toxic compounds from the cigarette passed to the butt during smoking. The adsorption efficiency of methylene blue by SCB was of 52%, and the toxicity of methylene blue solution (0.25%) on C. elegans was reduced from 100% to 75% after treatment with SCBs. In conclusion, the CBs are able to adsorb toxic compounds from the cigarettes and pollutants from the water environment, and this feature can be used to clean waste waters. GRANT 115 2017, Vicerrectoria de Investigaciones, Universidad de Cartagena.

2014 Alterations in Neuro and Interrenal Steroidogenic Responses in African Catfish (Clarias gariepinus) following Long-Term Exposure to Waterborne Di-(2-ethylhexyl) Phthalate

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We have investigated the effects of long-term exposure to waterborne di-(2-ethylhexyl) phthalate (DEHP) on sex and organ-specific steroidogenic responses in Clarias gariepinus exposed to waterborne DEHP at 0 (control), 10, 100, 200, and 400 µg/L for 84 days. Alterations in gonad and head kidney steroidogenic protein (STAR) and enzyme gene expressions for P450scC, cyp17, cyp19a1, 3β-, 11β-, 17β- and 20β- hydroxysteroid dehydrogenase (HSD) and 17β-HSD were analyzed using real-time PCR. Star, P450scC, and CYP19 protein levels were analyzed using immunohistochemical method with specific antibodies. Cellular steroid hormone (E2 and T) levels were analyzed using enzyme immunoassay. We observed alterations in key steroidogenic protein and enzyme genes that were apparently dependent on DEHP concentration, time, and organ. Generally, these responses increased significantly at 10 µg/L compared to control group, and decreased thereafter in an apparent concentration-specific manner, for most variables, except for T and CYP19 protein, that decreased with increasing DEHP concentration. Interestingly, all measured responses showed a higher gonadal sensitivity compared with the head kidney, indicating organ-specific response pattern. The alterations in steeroidogenic genes and proteins demonstrated in the present study could have severe implications on the production of steroid hormones with probable downstream negative effects on adaptation, sexual maturation and reproduction.

2015 Champoton River’s Water and Soluble Soils Components Toxicity by L. sativa Bioassay


The aim for this study is to evaluate toxicity indicator in seed germination and root elongation in lettuce seeds exposed to water and soils-water solutions of Champoton’s river as alternative to determine toxicity in water. The Champoton River is localized at Campeche State of Mexico country, the most important activity in the river basin is agriculture of sugar cane, also fishing and tourism. The sampling was carried out in...
two sites, CR2 was localized upstream from the most popular swimming area and CR1 was downstream. For each sampling site, 500 mL water sample at 10 cm of deep to surface and 10 cm² of soil were collected. The experiment consisted in 2 water and 2 water-soil samples, 3 treatments per sample at 100, 50 and 25% of initial solution concentration with duplicate and control of distilled water for a total of 30 experimental units. For each unit, 25 seeds of Lactuca sativa were placed in a 90 mm diameter polyethylene containers, with filter paper in the bottom as the support. Afterward 10 mL of the different solution concentration were applied. All units were kept in controlled ambient temperature of 24 °C ±1, for 120 hours. In general, no phytotoxic effects were observed on the percentage of germination among the 4 different samples, there was not significant difference with respect to the control, however, the water solutions for both sites caused a 76% reduction in root elongation, meanwhile water-soil dilutions caused 35%. The inhibitory concentration 50 (IC₅₀) of water sample of CR2 site in roots elongations was 65% and 85% at CR2 site. These results indicate that further studies of toxicity are necessary due to the presence of possible toxic agents in river water.

### 2016 Repellent Activity of Four Colombian Essential Oil on Tribolium castaneum Herbst

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Investigations have been carried out on essential oils and their components as repellents and fumigants, against insects from stored products as the weevil of Tribolium castaneum Herbst. They are the most widespread and destructive stored product pests, causing damage to storage grains directly by feed and indirectly by the secretion of quinones that are responsible for human allergies. The objective was to evaluate the volatile chemical composition of the essential oil from four plants in the department of Chocó: Croton chocoanus, Croton cabi sp, Croton pacurita, Piper pacurita, Piper hispidum and to evaluate their repellent activities against the weevil of the flour Tribolium castaneum. The extraction of essential oils was done by the hydrodistillation method, the volatile chemical composition was carried out using gas chromatography coupled to mass spectrometry and the repellent activity was evaluated by the method. Each of these tests was carried out in order to analyze the percentage of repellency of the essential oils from the species tested with respect to the commercial repellent. The principal compounds found were: Croton chocoanus Croizat, β-linalool (5.7%), α-terpineol (4.0%), 4-O-H Eudesmane (19.9%), aristolene (5.3%), trans-calamenene (7.7%), Croton cabi sp: linalool (28.0%), eucalyptol (13.35%), caryophyllene oxide (8.16%); Croton pacurita: Spatulenol (12.78%), Aromandendrene (8.09%); Piper hispidum:3-carene (35.3%), Limonene (27.1%). Essential oil (Croton cabi sp and Piper hispidum) show the majority repellent activity (90% and 84%, respectively at 1% of concentration), followed by Croton pacurita (83%) and Croton chocoanus (81%). The results show that the essential oils presents more repellent activity against Tribolium Castaneum than the commercial repellent (Ethyl butylacetaminopropionate) its repellent activity at 1% of concentration and two hours of exposure was 70%. Piper hispidum and ethanol stem bark extract of Khaya senegalensis (KNE) was also assessed. The cells were treated with graded concentrations of AFB₃, SA, curcumin, and K2S for 25, 48 and 72 h. At optimum cytotoxic concentrations, cells were exposed to toxicants with influence of non-toxic concentrations of curcumin and K2S determined. Cytotoxicity was assessed by Hoechst/PI staining technique and changes in adenosine triphosphate levels determined using Promega’s CellTiter-Glo Assay. Pre-treatment of cells with curcumin and K2S before exposure to AFB₃ reduced cytotoxicity by 3.5- and 2.9-folds, while post-treatment resulted in 1.1- and 1.3-folds reduction, respectively. Pre-treatment of cells with curcumin and K2S before exposure to SA reduced cytotoxicity by 3.8 and 3.0-folds, while post-treatment resulted in 1.2- and 1.3-folds reduction, respectively. Overall, pre-exposure of the cells to curcumin and K2S exhibited cytoprotective effects with inferred tendencies to prevent carcinogenesis.

### 2017 Curcumin and Khaya senegalensis Mitigate Cytotoxicities Induced by Aflatoxin B₁ and Sodium Arsenite in HUC-PC Urinary Bladder Cells

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The inadvertent co-exposure to certain environmental/occupational toxicants, such as aflatoxin B₁ (AFB₁) and sodium arsenite (SA), in some regions is inevitable. Hence, the need to assess the efficacy of easily accessible, affordable, and relatively safe agents such as phytochemicals with known ethnomedicinal applications. Cytotoxic interaction between AFB₁ and SA in bladder cells was investigated. Cytotoxicity effect of curcumin and ethanol stem bark extract of Khaya senegalensis (KNE) was also assessed. The cells were treated with graded concentrations of AFB₁, SA, curcumin, and K2S for 25, 48 and 72 h. At optimum cytotoxic concentrations, cells were exposed to toxicants with influence of non-toxic concentrations of curcumin and K2S determined. Cytotoxicity was assessed by Hoechst/PI staining technique and changes in adenosine triphosphate levels determined using Promega’s CellTiter-Glo Assay. Pre-treatment of cells with curcumin and K2S before exposure to AFB₁ reduced cytotoxicity by 3.5- and 2.9-folds, while post-treatment resulted in 1.1- and 2.6-folds reduction, respectively. Pre-treatment of cells with curcumin and K2S before exposure to SA reduced cytotoxicity by 3.8 and 3.0-folds, while post-treatment resulted in 1.2- and 1.3-fold reduction, respectively. Overall, pre-exposure of the cells to curcumin and K2S exhibited cytoprotective effects with inferred tendencies to prevent carcinogenesis.

### 2018 Effect of Arsenic on Thermogenesis and Metabolic Health

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The prevalence of type 2 diabetes (T2D) has nearly doubled since 1980. Poor nutrition, sedentary lifestyle, and obesity are among the significant risk factors for the development of this metabolic disease. Environmental pollutants however, also have the potential to alter glucose homeostasis and lead to the development of T2D. Arsenic is one of these chemicals, with epidemiological studies worldwide supporting this association. While the precise mechanism of action by which arsenic exhibits its diabetogenic effects remains unclear, we propose arsenic impairs thermogenic tissues involved in insulin sensitivity and glucose tolerance. The objective of this study was to address the effects of chronic arsenic ingestion on cellular metabolism in the adipose depot associated with thermogenesis. Since cold is a key activator of these thermogenic tissues, we hypothesize arsenic alters metabolic heat production in mice exposed to settings with decreased temperatures. To test this, we administered 300 parts per billion (ppb) of sodium arsenite to C57BL/6J male mice beginning at 5-6 weeks of age, and assessed their metabolic phenotype via the use of indirect calorimetry and body composition analyzers. After 15 weeks of exposure, arsenic treatment revealed significantly decreased lean body mass despite unchanged whole body adiposity. When challenged by cold tolerance testing (e.g. at 30, 23, 12, and 4 degrees Celsius), arsenic-treated mice showed significantly lower energy expenditure and oxygen consumption parameters. Chronic arsenic treatment also decreased core body temperatures during acute cold exposure. Caudal assessment revealed greater unilocular lipid droplets in arsenic exposed mice, suggesting a decrease in brown-like adipocytes. These findings support the hypothesis that continuous low-dose arsenite exposure disrupts energy homeostasis and adaptive thermogenesis in vivo.

### 2019 Neurobehavioral Effects of Neonicotinoids on Embryo-Larval Zebrafish at Environmentally Relevant Concentrations

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Neonicotinoids are common surface water contaminants in both urban and agricultural landscapes. Mean stream concentrations in the US were recently reported at 175 ng/L and 66 ng/L for imidacloprid and clothianidin, respectively (Bradley et al., 2017). Neurobehavioral effects on larval fish are known to occur at concentrations higher than those reported; however, behavioral effects have yet to be investigated at environmentally relevant concentrations. For this ongoing study, the developmental neurobehavioral effects of neonicotinoid exposure on embryo-larval zebrafish (Danio rerio) were assessed using a larval zebrafish behavior assay. Three of the most common neonicotinoids (clothianidin, imidacloprid and thiamethoxam) and two environmental imidacloprid metabolites (desnitro-imidacloprid and 6-chloronicotinamide) were assessed individually and as part of environmentally relevant mixtures. Embryos were exposed to neonicotinoids at 1000, 100, 1, 0.1, 0.01 µg/L starting at 4 h post-fertilization (hpf) to 3 d post-fertilization (dpf). Behavior assays assessing swimming behavior (distance traveled and velocity) were conducted at 5 dpf. Data were analyzed using one-way ANOVA with Tukey HSD post hoc test. Zebrafish larvae traveled a significantly greater distance at 1000 µg/L Imidacloprid (p < 0.05) compared to 100, 10, 1, 0.1, 0.01 µg/L. All treatments groups traveled greater distance than the control group. Velocity of larva exposed to 1000, 100, 1 µg/L imidacloprid were significantly higher than those in the lowest treatment group 0.01 µg/L. All treatments groups traveled greater distance than the control group. Velocity of larva exposed to 1000, 100, 1 µg/L imidacloprid were significantly higher than those in the lowest treatment group 0.01 µg/L and control group. The results for the other neonicotinoids, imidacloprid metabolites, and mixtures are forthcoming; however, the preliminary imidacloprid data indicates that larval exposure to neonicotinoids causes behavioral changes at 1000 µg/L and may also cause behavioral changes at lower, more environmentally relevant concentrations.
Metals are one of the most abundant pollutants in river sediments in countries with mining activity, and their toxicity could differ when they are in a complex mixture. In Colombia, San Jorge River is an important freshwater source that receives polluted waters from nickel mining. In this study, sediment samples were collected in twelve sites along the San Jorge River and analyzed for 47 metals. Sediment metal concentrations were compared with the threshold effect concentrations, TEC, the midpoint effect concentration, MEC, and the probable effect concentration, PEC, for freshwater sediments. The level of metal contamination was determined by calculating the contamination factor, fi; pollution load index, PLI; and the potential ecological risk index, RI. Toxicity assessment was carried out exposing Caenorhabditis elegans to pore water samples obtained by centrifugation, evaluating survival, growth, locomotion, and changes in the expression of stress response genes using gfp transgenic strains. Samples taken near the nickel mine showed Cr and Ni concentrations above the PEC. According to fi, most sampling sites have high contamination by B, Se, Sh, and W. Regarding RI, eight locations were moderately polluted by Cd. Pore water samples affected the growth, locomotion, and survival of C. elegans. Samples from Boc de Ure increased the expression of mit-2 and sod-4 compared to control. Ni and Cr were associated with survival, and the expression of stress response genes, while V, Sc, Co, Ga, Ge, Se, Y, Zr, Nb, Mo, Mo, Eu, Tb, Dy, Ho, Er, Tm, Yb, Lu, Hf, and Ta were strongly correlated with all toxicological effects on C. elegans. In conclusion, the presence of metals in San Jorge River sediments was associated with the toxicity on the biological model C. elegans. Colciencias 110771451049.

Microplastic pollution is a growing problem in the oceans of the world, as these particles impact marine biota at most trophic levels. Despite their widespread distribution, few studies have been carried out in the Caribbean. The objective of this research was to determine the distribution of microplastics on beaches and fish from Cartagena, Colombia. Pellets were obtained from two different places, classified according to color and characterised based on their surface features and IR spectra. White pellets, mostly composed of polyethylene or polypropylene were the most abundant, although some displayed signs of degradation with oxidation-related characteristics. Black pellet samples were subjected to extraction with organic solvents, and the GC-MS analysis revealed the presence of several high molecular weight hydrocarbons, eicosane, heneicosane, tetracosane; terpenoids, organometallic compounds, hormones, pesticides and phthalates, among others. The stomach content of several carnivorous fish species was scrutinized and showed the presence of microplastic fibers of different nature. Water- and methanolic-based extracts from granular pellets exhibited several toxicological effects on Caenorhabditis elegans, suggesting these particles release and accumulate chemicals than can be leached inside the body of organisms that eat them. Both pellets and fibers represent toxicological hazards for marine life, and therefore different actions must be taken to avoid their incorporation into the oceans. UniCartagena (2016-2017), Colciencias, 647-2014.

Panama Channel has been a milestone in world history as it has shortened the distance and navigation time between nations. Despite these benefits, there are environmental risks associated with the emission of air and water pollutants by the shipping and associated industrial activities. In order to determine the relationship between the composition of trace elements and the toxicity of the surface sediments at Panama Channel basin, sampling was carried out in twelve points from November to December 2016 and analyzed for 47 trace elements by ICP-MS, and total Hg in a Direct Mercury Analyzer. Sediment fractions were also extracted with water and their toxicity evaluated on wild type and mutant strains of Caenorhabditis elegans. Results showed some environmentally-relevant elements had the greater concentrations in sediments, decreasing in the order Sr>V>Zn>Ba>Cu>B>Zn>Cr>Hg. Sediment aqueous extracts inhibited locomotion of C. elegans, in particular the sample from Gatun Lake, and induced expression of oxidative stress and metal exposure-related genes. A multiple linear regression model suggested the relative expression of metallothionein-2 is a function of As, Sn and Co concentrations. In short, sediments from Panama Channel basin possess high potential to activate toxicity mechanisms associated with the production of metallothioneins and oxidative stress, processes that can negatively affect the viability of benthic organisms. UniCartagena (2016-2017), Colciencias, 647-2014.
Breast cancer is a heterogeneous disease and accounts for 25% of all cancer cases. Current treatment options target either the estrogen receptor or the human epidermal growth factor 2 receptor. To date there is no FDA-Approved targeted treatment for triple negative breast cancer (TNBC), which has a poor prognosis. Hence, this study focused on second-generation curcumin analogue, RL71, in a nanomicellar form for TNBC treatment. Previous studies have shown RL71 to have submicromolar cytotoxicity in TNBC cells, however, no regression in tumor growth was demonstrated in vivo following daily oral administration for 70 days. Styrene-co maleic acid was used to encapsulate RL71 (SMA-RL71), to take advantage of the enhanced permeability and retention effect, and was administered intravenously twice a week for 24 days in a xenograft model of TNBC. Nicotine, a major component of tobacco, significantly increased proliferation between the effect of SMA-RL71 (10 mg/kg) and duration of treatment on the suppression of tumor growth via molecular mechanisms including anti-angiogenic and pro-apoptotic effects, as well as inhibition of EGFR and AKT/mTOR/4EBP1 signaling. GNL1 is a member of the HSF1 transcription factor family. GTPases including GNL3 and of subfamilies GNL1 and GNL3 are associated with breast cancer and localized in the nucleus which promotes G1 to S phase transition of cells or cell proliferation by the wnt/beta-catenin pathway, respectively. However, GNL1 is a nucleocytoplasmic shuttling protein localized in the nucleus at the G2-phase of cell cycle but its function is unknown. The effect of SMA-RL71 on the protein expression of GNL1 was investigated in vivo and in vitro due to previous work showing an increase in gene expression in vitro. GNL1, was downregulated upon SMA-RL71 (10 mg/kg) treatment in TNBC tumors. Its expression was also decreased 55% in MDA-MB-231 and 82% in HSS578i cells treated with SMA-RL71 (1 μM) for 24 h. Furthermore, to determine if GNL1 could be a potential therapeutic target, a G-protein inhibitor, Brefeldin-A was used. Brefeldin-A showed an IC50 of 56 nM and 49 nM on MDA-MB-231 and HSS578i TNBC cells, respectively using sulforhodamine B assay. Brefeldin A (0.1 μM) further inhibited the expression of GNL1 protein by 48% in MDA-MB-231 and 45% in HSS578i by western blotting after 24 h of treatment. GNL1 may therefore play important role in tumorigenesis and serve as a potential therapeutic target for TNBC.

Orally Administered Nicotine Effects on Rat Urinary Bladder Proliferation and Carcinogenesis

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Tobacco smoking is a major risk factor for human cancers including urinary bladder carcinoma. Cigarette smoke inhalation in mice and orally administered nicotine in rats and mice increased urothelial cell proliferation. Nicotine, a major component of tobacco, induced proliferation in multiple cell types in vitro. In the present study, the enhancing effects of nicotine on F344 rat bladder carcinogenesis induced by N-butyl-N-(4-hydroxybutyl)nitrosamine (BNB) were examined. Nicotine administered in drinking water for 32 weeks following 4 weeks of BBN treatment significantly increased the incidence of bladder tumors and the number of the BBN-induced bladder carcinomas dose-dependently. Ki67 and PSTAT3 labeling indices and expression of nicotinic acetylcholine receptor alpha 7 (nAChRα7) in non-tumor bladder urothelial lesions were significantly increased by nicotine, but the TUNEL assay for apoptosis showed no increase. In a 4-week study, nicotine did not express nicotine receptors below micromolar concentrations, nor in human RT4, T24 or UMUC3 urothelial carcinoma cells (express nicotine receptors). However, nicotine slightly, but statistically significantly, increased cell proliferation at micromolar concentrations in human urothelial carcinoma cells. These data suggest that nicotine enhances urinary bladder carcinogenesis by inducing cytotoxicity with regenerative proliferation and possibly direct mitogenesis, which may involve nAChR and STAT3 signaling. The role of nicotine receptors appears uncertain.

SMA-RL71 Downregulates GNL1 following Tumor Suppression in a Xenograft Model: A Potential Therapeutic Target in Triple Negative Breast Cancer


Although induction heating devices and wireless power transfer systems utilizing intermediate-frequency magnetic fields (IFMFs) are widely used, assessment of health risks associated with exposure to these magnetic fields has been insufficient. Previous studies involving rats demonstrated the absence of toxicities for up to 18 months. The purpose of this study was to assess the carcinogenicity of these IFMFs, because other lines of evidence have indicated a lack of carcinogenicity in both male and female B6C3F1 mice. In addition, the tumor ratio (TR) of 0.005 was used as an endpoint for tumor incidence, and the differences were significant. Mice were divided into nine groups with four replications each. The treated groups were exposed to a 20-mW/m² vertical field with a uniform horizontal intensity of 150 μT. A 4-mm cellulose acetate filter was used to filter out the 50-Hz power line. Four horizontally layered Merritt-type coils produced a very uniform vertical field with a sinusoidal waveform and the adopted field strength was 7.4-fold greater than that in the exposure guidelines for the public set by the International Commission on Non-Ionizing Radiation Protection in 2010. Experimental procedures were performed, including histopathological evaluations, were conducted by a blinded GLP-licensed laboratory to assure toxicological quality. In accordance with the Standards Related to the Care and Management of Experimental Animals, moribund animals were euthanized during the exposure, and surviving mice after the exposure were sacrificed for complete necropsy. Tissues and organs were excised and examined macroscopically and microscopically. Histopathological examinations revealed spontaneous neoplastic lesions, either benign or malignant, in field- and sham-exposed male and female mice. Observed neoplastic lesions included hemangiosarcomas in the spleen and thymus, squamous cell papillomas of the stomach, and malignant lymphomas. The frequencies of the neoplastic lesions were 1 to 3 per group. No statistically significant differences were found between the field- and sham-exposed groups. In the positive control group, malignant lymphoma bearing metastasis manifested in 8 out of 10 males and in all 10 females. The results indicate no carcinogenicity of IFMF exposure in rhesi2 transgenic mice.

Toxicogenomics Study of Pentabrominated Diphenyl Ether in Rat Liver during Early Life Exposure

K. R. Shockley. NIHES, Research Triangle Park, NC. Sponsor: J. Dunnick

Pentabrominated diphenyl ethers (PBDEs) have been used as flame retardants in polyester foams and other household products, leading to continued widespread exposure in the United States. Early-life exposure to PBDEs has been associated with loss of Intelligence Quotient and altered thyroid hormone levels. In 2-year studies, a PBDE mixture (DE-71) caused liver tumors and liver fatty change in male and female Wistar Han rats and B6C3F1/N mice. The PBDE mixture reduced serum thyroid hormone (T4) levels in a 13 week study in male and female F344/N rats, and induced genes involved in xenobiotic metabolism and reduced genes associated with lipid metabolism in rats in a separate study. In humans, the most prevalent PBDE congener is pentabrominated diphenyl ether-47 (PBDE-47). Here, PBDE-47 and PBDE mixture liver toxicity was characterized at PND22 in Wistar Han rat pups after in utero/postnatal exposure (0, 0.1, 15, or 50 mg/kg; dams, GD6-21; pups, PND12-PND21; oral gavage daily dosing). We found that PBDE-47 induced liver fatty change and T4 levels, similar to that previously observed with exposure to the PBDE mixture. Global gene expression patterns in the liver were obtained using the Affymetrix Rat Genome 230 2.0 Array platform. There were 628 differentially expressed gene transcripts between control and BDE-47 (50 mg/kg): at a false discovery rate threshold of 5%. These transcriptional changes were associated with the induction of cytochrome p450 enzymes that metabolize xenobiotics to more water-soluble compounds, Nr2f2-mediated oxidative stress response and ABC transporters involved in xenobiotic exporter systems. Downregulation of ABC liver transport genes provided a plausible mechanism for lipid accumulation, characterized by a treatment-related
increase in hepatic steatosis. The upregulation of the Nrf2 antioxidant pathway and changes in metabolic transcripts after PBDE-47 and PBDE mixture exposure seen in this study suggest that PBDE-47, like the PBDE mixture (National Toxicology Program 2017, Technical Report 589), might also be a liver carcinogen after continued long-term exposure.

2029 The Role of EGFR and AKT Activation in Cadmium-Induced Prostate Carcinogenesis

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Cadmium is a non-essential metal and significant correlations between chronic cadmium exposure and the risk of human prostate cancer have been reported. Although an association has been established, the molecular mechanism by which this metal induces cellular transformation has yet to be delineated. One underexplored process that may be affected by cadmium is EGFR/AKT activation and autophagy. Autophagy promotes cancer cell survival (oncogenic autophagy) by protecting cells from nutrient deprivation, starvation, and oxidative stress. The aim of the study is to understand the molecular mechanisms of cadmium-mediated prostate carcinogenesis by focusing on the unexplored roles of EGFR activation on autophagy. Acute exposure to cadmium significantly inhibited the growth of RWPE-1 cells. In contrast, CTPE cell growth was not significantly affected, suggesting that these cells are resistant to cadmium. Induction of EGFR and AKT-activation with concomitant upregulation of autophagy markers were evident in CTPE cells. These molecular events correlated with the induction of autophagy. Similarly, immunohistochemical staining showed increased expression of EGFR, AKT, Plac8, LC3B and Lamp2 in cadmium CTPE tumors confirming our in vitro findings. CTPE cells challenged with EGFR and AKT inhibitors demonstrated significant growth inhibition and downregulating EGFR, AKT and autophagy signaling. Autophagy inhibitors however, did not affect EGFR and AKT expression resulting resistance in CTPE cells. Finally, the levels of EGFR/AKT and other autophagy proteins in human prostate cancer were analyzed, which correlated with aggressiveness of the prostate cancer. The results suggest that EGFR and AKT activation may responsible for pro-survival function of autophagy, inhibition of these signaling may inhibit cadmium-induced prostate carcinogenesis.

2030 Novel Strategies for the Treatment of Crizotinib Resistant EML4-ALK+ Lung Cancer


Receptor tyrosine kinases (RTKs) have been discovered to play a role in the development of cancer through the generation of an oncogenic RTK. The anaplastic lymphoma kinase (ALK), which usually fuses with EML4 produces a specific subtype of lung cancer found in 2-7% of patients. Crizotinib is the first-line treatment for ALK+ lung cancer with a greater progression free survival and overall response rate compared to standard chemotherapy. However, acquired resistance usually develops within 12 months. We investigated the antitumor activity of two distinct drug classes; novel curcumin derivatives (RL66 and RL118) and the combination of crizotinib with a MEK inhibitor, selumetinib. The sulfonofuridine B assay was performed to investigate cytotoxicity. ALK+ H3122 cells exhibited greater sensitivity to RL66 and RL118 with IC50 values of 0.97 and 0.70 µM, respectively, when compared to ALK- A549 cells (2.65 and 1.15 µM, respectively). Interestingly the crizotinib resistant cells were sensitive to RL66 and RL118 (2.15 and 1.44 µM, respectively) when compared to crizotinib, which had a 13-fold increase in the IC50 compared to the parental H3122 cells. We concluded from these results that the mechanism of RL66 and RL118 toxicity does not involve direct effects on ALK. We also investigated the effect of the combination of crizotinib and selumetinib in H3122 and A549 cells. The combination had a synergistic cytotoxic effect. In the ALK- A549 cells no synergism with the combination was found showing the combination is specific to ALK. Further analysis using crizotinib resistant cells demonstrated that the combination of crizotinib and selumetinib was more potent than crizotinib alone, allowing a synergistic growth suppression of the cells. Western blotting and densitometry revealed that the protein expression of p-ALK and p-ERK decreased with the combination compared to the control treatment. From these results we can conclude that the combination of the two drugs has potent cytotoxic effects in parental ALK+ cells and crizotinib resistant cells. RL118 has a greater anticancer effect compared to RL66 and the mechanism of these compounds is under further investigation. Also the combination of crizotinib and selumetinib shows a promising therapy for crizotinib resistant ALK+ lung cancers.

2031 A Saturated Fat Diet Promoting Hepatic Cancer in a Mouse Model Is Associated with Increased Hepatic Expression of the Proto-Oncogene AGAP2

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The dietary effects of different fat types on mechanisms driving the progression of hepatic tumors is poorly understood. A mouse model of chemical carcinogenesis was used in the current study. Male C57BL/6 mice were given a single 10 mg/kg i.p. injection diethylnitrosamine (DEN) on postnatal day 13. The mice were subsequently fed at low fat (10%) control diet or various high fat (35%) diets. The fats in the high fat diets were of four different types: corn oil (polyunsaturated omega-6 fat), cocoa butter (saturated fat), olive oil (monounsaturated fat), and corn oil + DHA (omega-3 and omega-6 fats). We previously observed that feeding the saturated fat diet with cocoa butter for 30 weeks promoted tumorigenesis specifically relative to the other high fat diets (P<0.05). We subsequently hypothesized that the cocoa butter diet alters expression of genes promoting tumorigenesis in the liver. RNA-Seq analysis of a subset of the mice livers demonstrated the expression of 7 genes, AGAP2, VDli, lild2, Cyp4a14, Cyp4a16, Cyp5a11, and Chnn4 were altered at least 2-fold in cocoa butter fed males compared to males fed other high-fat diets (P<0.05). We performed qRT-PCR analysis of these genes from all the mice in the study, which confirmed the altered expression as indicated by RNA-Seq. AGAP2 (ArfGAP with GTPase domain, anykinin repeat and PH domain) 2 seemed the most interesting gene, as it is a proto-oncogene, i.e. a gene that promotes cancer if over-expressed. It encodes a GTP-binding protein that is a phosphoinositide 3-kinase enhancer (PIKE). Furthermore, AGAP2 had highest expression in males fed the cocoa butter diet and showed the highest correlation among the 7 genes (R= 0.494, p < 0.001) with the number of visible liver tumors. We also extracted liver RNA from mice fed high-fat diets for just 15 weeks before visible tumors appear. The highest expression of AGAP2 at 15 weeks was likewise observed in males fed the cocoa butter diet, indicating that increased AGAP2 expression is a consequence of the dietary regime and not the cancer phenotype. We conclude that AGAP2 expression is stimulated by the cocoa butter diet, indicating that increased AGAP2 expression is a consequence of the dietary regime and not the cancer phenotype. We conclude that AGAP2 expression is stimulated by the cocoa butter diet, indicating that increased AGAP2 expression is a consequence of the dietary regime and not the cancer phenotype. We conclude that AGAP2 expression is stimulated by the cocoa butter diet, indicating that increased AGAP2 expression is a consequence of the dietary regime and not the cancer phenotype. We conclude that AGAP2 expression is stimulated by the cocoa butter diet, indicating that increased AGAP2 expression is a consequence of the dietary regime and not the cancer phenotype.

2032 Aberrant DNA Methylation in Radon or Cigarette Smoke-Induced Maglinant Transformation in BEAS 2B Cell

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It is well known that cigarette smoking and/or radon induce malignant transformation in lung cells. To investigate the mechanisms underlying lung carcinogenesis induced by cigarette smoke (CS), radon (Rn); or Rn followed by CS using BEAS-2B cell line derived from human bronchial epithelial cells. BEAS-2B cells were exposed to either R (20,000 Bq/m3) for 30 min or CS (20%) for 10 min or Rn followed by CS for 40 min. Global and gene-specific DNA methylation modifications were measured by microarray and methylation-specific polymerase chain reaction (MSP). Cell cycle and apoptosis were determined by flow cytometry, while soft agar colony formation was conducted to assess the characteristics of malignant transformation. Data demonstrated global hypomethylation as well as gene-specific DNA methylation alterations in all treatment groups compared to unexposed control cells. In addition, Rn and CS produced DNA hypermethylation of protein tyrosine phosphatase receptor type M (PTPRM) and ectodysplasin A2 receptor (EDAR2), two genes related to malignant transformation. In all treatment conditions, cell proliferation and survival of malignant cells was increased, while apoptosis was initially first passage elevated but decreased at passages 5-15. Our results indicate that aberrant DNA methylation plays an important role in Rn- and/or CS-induced malignant transformation. In addition, BEAS-2B cell line may be used as an in vitro model to investigate mechanisms underlying malignant transformation induced by ambient environmental contaminants.
Obesity is a global epidemic with a predicted rate of 42% in the USA by 2050. Epidemiological studies show that obesity is a risk factor for developing cancer, however; the molecular mechanism has not been fully elucidated. Our published data demonstrate that fibroblast growth factor-2 (FGF2) released from fat cells (adipocytes) in the visceral adipose tissue (VAT) induces transformation/tumorigenicity in the skin and mammary epithelial cells. Specifically, FGF2 released from VAT stimulates epithelial cell growth in soft agar by inducing the proto-oncogene c-Myc. Growth in soft agar is a measure of transformation/tumorigenicity; neither transformation nor c-Myc induction in epithelial cells was reversible. c-Myc overexpression can initiate a process of genetic instability linked to tumor initiation. Our discovery of this novel direct path of VAT-stimulated tumorigenesis adds mechanistic insight to our earlier discovery that VAT secretions promote UV-induced non-melanoma skin cancer. The objective of our current study was to determine the mechanism by which FGF2 stimulates malignant transformation. We hypothesized that FGF2 from VAT induces c-Myc and subsequent genomic instability in epithelial cells leading to increased carcinogenesis. To test hypothesis we generated a filtered conditioned-medium from the human VAT, treated MCF-10A (mammary epithelial) and J86 P+ (skin epithelial) cells and measured several downstream mediators of FGF2 and FGF2 receptor. Following VAT treatment, epithelial cells demonstrated induced c-Myc protein expression along with ROS accumulation, elevated γ-H2AX foci, and increased micronucleus (MN) formation. We found that inhibition of c-Myc attenuated VAT-induced neoplastic transformation of MCF-10A and J86 P+ cells, while constitutive activation of the c-Myc induced spontaneous neoplastic transformation of J86 P+ cells. Collectively, our data suggested FGF2 released from VAT interacts with FGFFR-1 and activates c-Myc. The role of c-Myc in the formation of MN and DNA damage is under investigation. Determining the impact of excess VAT on cancer will lead to strategies to help prevent adiposity-associated cancers and identify individuals at risk for disease or individuals that may be susceptible to compound-induced genotoxicity due to DNA damaging environmental exposures.
aerosol for 4 h/day for 8 weeks, at a target concentration of 40 mg/m³. Lung nodules were enumerated at 30 weeks post-initiation. GMA-SS and GMA-MS fumes significantly promoted lung tumor multiplicity in A/J mice initiated with MCA (16.11 ± 1.18; 21.86 ± 1.50, respectively) compared to MCA/air-exposed mice (7.93 ± 0.82; 8.34 ± 0.59, respectively). Oropharyngeal aspiration of GMA-SS and its component metals showed that GMA-SS fume was more pneumotoxic than the individual components. Component Fe₂O₃ was the most toxic and also the only metal to promote lung tumors in A/J mice. In conclusion, this study demonstrates that inhalation of GMA-SS and GMA-MS welding fume as well as Fe₂O₃ promotes lung tumor formation in vivo and provides support for the epidemiological study that shows welders, using mild and/or stainless steel, are at an increased risk for lung cancer.

**2038 Direct Formalin Fixation Induces Widespread Genomic Effects in Archival Tissues**


Recent advances in next generation sequencing have dramatically improved transcriptional analysis of degraded RNA from formalin-fixed paraffin-embedded (FFPE) samples. However, little is known about potential genomic artifacts induced by formalin fixation, which could affect toxicological and clinical studies being conducted in FFPE samples. Previously, we identified a consistent shift in RNA-sequencing profiles between matching frozen and FFPE samples. We hypothesized that this shift was due to a core set of transcriptional changes induced when fresh tissue is fixed in formalin. To test this idea, liver samples were collected from C57BL/6 mice treated with 600 ppm of pentoxyresin, parabital (PB) or vehicle control (Con) for 7 days. Samples were divided into the following conditions: 1) fresh-frozen (FR); 2) directly fixed in 10% buffered formalin for 18 hours and processed to FFPE (FIX); and 3) processed as for FIX but initially frozen (FR-FIX) (n=6/group/condition). The FR-FIX group served as a control for tissue processing and sequencing after collection. Total RNA libraries were prepared and ribo-depleted prior to sequencing on an Illumina Hi-seq 2500. Reads were aligned using Star (2.4) and analyzed in Partek Flow (6.0). Direct fixation (FIX vs. FR) resulted in 2946 differentially expressed genes (DEGs), 98% of which were down regulated. Freezing prior to fixation (FR-FIX vs. FR) resulted in 95% fewer DEGs, indicating that the majority of formalin effect occurred at the time of fixation (i.e., as a transcriptional response) rather than during tissue processing or sequencing. Comparative analysis of this formalin-induced gene set with two independent studies in Ingenuity Pathway Analysis identified consistent enrichment in oxidative stress, mitochondrial dysfunction, and transcription elongation pathways. However, direct fixation did not have a clear impact on chemotherapeutic resistance, PB treatment induced 180 DEGs within the FIX group and 159 in the FR-FIX group of which 120 were shared. The DEGs in each list were consistent with CAR/PXR activation and PB exposure, suggesting that formalin fixation did not confound the chemical response. Our results highlight distinct transcriptional effects of formalin fixation that could impact RNA-sequencing studies using FFPE samples. This abstract does not reflect US EPA policy.

**2039 Effect of Resveratrol on Gut Microbiome in Colorectal Cancer**

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Colorectal cancer (CRC) is a heterogeneous disease with recognizable clinical and molecular features, with wide range of prognostic and treatment responses observed among CRC patients worldwide. Currently, the treatment of patients suffering from CRC, and most tumors involves the surgical removal of the cancer and chemotherapy that are not effective in most cases and have adverse side effects or increase the toxicity. In current study, we investigated the effects of resveratrol (RES), a natural component found in grapes, strawberries and raspberries on murine azoxymethane-dextran sodium sulfate (AOM-DSS) induced CRC model. Our data shows that administration of RES alleviates symptoms in AOM-DSS group, with normal body weight, normal food intake, and normal survival of the mice. The histopathological changes in the colon tissues were analyzed by H&E staining. We observed normal colon architecture without evidence of in vivo tumourigenesis, goblet cell hyperplasia, or inflammation. These results suggest that RES has a protective effect on CRC development. In conclusion, this study demonstrates that RES supplementation may be a potential therapeutic strategy for the prevention and treatment of CRC.

**2040 Cells That Escape Cr(vi)-Induced Cell Death Exhibit Chromosome Instability**

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Hexavalent chromium (Cr(VI)) compounds are established human lung carcinogens; however, the mechanism remains poorly understood. Cr(VI) induces DNA damage which under normal circumstances triggers the protective apoptotic machinery to avoid transformation and carcinogenesis. Evasion of apoptosis is a hallmark of carcinogenesis, however it is unknown how Cr(VI)-damaged cells are able to escape cell death and become tumorigenic. In the current study we exposed human lung cells to low concentrations of zinc chromate continuously for 6 months. Because at various intervals during treatment we assessed growth parameters using a colony forming assay. We also monitored changes in chromosome number and structure of surviving cells using traditional karyotyping methods. We found that Cr(VI)-treated cells produced a decrease in cell death in the first 25 days of exposure. The lower concentrations (0.0125 and 0.025 ug/cm²) showed decreased plating efficiency relative to the control, indicative of cell death, for the entire length of treatment. Interestingly, the highest concentration, 0.05 ug/cm², initially caused significant cell death, but was followed by a period of enhanced survival at day 70 of treatment, and then decreased plating efficiency after day 120 which remained throughout exposure. Although plating efficiency decreased, cell growth was accelerated in the high treatment group. Additionally, cells that escaped particulate Cr(VI)-induced cell death exhibited significant amounts of both structural and numerical changes. Control cells at all time points showed normal chromosomes; at day 5 there were 24, 26, and 30 percent of cells with normal karyotypes at 0.0125, 0.025 and 0.05 ug/cm² zinc chromate, respectively; at day 70 there were 46, 52, and 48 percent of cells with normal karyotypes; at day 180 there were 18, 72, and 84 percent of cells with abnormal karyotypes. Increases in aneuploidy earlier were observed at earlier time points compared to structural alterations. These data support a hypothesis that Cr(VI)-treated cells can evade apoptosis and transform into chromosomally unstable cells yet continue to survive. These cells have the potential to become carcinogenic. Further work will elucidate the mechanism behind the evasion of apoptosis. By considering both mutagenic and epigenetic alterations, this work will contribute to the understanding of carcinogenicity of Cr(VI).

**2041 In Vitro Investigations of Adjuvant Chemotherapy for Prevention of Breast Cancer Tumor Recurrence**

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Breast cancer is the most common cancer among women. Once identified and tumor excised, the risk of local breast cancer recurrence (or return of cancer within the breast) depends on tumor characteristics. Adjuvant therapies are used to ensure that remaining microscopic disease will be eradicated, and to help extend survival time of the patient. A standard clinical regimens is a combination of cyclophosphamide, adriamycin (doxorubicin) and 5-fluorouracil (CAF) that is administered for four months. Despite the initially successful multimodal therapy, tumor recurrence remains a major cause of mortality in breast cancer patients (1). Hence, better treatment options are necessary. In this study, responses from murine H8N8 and H8N8 T3.2 cells were investigated via two different assays: via image-based live-cell analysis and an impedance-based cell monitoring system. The H8N8 cells are an immortal mammary carcinoma cell line with tumor stem cell properties, and the H8N8 T3.2 cells are a recurrent tumor variant. H8N8 T3.2 cells were established from a solid breast tumor that received the CAF clinical regimen in vivo. For further in vitro tumor recurrence investigation,
In Vitro Anticancer Potential of a Specific Polyphenol Mixture of Curcumin, Quercetin, Cruciferous Plants, and Green Tea Extracts, and Resveratrol on Human Prostate Cancer Cell DU-145

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In vitro anticancer potential of a specific polyphenol mixture of curcumin, quercetin, cruciferous plants and green tea extracts and resveratrol on human prostate cancer cell DU-145. Prostate cancer is one of the most common diagnosed cancers in men aged 50 and older. Metastasis is a major cause of mortality. Current treatments include surgery, hormone therapy, radiation therapy and chemotherapy, all of which are associated with adverse side effects.

We investigated the effect of a specific polyphenol combination (PB) of curcumin, quercetin, green tea extract, cruciferous and essential oil, on viability, invasion, MMPs secretion, cell migration and morphology on human prostate cancer cell lines. Human prostate cancer cell cell DU-145 (ATCC) was cultured in DMEM media and supplemented with bovine serum and antibiotics in 24-well tissue culture plates. Cell viability, invasion, MMPS secretion, cell migration and morphology were measured using Western blotting, zymography, cell invasion using Matrigel, cell migration by scratch test, and H&E staining.

PB at 0, 10, 25, 50 and 100 µgm/ml concentration, respectively. Zymography demonstrated expression of only MMP-9 which was inhibited by PB in a dose response fashion with total inhibition at 50 µg/ml. PB at 10, 25, 50 and 100 µg/ml concentration, respectively. Zymography demonstrated expression of only MMP-9 which was inhibited by PB in a dose response fashion with complete block at 50 µg/ml. H&E staining showed apoptotic changes at 50 and 100 µg/ml concentration. These results suggest therapeutic potential of PB in the treatment of prostate cancer.

Ligand Activation of PPARβ/δ Inhibits UV-Induced Skin Cancer by Targeting p53 Mutant Cells

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Non-melanoma skin cancer is the most common type of cancer with an estimated 2.5 million new cases each year. 90% of non-melanoma skin cancers result from UV-induced skin cancer. UV-induced skin cancer was examined in this study. Female SKH-1 mice (wild-type or Pparβ/δ-null) were UV exposed (180 mJ/cm²) 3 times/week followed by topical application with vehicle control, the highly specific PPARβ/δ ligand (1.6-fold), whereas no increase in proliferation was observed in CAR-KO mice. The selective repressive ligand also inhibited growth of DLD1 spheroids but this effect was not found in RKO spheroids. Interestingly, DLD1 cells had a reduction in the APC gene while RKO cells did not. This suggests that selective repression of PPARβ/δ may preferentially target cells with APC mutations, a common risk factor for human colon cancer. Combined, these results suggest that ligand activation and/or selective repression of PPARβ/δ is a potential cancer therapy and invasion, but the detailed mechanisms need further investigation.

Carinogenesis, p53 mutant cells may be particularly susceptible to inhibition of the ER stress response pathway because of increased protein production. Cytosolic expression of ER stress response protein, IRE1α, was decreased in wild-type UVB-induced skin samples compared to Pparβ/δ-null samples. It was also found that nuclear expression of p107, a transcription factor that represses the cell cycle, was increased in wild-type UVB-induced skin samples compared to Pparβ/δ-null samples. Results from these studies provide strong evidence that ligand activation of PPARβ/δ inhibits UVB-induced skin cancer by targeting TP53 mutant cells and inhibiting UVB-induced DNA damage. Supported by CA124533, and CA140369.

2044 PPARβ/δ Inhibits Proliferation and Invasion of Human Colon Cancer Cell Lines

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Whether PPARβ/δ promotes or potentiates colon cancer is not fully understood. While some studies have shown that the expression level of PPARβ/δ is reduced in human colon tumors compared to normal tissue control, and is negatively correlated to survival of colorectal cancer patients, others have suggested the opposite. In this study, the hypothesis that ligand activation of PPARβ/δ inhibits human colon cancer cell line proliferation and reduces their potential of migration and invasion was examined. Ligand activation of PPARβ/δ inhibited growth of HT29 cells, and selective repressive transcription of PPARβ/δ inhibited growth of DLD1, HCT116 and HT29 cells. Additionally, both ligand activation and selective repression of PPARβ/δ inhibited migration of DLD1 cells, with only ligand activation inhibited RKO cell migration. Selective repression of PPARβ/δ reduced expression of EMT markers in RKO and HCT116 cells, further suggesting an anti-invasion role of PPARβ/δ in these human colon cancer cell lines. The selective repressive ligand also inhibited growth of DLD1 spheroids but this effect was not found in RKO spheroids. Interestingly, DLD1 cells harboring a mutation in the APC gene while RKO cells do not. This suggests that selective repression of PPARβ/δ may preferentially target cells with APC mutations, a common risk factor for human colon cancer. Combined, these results suggest that ligand activation and/or selective repression of PPARβ/δ is a potential cancer therapy, but the detailed mechanisms need further investigation. Supported by CA142533, and CA140369.

2045 Driving into the Future with CAR: Moving from Current Mode-of-Action to Predictive Approaches for Addressing Nuclear-Receptor Mediated Hepatocarcinogenesis

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There are several well-characterized modes of action (MoA) for hepatocarcinogenesis, and the analysis of robust mechanistic data can provide a reasonable basis for extrapolation of a rodent MoA to human relevance. While in the past MoA programs were triggered in response to observed liver tumors in cancer bioassays, predictive approaches are now being undertaken to better characterize carcinogenic potential early in discovery testing programs. As a case study, the agrochemical nitrapyrin, which induces hepatic tumors in mice, was put through a series of MoA experiments and proof of principle predictive toxicogenomics experiments to serve as a backdrop for future predictive approaches. In the MoA experiments, nitrapyrin induced a dose-related increase in the Cyp2b10/CAR (constitutive androstane receptor) transcript and protein, an increase in hepatocellular proliferation, and ultimately, tumors. Nitrapyrin exposure in wild-type 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 tumour formation. Furthermore, nitrapyrin exposure induced a clear, concentration-responsive increase in cell proliferation in mouse, but not human, hepatocytes in vitro. These data support a MoA characterized by CAR activation, which due to qualitative differences between mice and humans is not relevant for human health risk assessment. New approaches to predict toxicity are now being embraced by employing toxicogenomics. A retrospective in vivo toxicogenomics experiment with nitrapyrin was conducted, where rats were exposed to nitrapyrin for 28-days and RNASEq was performed on treated and control liver samples. The toxicogenomics data reveal that CAR-mediated hepatocarcinogenesis may be observed in rodents, as indicated by hierarchical
clustering of gene set enrichment where nitrapyrin clustered closely with other CAR hepatocarcinogens, including phenobarbital. These data support the utility of predictive toxicogenomic approaches to characterize potential hepatotoxicity, which can be used to guide early-stage discovery molecule decisions and proactively drive future MoA testing programs.

2046 Exosomal ID3 Aids in Estrogenic Chemical Dependent Vascular Guiding of NRF1-Driven Secondary Breast Cancer Lesions

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During blood-borne metastasis, upregulation of endothelial cell adhesion molecules can accelerate metastatic processes of breast cancer stem cells (BCSCs) through increased adhesion of tumor cells to the endothelium. In this study, we have examined whether exosomal ID3 aids in estrogenic chemical dependent vascular guiding of NRF1-driven secondary breast cancer lesions. Besides NRF1 mediated stochastic transcriptional programming of BCSCs, our studies support a novel concept that ID3 expressing vascular EndSCs are not just contributing in new blood vessel formation, but also increased the adhesion and transmigration of BCSCs. Differential effects were demonstrated with endocrine disruptors PCB 153 and PCB 77. Only PCB 153 treatment exacerbated growth of BCSC tumors spheroids in vivo mimicking deviant microvascular ID3+ endothelium and NRF1+ BCSCs in tissue culture. PCB 153 also increased tumor cell adhesion to microvascular endothelium and transendothelial migration of BCSCs. Exosomal ID3 from endothelial cells aids in the propensity of mesenchymal NRF1+ BCSCs to specify and commit to a particular lineage. Organized growth of organ-specific ID3+ EndSCs accompany and guide a specific subtype of mesenchymal NRF1+ BCSCs to spread to a colonizing organ site. ID3+ EndSCs not only supported the growth of BCSC tumorspheres, but guided the in vivo migration of tumor stem cells to the brain in the zebrafish embryo. These studies provide convincing support to our postulate that gain-of-function by NRF1 in BCSCs and ID3 in EndSCs will lead to stochastic transcriptional programming of their target genes controlling the specification and guidance of BCSCs to spread to distant sites in exposed individuals. Moreover, the effect of the estrogenic chemical PCB 153 has expanded our understanding of environmental exposure to endocrine disruptors may influence the specification of NRF1+ BCSCs and their guidance by ID3+ EndSCs to secondary target organs. Thus, establishing a role for NRF1/ID3 in speciation and guidance of BCSCs to spread to secondary target organs will directly link environmental exposure to estrogenic chemicals, including PCB153.

2047 Bortezomib Inhibits Growth of Multiple Myeloma Cells through Downregulation of Specificity Protein Transcription Factors

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Bortezomib is a member of a relatively new class of drugs that act as proteasome inhibitors and it is the first proteasome inhibitor approved by FDA for treatment of multiple myeloma (MM). Several studies demonstrated that Sp1 Transcription Factors (TFs) is overexpressed in multiple myeloma cells and Sp1 regulates expression of genes important for cell growth, proliferation, and survival, however, the role of Sp1 and other Sp TFs has not been thoroughly investigated in Multiple myeloma cells and interactions of Bortezomib and Sp TFs have not been determined. Initial studies shows that not only Sp1 but also Sp3 and Sp4 are highly overexpressed in multiple myeloma cells. Treatment of cells for 24 and 48 hours with 10, 15 and 20 nM Bortezomib significantly inhibited cell viability. Moreover, treatment of the cell lines with 10, 15 and 20 nM of Bortezomib induced Annexin V staining and several markers of apoptosis including cleavage of caspase-8, caspase-3 and cPARP. Using these same treatment protocol we show that Bortezomib also downregulates expression of Sp1, Sp3 and Sp4 and several pro-oncogenic Sp-regulated genes. RNA interference (RNAi) studies show that the knockdown of Sp1, Sp3 and Sp4 individually resulted in inhibition of cell growth and induction of apoptosis in untreated 2218 and ANBL-6 cell demonstrating that not only Sp1 but also Sp3, Sp4 and Sp4 contribute to MM cell growth and survival. In addition, we also observed that Bortezomib-mediated downregulation of Sp proteins involved activation of caspase-8 and the mechanism of this effect is currently being investigated. Our results demonstrate for the first time that the anticancer activity of Bortezomib is due, in part to Sp downregulation.

2048 IDH Mutation-Inspired Oxidative Therapy to Higher Grade Gliomas

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Glioblastoma multiforme (GBM) is the deadliest brain cancer among gliomas with a median survival below one year. Despite the Spartan efforts taken to advance the therapeutic strategies, the prognosis remains grim for this devastating disease with the current combination therapy, mostly because of the resistance offered by the overexpression of 6-methyl guanine methyl transferase (MGMT). MGMT is a DNA repair enzyme, which by its action, removes the methyl adducts induced by TMZ, leading to resistance. Attempts to inhibit MGMT with pseudo substrates failed mostly because of toxicity. MGMT is a redox sensitive protein owing to the cysteine in its active site. Whole genomic sequencing disclosed the existence of mutations in isocitrate dehydrogenase 1/2 (IDH1/2) in majority of low grade gliomas. Mutations in IDH causes loss of normal and gain of new enzymatic activity causing accumulation of the oncometabolite, D-2 hydroxylglutate (D-2HG), leading to oncogenesis. In addition, D-2HG accumulation leads to loss of redox balance. It is established in clinic that glioma patients harboring IDH mutations respond well to chemotherapy. We hypothesize that, small molecules that mimic α-KG, can replicate the beneficial aspects as seen in the case of IDH mutations, in GBM in vivo or in vivo tumor models. We synthesized a library of molecules that mimic α-KG, out of which DMG, showed synergistic cytotoxicity in combination treatment with TMZ and BCU2 against various GBM cells. Enzymatic assay to see its role against α-KG dehydrogenase enzyme revealed its potent inhibitory role which further perturbs the redox balance. Induced ROS levels found to induced loss mitochondrial membrane integrity. Later, western and in vivo activity assay showed dose dependent inhibition of MGMT in ROS dependent manner. We then performed extracellular flux analysis and found a significant decrease in oxidative consumption rate (OCR) with in situ or pretreatment with DMG. Proteomic analysis using LC-MS/MS, ELISA studies to measure ATP, NADH revealed metabolic and energy stress. Immunofluorescence studies confirmed the nuclear translocation of cytochrome C and apoptosis inducing factor and inhibition of ROS mediated AMPK activation. Notably, the efficacy of DMG was evaluated in vivo against subcutaneous xenografts developed in nude mice. DMG treatment resulted in a significant reduction in tumor growth. Supported by CPRIT grant RP130266 and RP170207 to KSS.

2049 Mode-of-Action Analysis for Uterine Adenocarcinomas Associated with High Dietary Ingestion of the Insecticide Aldofypropen

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Aldofypropen (CAS # 915972-17-7) is a novel insecticide that acts as a TRPV channel modulator in the chordotonal organs of target insects. In two carcinogenicity studies with Fischer rats, an increased incidence of uterine adenocarcinomas was observed (incidences of 4/50, 1/50, 2/50 and 10/50 in the first study at doses of 0, 1000, 3000 ppm respectively). The key events responsible for the uterine tumors were investigated through a series of mechanistic and toxicokinetic studies with Aldofypropen. The IPCS framework was used to analyze the relevance of the rat uterine tumors to humans. The results indicate that the uterine tumors were mediated through a mode of action (MoA) of dopamine enhancement, subsequent hormonal alterations, endometrial hyperproliferation, and promotion of uterine adenocarcinomas. Specifically, the findings of dopamine receptor 2 agonism, decreased serum prolactin levels, decreased mammary gland dilation (indication of delayed entry into senescence), and a late onset of endometrial hyperplasia and progestin dominated changes in hormone profiles consistent with endometrial adenocarcinomas in rat uteri. This MoA is considered to be rat-specific and not relevant to pathophysiologic conditions in human. Furthermore, both of the doses where tumors were observed in rat, 1000 ppm (50.4 mg/kg bw) and 3000 ppm (146.9 mg/kg bw), exceed the kinetically derived maximum tolerated dose (KMD) of 15 mg/kg bw established for Aldofypropen. Effects that occur at doses above the KMD, regardless of MoA, are quantitatively not
relevant to humans when the expected human exposures are below the KMD. Exposures to Afidopyropen are expected to be thousands-fold lower than the established KMD. In conclusion, the tumor incidence associated with high doses of dietary Afidopyropen in the rat is not expected to pose a carcinogenic risk to humans.

**2050 Nfe2l1 Silencing in Insulinoma Cells Causes the Emergence of an Aggressive and Chemo-Resistant Tumor Phenotype Via Metabolic Reprogramming**

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Nuclear factor erythroid 2 like 1 (NFE2L1, also known as NRF1) is a transcription factor known to be key to embryonic development, maintenance of the ubiquitin-proteasome system, and regulation of the cellular antioxidant response. We previously found that pancreatic β-cell-specific Nfe2l1-knockout mice showed severe hyperinsulinemia, and silencing of Nfe2l1 in MIN6 β-cells and mouse islets resulted in elevated basal insulin release and altered glucose metabolism. In the present study, we examined the tumorigenicity of Nfe2l1 deficient insulinoma MIN6 cells (Nfe2l1-KD) and their sensitivity to chemotherapy. We found that Nfe2l1-KD insulinoma cells grew faster and were more aggressive than Scramble cells in vitro. Insulinoma arising from Nfe2l1-KD cells in an allograft transplantation model was more aggressive and chemo-resistant. This conclusion was confirmed by streptozotocin (STZ) administration in an allograft transplantation model in diabetic Akita background mouse. In addition, we found that Nfe2l1-KD cells were resistant to chemotherapeutic drugs STZ and 5-fluorouracil-induced damage, which was linked to binding of hexokinase 1 with mitochondria, enhancing mitochondrial potential membrane closing mitochondrial potential transition pore. Overall, both in vitro and in vivo data from Nfe2l1-KD MIN6 insulinoma cells indicate a previously un-appreciated action of NFE2L1 in suppression of tumorigenesis and sensitizes cells and their derivative tumors to chemotherapeutic-induced damage and apoptosis, likely via metabolic reprogramming. These data indicate that NFE2L1 could potentially play an important role in the carcinogenic process and impact chemotherapeutic efficacy, at least within a subset of pancreatic endocrine tumors.

**2051 Xenobiotic Nuclear Receptors Expression in High Fat Diet-Induced Non-Alcoholic Fatty Liver Disease: A Time-Course Study**

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Non-alcoholic fatty liver disease (NAFLD) is a common chronic liver disease. The pathologic progression of NAFLD includes steatosis, steatohepatitis, fibrosis, cirrhosis and possible hepatocellular carcinoma (HCC). Nuclear receptors regulate physiological and pathological pathways in response to environmental and endogenous stressors. Modification of hepatic nuclear receptor expression during NAFLD, which has been suggested to modulate metabolism of nutrients and chemicals in the liver. The current study was designed to systematically characterize the time-dependent modulations of nuclear receptors including peroxisome proliferator activated receptors (PPAR), constitutive androstane receptor (CAR), pregnane X receptor (PXR), liver X receptor (LXR), and farnesoid X receptor (FXR) in the progression of NAFLD. Male C57BL/6 mice were fed a high fat diet (HFD) to induce NAFLD. Hepatic steatosis was detected after 8 weeks on HFD. Further progression to fibrosis was seen after 24 and 32 weeks on diet as evident by Sirius red staining and a 6-8 fold increase of Col1a1 and Timp1 expression in the liver. DNA synthesis level (Brdu labeling index) significantly increased up to 6-fold in the liver with steatohepatitis. Transcriptome analysis of liver samples after 16 weeks on HFD increased the expression of PPARα, CAR, and PXR (p < 1.0e-10) compared to normal chow fed control mice. In the time-course analysis, PPARα was activated (4 fold) as indicated by Cyp4a10 gene expression and a significant increase in acyl CoA oxidase activity. PPARα mRNA was moderately elevated while PPARδ was down regulated to 20-40% comparing to controls. PXR target gene Cyp3a11 was consistently increased 3-4 fold in addition to the increased microsomal Cyp3a enzymatic activity in the stages of steatois, steatohepatitis and fibrosis. In contrast, activation of CAR related genes was not as robust as the PPAR related genes. LXR target genes Abcg5 and Abcg8 were also significantly elevated (2-3 fold) that correlated with the development of hepatic steatosis, indicating its role in lipid and cholesterol metabolism. The mRNA of FXR was downregulated at 24 and 32 weeks after high fat diet treatment, which may correlate with hepatic fibrosis. This study provides a systematic characterization of the changes in important hepatic gene expression pathways that correlated with biochemical and pathologic changes related to the progression of NAFLD induced by HFD.

**2052 Correlation between Genome-Wide Histone Acetylation Changes and Repression of Gene Expression by Ochratoxin A**

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Ochratoxin A (OTA) is a potent renal carcinogen but its mechanism has not been fully resolved. Using a novel mass spectrometry approach employing chemical 15C-acetylation of unmethylated lysine residues for quantitative analysis of site-specific alterations in histone acetylation in human kidney epithelial cells (HK-2) exposed to OTA, we previously established that OTA causes global reduction of histone acetylation, including loss of acetylation at histone H3 lysine 9 (H3K9), a well-known euchromatic hallmark that is typically elevated at promoter regions of transcriptionally active genes. Considering that the predominant transcriptional response to OTA in vitro and in vivo is down-regulation of gene expression, we employed ChIP-Seq to test if OTA mediated changes in gene expression may at least in part be mechanistically linked to H3K9 hypoacetylation at respective promoter sequences. Integrated analysis of OTA mediated genome-wide changes in H3K9 acetylation with published gene expression data demonstrated that among OTA responsive genes almost 80% of hypoacetylated genes were down-regulated, thus confirming an association between H3K9 acetylation status and gene expression of these genes. However, only 7% of OTA-repressed genes showed loss of H3K9 acetylation within promoter regions. Interestingly, however, GO analysis and functional enrichment of down-regulated genes showing loss of H3K9 acetylation at their respective promoter regions revealed enrichment of genes involved in the regulation of transcription, including a number of transcription factors that are predicted to directly or indirectly regulate the expression of 98% of OTA repressed genes. Thus it is possible that histone acetylation changes in a fairly small set of genes but with key function in transcriptional regulation may trigger a cascade of events that may lead to overall repression of gene expression.

**2053 Atovaquone: An Anti-Protozoal, Anti-Malarial Drug Suppresses Breast Tumor Growth by Inhibiting Her2/β-Catenin Signaling**

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Breast cancer is the second highest cause of cancer-related mortality in women, HER2 is an oncoprotein overexpressed in about 30% of breast cancer patients and β-catenin, a proto-oncogene plays a pivotal role in breast cancer metastasis and drug resistance leading to poor prognosis. In the current study, we evaluated the anti-cancer effects of an anti-protozoal drug, atovaquone against several breast cancer cell lines such as MCF-7, 4T1, HCC1806, C66, SKBR3 and T47D. Our results showed that atovaquone treatment induced apoptosis as exhibited by PI/annexin assay and cleavage of caspase-3 and PARP, and inhibited the growth of all the breast cancer cell lines tested. Similar observations were made in mice with orthotopic breast cancer cell lines. In addition, atovaquone treatment significantly reduced the expression of HER2, β-catenin and its downstream molecules such as pGSK-3β, TCF-4, cyclin D1 and c-Myc in these cell lines. Treatment of HCC1806 cells with 5 µM atovaquone resulted in significant reduction in mammosphere forming efficiency (MFE) of breast cancer cells. Mammosphere-forming cells are known to exhibit cancer stem cell (CSC)-like properties together with drug resistance. Efficacy of atovaquone was evaluated in the in-vivo tumor model by orthotopic implantation of two highly aggressive 4T1 and C66, triple negative breast cancer cells in the mammary fat pads of female mice. Our results demonstrate that atovaquone administration suppressed the growth of C66 and 4T1 tumor by 70% and 60% respectively. Atovaquone treated tumors collected from orthotopic model exhibited reduced HER2, β-catenin and increased apoptosis when analyzed by western blot. Atovaquone is highly safe, non-toxic and well tolerated drug in humans. Moreover, we have found in our study that the intended dose of atovaquone to induce anticancer effects is about 6.6 fold less than its highest anti-malarial dose. Conclusively, our results indicate that atovaquone effectively reduces the growth of primary breast tumors by inhibiting HER2/β-catenin signaling. Most importantly, atovaquone is already in
clinical use with an established safety record therefore, any positive findings from our studies will prompt further clinical investigation into repurposing atovaquone for the treatment of advanced breast cancer patients. Supported in part by R01 grant CA 129038 awarded by National Cancer Institute, NIH.

2054 γ-H2AX Formation Induced by the Bladder-Carcinogenic Aromatic Amines o-Toluidine and o-Anisidine in the Urinary Bladder of Rats

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Although aromatic amines are widely used as raw materials for dyes, there have been concerns about carcinogenicity, particularly in the urinary bladder. In the present study, we examined the formation of γ-H2AX, a biomarker of DNA damage, in the urinary bladder of rats using immunohistochemistry to detect early changes induced by five monocyclic aromatic amines that exhibit similar structures. Six-week-old male F344 rats were administered 0.4% or 0.8% o-toluidine, 0.3% or 1% o-anisidine, 0.4% 2-xylyl, 0.2% p-toluidine or 0.6% aniline in their basal diet for 4 weeks. Five animals in each group were sacrificed at day 2 and weeks 1, 2, 4, and 6, and histopathological and immunohistochemical analysis was performed. In the 0.8% o-toluidine group, we observed sequential progression of bladder lesions, characterized by intrumoral hemorrhage and interstitial necrosis at day 2 and formation of granulation tissue with mononuclear cell infiltration at week 1, to diffuse hyperplasia at weeks 2 and 4. In the 1% o-anisidine group, simple hyperplasia without obvious infiltration of inflammatory cells was detected from weeks 2 to 4 whereas γ-H2AX-positive bladder epithelial cells in the 1.0% o-anisidine group were significantly increased in a time-dependent manner, transient increases of γ-H2AX-positive cells were detected at day 2 and week 1 in the 0.8% o-toluidine group. No apparent bladder lesions or significant increases in γ-H2AX formation were observed in the 0.4% 2-xylyl, 0.3% o-anisidine, 2.4-xylyl, p-toluidine, and aniline groups. Results of this study suggest that o-toluidine and o-anisidine may induce in vivo genotoxicity in the urinary bladder of rats. Moreover, histopathological examination revealed different mechanisms of bladder mucosal injury associated with both compounds.

2055 Using Transgenic Zebrafish Larvae for Xenotransplantation of Human Breast Cancer Cells and Screening of Natural Compounds for Anti-Cancer Activity

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Zebrafish are widely accepted as a model organism for developmental research and human disease pathways. Transgenic zebrafish are used for xenotransplantation human cancer cells to study the mechanisms involved in growth, proliferation, and metastasis of cancer cells in an amenable tumor microenvironment. Zebrafish xenografts also offer the opportunity for high-throughput screening of potential anti-cancer compounds for toxicity and therapeutic efficacy. In our xenotransplantation studies, estrogen receptor positive MCF-7, and human triple negative breast cancer cells were used to model benign and malignant types of breast cancer, respectively. A lipophilic red fluorescent dye (CM-DiI) was used to label cells and traditional chemotherapy agents were used to determine in vitro cell viability. We also measured cytotoxicity of extracts from the plant Tinospora crispa supported by the American Beverage Association.

2056 Topical Application of Mycotoxin-Alternariol Induces Inflammation and Tumor Initiation in Swiss Albino Mouse Skin

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Alternaria alternata produces various secondary metabolites including Alternariol (AOH), which is a natural contaminant processed refrigerated and stored food and feed products. Although preferably humans are exposed to mycotoxins through dietary exposure, however, exposure through dermal route can not be ruled out. Considering this possibility, WHO has highlighted the need for toxicological studies regarding dermal exposure of mycotoxins. Susceptible populations for dermal exposure of mycotoxin may include agricultural field workers and elderly persons with impaired skin barrier functions. In the present study, dermal toxic potential of AOH was assessed using mouse as an animal model. Swiss Albino mice (n= 5/group) were topically exposed with different doses of AOH from weeks 4 to 12 whereas γ-H2AX-positive cells were counted at day 2 and week 1 in the 0.8% AOH group. No apparent bladder lesions or significant increases in γ-H2AX formation were observed in the 0.8% o-toluidine or 0.3% 2,4-xylidine, 0.8% aniline in their basal diet for 4 weeks. Five animals in each group were sacrificed at day 2 and weeks 1, 2, 4, and 6, and histopathological and immunohistochemical analysis was performed. In the 0.8% o-toluidine group, we observed sequential progression of bladder lesions, characterized by intrumoral hemorrhage and interstitial necrosis at day 2 and formation of granulation tissue with mononuclear cell infiltration at week 1, to diffuse hyperplasia at weeks 2 and 4. In the 0.8% o-toluidine group, simple hyperplasia without obvious infiltration of inflammatory cells was detected from weeks 2 to 4 whereas γ-H2AX-positive bladder epithelial cells in the 1.0% o-anisidine group were significantly increased in a time-dependent manner, transient increases of γ-H2AX-positive cells were detected at day 2 and week 1 in the 0.8% o-toluidine group. No apparent bladder lesions or significant increases in γ-H2AX formation were observed in the 0.4% 2-xylyl, 0.3% o-anisidine, 2.4-xylyl, p-toluidine, and aniline groups. Results of this study suggest that o-toluidine and o-anisidine may induce in vivo genotoxicity in the urinary bladder of rats. Moreover, histopathological examination revealed different mechanisms of bladder mucosal injury associated with both compounds.

2057 Assessing Responses of Mouse Lung and Liver to 4-Methylimidazole Using Whole Genome Gene Expression Analysis after Oral Dosing for 2, 5, and 28 Days

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4-Methylimidazole (4-MeI) increased lung tumors in 2-year studies in female and male mice. Changes in gene expression were evaluated in lung and liver of B6C3F1 mice exposed to 4-MeI for 2, 5, or 28 days using RNA-seq. The magnitude of change in differential expressed genes (DEGs) were small for any one gene, but relatively large numbers of DEGs were seen at 2-days in both tissues followed by decreasing numbers at longer exposures. Pathway enrichment was determined using specific selection criteria - both fold-change expression (|FC|) and a false discovery rate (FDR) - or by ranking genes in order of fold-induction and iteratively calculating enrichment for gene lists of different sizes. There were some significant differences in enrichment patterns in male and female lung at 2-days. However, several changes, including regulation of circadian clock genes at 2-days, downregulation of mitochondrial functions at later times, and various effects on G-protein coupled receptor (GPCR) signaling pathways were present in both males and females. The liver had upregulation of mitotic pathways at early times and downregulation of various metabolic pathways and GPCRs at later times. Based on (1) structural similarities of 4-MeI with histamine, (2) known short-term CNS and testicular toxicity of 4-MeI, (3) changes in arachidonic acid metabolism pathways, (4) rapid accommodation of the gene expression changes and (5) changes in GPCR pathways, it is hypothesized that 4-MeI may interact with histaminergic GPCR functions that are associated with numerous biological activities and functions. This study was supported by the American Beverage Association.

2058 Xenotransplanted larvae will be exposed to established chemotherapy drugs (e.g. 4-hydroxytamoxifen) to validate the effect of the chemotherapeutic drugs in vivo and subsequently to novel natural products to screen in vivo safety and potential efficacy of anti-cancer compounds. Supported by UM ORSP and UM GSC.

2059 Innovative Research (AcSIR), Chennai, India.

2060 Skin
While second-hand smoke exposure (SHS) is a risk factor for lung cancer, it is unclear whether there is an association between in utero SHS exposure and this lung disease. We previously showed in 15-week old mice exposed solely to in utero SHS that Dnmt3a, which 1) is responsible for de novo DNA methylation, 2) plays a key role in epigenetic mechanisms, and 3) whose deficiency in mice is associated with lung cancer, was significantly down-regulated. The current studies suggest that in utero SHS exposure alone may predispose to respiratory morbidity, including lung cancer. Here, we hypothesized that in utero SHS exposure promotes the development of carcinomas in a urethane-induced lung cancer model. Pregnant BALB/c mice were exposed from gestational days 6-19 to 10 mg/m³ of SHS or filtered air. From 13-16 weeks of age, male offspring were treated with 4 weekly injections of urethane or saline. At 44 and 58 weeks of age, mice were sacrificed and broncho-alveolar lavage fluids were collected. Tumors were analyzed for number, volume, and histopathology (adenoma; carcinoma; metastasis). Additionally, lung cancer-related gene expression was assessed. At 44-week of age, mice exposed in utero to SHS and treated as adults with urethane (SU), exhibited significantly (p<0.05) higher number of large tumors, increases in tumor volume, percentages of lymphocytes and levels of oxidative stress in the lungs, compared to their respective air-urethane controls (AU). Compared to 44-week old mice at 58-week of age the SU treatment significantly increased the number of intrapulmonary metastases. SU cancer-dysregulated genes were part of functional clusters associated with non-small cell lung cancer (Cdkn2a, Egr, Rb1), tumor suppressors (Cadm1, Cdkn2a, Dicl1), and Ras signaling pathway (Rassfl1, Hgf, Vegfa). Furthermore, SU vs. AU showed 15 differentially expressed cancer-related genes. Among these, Mmp1a and Mmp9 were up-regulated, and 10 were down-regulated, including Dg2 and Sostd1, both tumor suppressor genes. Our data provide in vivo evidence that in utero SHS aggravates urethane-induced lung cancer by dysregulating the expression of genes related to tumor suppressors, Ras signaling pathway and extracellular matrix remodeling, which play key roles in cancer progression and metastasis.
Methyl acrylate (MA), ethyl acrylate (EA), n-butyl acrylate (BA), and 2-ethylhexyl acrylate (EHA) are well studied industrial materials by conventional toxicological methods. Credible available data from in vitro, in vivo, and in silico experiments reveal the similarities and differences in biological response as the alkyl moiety of these esters changes. The genotoxicity, carcinogenicity and new mechanistic data available for these compounds are summarized to illustrate use in hazard classification and in support of development of modes of action (MOA) or adverse outcome pathways (AOP) relevant for human health assessments. This analysis is used to demonstrate the applicability and limitations of these types of data for materials, such as these esters, whose chronic effects are considered primarily related to sustained irritation, with or without a genotoxic component. Irritation severity, dose related impact on metabolism (rates, preferred pathways, and resulting by-products), and derivation of the maximum tolerated dose (MTD) at target organ sites are used to support or discount the proposed mechanisms leading to the observed chronic outcomes in the various test animals or systems and to ascertain the appropriate framework for determination of the relevance for humans. The contribution of genotoxicity to a mechanism is dependent upon the ability of a material or a relevant metabolite to cause the generation of a DNA adduct or mutation at the target DNA site. This exercise illustrates both the utility of supportive data from a family of materials as well as the need for compound specific data when conditions cause an alteration in the primary homeostatic pathway to occur. Critical review of the support for proposed pathways helps determine if the existing toxicological data are adequate or may indicate where additional data could serve to improve the confidence in the assessment of the impact on human health.

Twelve Weeks Exposure to Dimethylnitrosamine (DMN) Induces Rare Forms of Spleen and Duodenal Cancers

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According to American Cancer Society (ACS), there will be an estimated 1,688,780 new cancer cases diagnosed and 600,920 cancer deaths in the US in 2017. Besides genetic etiology, other factors, such as environmental, food-borne and sedentary life style can precipitate causes of many cancers. Among cancer types, reports indicate that prostate, lung and colorectal dominate in men, whereas, breast, lung and colorectal dominate in women. Most cancers are categorized by the anatomic site and tissue of origin, although genetic information is frequently used to group cancers according to a tumor’s biological makeup. This results in the subdivision of some more common cancers into a collection of rarer cancers. Nearly 13% of all cancers are rare (1 out of 8) based on ACS’s definition (possible 208,000 new cases in 2017); commonest of the rare cancers of the digestive system is cancer of the small intestine, which is not well known. Here, we test this using epigenomic and transcriptomic alterations in a range of tissues. Whether Cd alters epigenetic reprogramming during human breast stem cell proliferation and differentiation is not well known. Here, we test this using epigenomic and transcriptomic profiling of Cd exposed patient-derived normal breast stem cells grown in three-dimensional cultures. We generated and analyzed two datasets, using ERRBS dsDNA methylation tools cultured in the presence of two physiologically relevant doses of cadmium chloride, 0.25 µM or 2.5 µM, or control. Cd exposed cells show transcriptomic alterations of cancer relevant pathways, including upregulation of genes involved in metal homeostasis and oxidative stress, and downregulation of genes involved in extracellular matrix formation and focal adhesion. Analysis of differentially methylated regions found enrichment for GO terms related to ion channel activity or metal transporter activity. There was also enrichment for differentially methylated genes related to cell migration or regulation of development. Many genes (141 genes with FDR < 0.1) are accompanied by changes in DNA methylation. For example MT1X, TXNRD1, EEF1A2, HMOX1 were upregulated in Cd treated cells and also hypo-methylated. PGBD3, APOD, LRP1, and COL6A1 were downregulated and hypermethylated. Some genes (MT1E, TKT, AKR1C3, AKR1C2) were both up and hypomethylated. These findings suggest alternative regulatory mechanisms for expression than gene promoter CpG methylation. These results show that Cd exposure in breast stem cells can induce epigenetic and transcriptional changes in genes and pathways associated with development and cancer progression.

Novel Non-Toxic Peptides for Cancer Treatment

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Cancer is a leading cause of death worldwide. Due to the limited therapeutic effect of both traditional and advanced anti-neoplastic agents there is a great need for safe and efficacious drugs for cancer. In the present study we propose a novel peptide, termed YH11, for treating solid tumors. Three types of highly metastatic tumor cells were used, D122 lung carcinoma, 4T1 mammary carcinoma and B16-F10 melanoma. The experimental systems included two procedures a) the local model - subcutaneous injection of tumor cells into the footpad or lateral thoracic-abdominal area of mice, b) the metastatic model - intravenous injection of tumor cells leading to lung metastasis after several weeks. YH11 demonstrated a marked anti-tumor effect at extremely low doses in both metastatic and local models using the three types of tumor cells. A significant reduction of 64% in tumor growth (local model) was observed in YH11-treated group (100ng/kg) inoculated with D122 lung carcinoma cells. A lower dose of 10ng/kg YH11 (onset of treatment - 7 days after cell inoculation) caused a pronounced effect in the metastatic model expressed by marked reduction in lung weight (88%), number of lung foci (67%) and histopathological parameters (67%). Similar results, at the same range of YH11 doses, were observed with 4T1 mammary carcinoma cells at the metastatic model. The peptide was also efficacious against B16-F10 melanoma cells at both metastatic and local models. Interestingly, YH11 exhibited its anti-tumor effect exclusively in in vivo systems but not in vitro. Namely, the peptide did not show anti-proliferative or cytotoxic effect in cell cultures. In order to elaborate the mechanism of action of YH11 we propose two hypotheses: a) Plasma of a healthy person contains a short half-life factor that protects the body against cancer cells. It serves as a sentinel that kills or inactivates tumor cells as they start to develop; b) YH11 increases levels of this plasma factor by blocking or slowing down its degradation resulting in augmentation of body response against tumor cells. LC/MS/MS analysis of human plasma incubated with YH11 resulted in the appearance of an additional peptide that showed marked anti-tumor activity in both in vitro cultured tumor cells and in vivo mouse models of metastatic mammary carcinoma and melanoma. Both this peptide and YH11 had no toxic effects in in vivo studies. In summary, the present study proposes two efficacious and safe candidates for cancer therapy.
Inorganic arsenic (iAs) is a ubiquitous environmental toxicant implicated in the induction of different diseases, including cancer. Chronic, low dose iAs exposure leads to changes in gene expression and epithelial-to-mesenchymal transformation. During this transformation, cells adopt a fibroblast-like phenotype accompanied by profound gene expression changes. While many mechanisms have been implicated in this transformation, studies that focus on the role of epigenetic alterations in this process are just emerging. Epigenetic regulation is a potential mechanism by which iAs causes cancer. To decipher this mechanism, we carried out high-resolution profiling of the epigenetic changes as cells go through iAs-mediated cellular transformation. We performed genome-wide analysis of both DNA methylation (MethyLMini-Seq) and hydroxymethylation (RRHP) patterns in iAs-transformed cells. Global DNA methylation was found to be globally the same; however the S-hydroxymethyl levels were globally increased. In both data sets there are changes at individual loci that lead to changes in gene expression seen in the iAs-induced transformed cells. Further analysis reveals that CTCF, a master transcription factor, shows differential occupancy, in the iAs-transformed cells, at strong and weak binding sites, controlling the expression of both TET and DNMT gene families. iAs disrupts CTCF binding through disrupting the eleventh zinc finger, which leads to lowered occupancy at weak binding sites, especially in the DNMT and the proximal TET promoters. However at strong binding sites, as in the distal TET promoters, binding is stronger, allowing this distal site to act as an enhancer, driving TET overexpression in the iAs-transformed cells. The differential binding drives differential expression, leading to the changes in global DNA modifications. These analyses provide the first step towards understanding the functional significance of epigenetic changes in iAs-mediated transformation. Additionally, they set a platform for the development of potential therapeutic agents in iAs-carcinogenesis.

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Structure-activity relationship (SAR) models for carcinogenesis typically use chemical descriptors and develop models based on chemicals that are classified as carcinogenic or non-carcinogenic. We report the application of an SAR approach using virtual biological descriptors (i.e., receptor interactions) for 12 tumor site rat carcinogen sets (i.e., clitoral gland, esophagus, hematopoietic system, kidney, large and small intestine, liver, lung, mammary gland, muscle, ovaries, skin, and uterus). Previous studies have demonstrated that similar receptor-based SAR models are capable of analyzing and predicting non-mutagenic carcinogens (i.e., through mechanisms other than DNA-reactivity). The first group, the Target-Site Carcinogen - Non-Carcinogen (TSC-NC) models were validated in a set of active chemicals that were not target site and inactive chemicals that were whole animal non-carcinogens. The second group, the Target Site Carcinogen - Non-Target Site Carcinogen (TSC-NTSC) was composed of active chemicals that again were carcinogenic to the target site but the inactive category was composed of carcinogens to any/all other sites except the target site. Furthermore, rather than traditional chemical descriptors, the novel SAR biological descriptors used herein were derived from the virtual screening for chemical-receptor interactions of carcinogens and non-carcinogens to a set of 9277 protein targets. Leave-one-out cross-validation of triplicate TSC models resulted in the models concordance value of 87% for the esophagus and 79% for the kidney. The TSC-NTSC triplicate models resulted in a range of concordance values for the small intestine model at 82% to the kidney model at 66%. Voting models, on the other hand, returned TSC-NC accuracy values between 95% for the esophagus and 79% for the kidney. The TSC NTSC voting results ranged between 93% for the small intestine and 75% for the liver. This new approach for organ-selective carcinogens, by contrasting carcinogenic agents between tumor sites and incorporating biologically-based descriptors (i.e., chemical-protein interactions) into the SAR models can enhance model predictivity, help bridge the gap between chemical exposure and carcinogenesis, and identify novel molecular targets for tumor site specific anticancer drug discovery.

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Structure-activity relationship (SAR) models for carcinogenesis typically use chemical descriptors and develop models based on agents that are classified as carcinogens or non-carcinogens. We report the application of a SAR approach using virtual biological descriptors (i.e., receptor interactions) for chemicals with anticancer activity. Previous studies have demonstrated that similar receptor-based SAR models are capable of analyzing and predicting non-mutagenic carcinogens (i.e., through mechanisms other than DNA-reactivity). The National Toxicology Program has tested nearly 600 chemicals. An important component of these long-term studies is data on agents that decrease the rate of spontaneous tumors in treated animals (i.e., anticarcinogenesis). Based on determination by the NTP, 96 chemicals were identified for structure-activity relationship modeling with anticancer activity to the rat mammary gland. For these analyses, a traditional fragment-based SAR approach was used along with new virtual receptor interaction models. The receptor models used a technique for developing SAR model descriptors based on virtual ligand binding affinity for agents to agonize receptors. Based on the NTP compounds that interacted with anticancer activity, three sets of models were developed by varying the makeup of the inactive category. These were mammary anticancerogens with non-carcinogens, mammary carcinogens, and non-mammary carcinogens. Averaged leave-one-out validations resulted in correct rate values between predicted in vitro activity and the 96 chemicals that were tested against the respective receptors. Based on these models, we hypothesize that estrogen and estrogen receptors (ERs) which negatively affect ER-mediated transcription. This pathway to abrogate sustained AR-mediated signaling in CRPC.

2072 Capsaicin Negatively Regulates Warburg Effect in Pancreatic Cancer and Enhances the Effect of Gemcitabine

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Tumor cell metabolism is considered to be a hallmark of tumorigenesis progression. Pancreatic cancer cells are shown to develop addiction towards the metabolic pathways by glycolysis and mitochondrial respiration to meet energy demands. Pancreatic tumor cells predominantly utilize cytosolic aerobic glycolysis for energy production (Warburg effect). Our results indicated that the extra-cellular acidification rate (ECAR), an indicator of glycolysis was significantly up-regulated in pancreatic cancer cells BxPC3, AsPC1, MiaPACA2 and P202 when compared to normal human pancreatic ductal epithelial (HPDE) cells. In this study, we have shown that capsaicin treatment inhibits the survival of AsPC-1, BxPC-3 and MiaPACA2 pancreatic cancer cell lines. We evaluated the effects of capsaicin on regulating glycolysis in pancreatic cancer cells. Capsaicin treatment reduced the extra-cellular acidification rate (glycolysis) in AsPC-1 and BxPC-3 cells in a concentration-dependent manner after 24h of treatment. Capsaicin treatment caused 86% and 55% down-regulation of glycolysis process in AsPC-1 and BxPC-3 cells respectively. Treatment of AsPC-1, BxPC-3 and MiaPACA2 with capsaicin for 24h inhibited the expression of glycolytic enzymes LDH-A, Enolase-1, Aldolase A and its upstream regulators such as HIF-1α, HIF-2α, HIF-1α and HIF-1α as evaluated by Western blot. Capsaicin treatment suppressed the activity of LDH-A in BxPC3 cells after 24h of treatment as evaluated by LDH-A enzymatic activity assay. Furthermore, treatment of AsPC-1 and BxPC3 cells with capsaicin in combination with gemcitabine displayed synergistic effects when compared to gemcitabine treatment alone in a cytotoxicity assay and western blot. Our study thus indicates that capsaicin inhibits pancreatic tumor growth by suppressing glycolysis and potentiates the effects of gemcitabine. Further mechanistic studies are in progress. Supported in part by R01 grant CA129038, awarded to (S.K.S.) by the National Cancer Institute.

2073 Riluzole Induces DNA Damage in GRM1+ Human Melanoma Cells through the Accumulation of Reactive Oxygen Species

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Riluzole Induces DNA Damage in GRM1+ Melanoma Cells through the Accumulation of Reactive Oxygen Species. Our group described the oncogenic activity of a normal neuronal receptor, metabotropic glutamate receptor 1 (GRM1), when aberrantly expressed in melanocytes, the pigment producing cells. Deregulated melanocytic cell proliferation gives rise to transformed melanocytes and progressive aggressive melanoma, the most aggressive form of skin cancer. The natural ligand of GRM1 is glutamate and it is well known that all cells, particularly cancer cells, depend on glutamate/glutamate for growth. We showed that GRM1 expressing cells establish an autocrine loop regulates in the extracellular space to constitute enzymatic activation of GRM1 and cell growth. Treatment of these cells with pharmacological reagents by reducing GRM1 expression by silencing RNA led to cell cycle arrest at the G2/M phase after 24 hours and most of the cells then enter the apoptotic G0 phase. One of the pharmacological reagents we have used is riluzole, which is FDA approved for the treatment of amyotrophic lateral sclerosis (ALS). One of the functions of riluzole is inhibition of glutamate release, which allows the drug to functionally act as an antagonist to GRM1. Riluzole treated melanoma cells arrested at the G2/M phase of the cell cycle is indicative of DNA damage. Examination of GRM1 treated melanoma cells revealed enhanced phosphorylated histone H2AX (pH2AX) and p53-binding protein (53BP1) levels, two protein markers for DNA double stranded-breaks (DSBs). Furthermore, elevated Reactive Oxygen Species (ROS) levels were also detected in riluzole-treated melanoma cells. Currently we are assessing if inclusion of an ROS scavenger such as N-Acetylcysteine (NAC) could alleviate and reduce ROS levels. We are also examining protein markers of single-stranded DNA breaks such as

2070 Fragment- and Receptor-Based Structure-Activity Relationship Models for Rat Mammary Anticarcinogens

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Structure-activity relationship (SAR) models for carcinogenesis typically use chemical descriptors and develop models based on agents that are classified as carcinogens or non-carcinogens. We report the application of a SAR approach using virtual biological descriptors (i.e., receptor interactions) for chemicals with anticancer activity. Previous studies have demonstrated that similar receptor-based SAR models are capable of analyzing and predicting non-mutagenic carcinogens (i.e., through mechanisms other than DNA-reactivity). The National Toxicology Program has tested nearly 600 chemicals. An important component of these long-term studies is data on agents that decrease the rate of spontaneous tumors in treated animals (i.e., anticarcinogenesis). Based on determination by the NTP, 96 chemicals were identified for structure-activity relationship modeling with anticancer activity to the rat mammary gland. For these analyses, a traditional fragment-based SAR approach was used along with new virtual receptor interaction models. The receptor models used a technique for developing SAR model descriptors based on virtual ligand binding affinity for agents to agonize receptors. Based on the NTP compounds that interacted with anticancer activity, three sets of models were developed by varying the makeup of the inactive category. These were mammary anticancerogens with non-carcinogens, mammary carcinogens, and non-mammary carcinogens. Averaged leave-one-out validations resulted in correct rate values between predicted in vitro activity and the 96 chemicals that were tested against the respective receptors. Based on these models, we hypothesize that estrogen and estrogen receptors (ERs) which negatively affect ER-mediated transcription. This pathway to abrogate sustained AR-mediated signaling in CRPC.

2071 Role of Aryl Hydrocarbon Receptor (AhR) and Estrogen Receptor Beta (ERβ) Crosstalk in Benzo(a)pyrene-Induced Colon Cancer

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Colorectal cancer (CRC) is the third leading cause of cancer-related deaths in the United States. Epidemiological evidence show estrogen might influence the incidence of CRC in women by acting in a protective role via estrogen receptor beta (ERβ) but the underlying mechanism is not known. Benzo(a)pyrene (BaP), a well-characterized toxicant has been proven to be one of the contributors to the development of sporadic colon cancer. Literature provides evidence of crosstalk between AhR and ERβ. In our study, we used CRC canine cell line AsPC-1 and BxPC3 cells expressing GRM1 as our model system. We showed increase in CYP181, SULT1A1 and GST when compared to males in various treatment groups. It is possible that the increase in Phase II enzymes may provide clearance of BaP, preventing polyp development in female PIRC rats. In future studies, by measuring the expression of other drug metabolizing enzymes (DME), along with measuring circulating estrogen levels, analyzing [BaP] metabolite and BaP-DNA adduct profiles, we will provide insight into how estrogen can protect females from developing CRC. Funded by NIH grants SR25GM059944-3, G12MD007586-29, 5SU54CA163069-04 and SROI CA142845-04.
phosphorylated Replication Protein A (pRPA) plus alkaline and neutral conditioned Comet assays to further elucidate the mechanism and types of DNA damage elicited by Riluzole. The brain is a common metastatic site of melanoma and riluzole is known to cross the blood-brain barrier, it is possible that riluzole could be used as an enhancer for γ-irradiation, a common approach to treat brain metastases.

2074 Development of a Novel RON-Targeted Antibody-Drug Conjugates Using Cysteine Bridging Technology for Potential Treatment of Pancreatic Cancer

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Therapeutics targeting known oncoproteins have been applied for pancreatic cancer treatment but clinical outcomes are not promising. Hence, there is an urgent need to identify novel targets and develop effective drugs to improve pancreatic cancer therapeutic index. Antibody-drug conjugates (ADC) represent a promising class of drugs for targeted cancer therapy. Here we developed a novel ADC targeting RON receptor tyrosine kinase for potential pancreatic cancer treatment. To this end, we have synthesized a bis-alkylating linker (BL), attached to a lysosomal protease-cleavable dipeptide with payload Monomethyl auristatin E (MMAE). The BL-MMAE was then conjugated to Zt/g4 (anti-RON mAb) through cysteine bridging technology to produce Zt/g4-BL-MMAE with a relatively homogeneous conjugation profile and an antibody to drug ratio of 1:4. Zt/g4-BL-MMAE showed significant improvement in drug conjugation homogeneity and serum stability over conventional ADCs prepared through maleimide-based linkers. In pancreatic cancer cell lines overexpressing RON, Zt/g4-BL-MMAE specifically targeted RON-expressing tumor cells and was effective in rapid induction of cell surface RON endocytosis. Functional analysis revealed that Zt/g4-BL-MMAE caused cell cycle arrest at G2/M phase, reduction of cell viability and subsequently resulted in massive cell death. The calculated IC50 is in the range of 1 to 2 µg/ml. We conclude that Zt/g4-BL-MMAE is a novel anti-RON ADC with excellent conjugation profile, serum stability, and selective cytotoxicity for pancreatic cancer cells. This work provides a pharmaceutical opportunity for evaluating potentials of RON-targeted ADCs in pancreatic cancer treatment in the future.

2075 Open Access Gradient-on-a-Chip to Study the Role of Environmental Factors in Breast Cancer Risk

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The common metabolic consequence of exposure to several environmental risk factors (diet, cigarette smoke, alcohol consumption, exposure to heavy metals and toxicants) associated with cancer oxidative stress (OS) created by an imbalance between reactive oxygen species (ROS) generated and cleared from the body. It is known that OS changes nuclear morphology and chromatin structure by stimulating global heterochromatin loss and induces H3K9me2 overexpression that suppresses gene expression. Yet, the nuclear mechanisms by which OS promotes carcinogenesis (a phenomenon greatly linked to modifications in gene transcription) remain to be understood. We have identified that the nuclear mitotic apparatus protein (NuMA), involved in chromatid organization and DNA repair, processes altered in cancer, interacts with a stress related transcriptional activator, lens epithelium derived growth factor (LEDGF) that is known to protect against OS, suppressed by H3K9me2 and is altered differentially in non-neoplastic cells versus preneoplastic triple negative breast cancer cells. Our central hypothesis is that a sustained gradient of OS influences NuMA-LEDGF expression, thus altering transcriptional regulation and triggering carcinogenesis. To test our hypothesis we used a 3D cell culture model of polarized mammary epithelium formed by human mammary non-neoplastic epithelial HMT-5322 S1 cells and exposed to acute OS by treating cells in 3D culture with 250 µM H2O2 for four hours that lead to a decreased expression of NuMA and yet this protein’s interaction with LEDGF was further strengthened. Using a gradient-on-a-chip that facilitates the continuous flow of a gradient of H2O2, as seen in vivo, in microfluidic channels diffused into 3D cultures of cells, we have shown that in preneoplastic triple negative breast cancer (TNBC) like S2 cells, nuclear morphology and cell response such as oxidative DNA damage to ROS are dose dependent.

To further investigate the effect of chronic OS on breast epithelium and carcinogenesis in association with NuMA-LEDGF pathway and an epigenetic impact, we plan to further modify our model to a risk-on-a-chip system to allow long term exposure of different microenvironmental factors such as ROS, and stromal factors to the normal tissue and study the synergism among these factors and related nuclear events that lead to cancer.

2076 Evaluation of T-Cell Receptor Codon Usage Reveals Immature Thymic Phenotype in ROYT Knockout Mice


ROYT, a sequence variant of ROY expressed exclusively in the thymus, is a nuclear hormone receptor and transcription factor that functions in thymocyte development and maturation. ROYT-/-mice develop thymic lymphoma with high penetrance and short latency, but the mechanisms underlying these changes are not well understood. In the present study, T-cell receptor alpha variable (TCRα) rearrangement was evaluated in constitutive (ROYT-/-) and inducible ROY KO mice by quantitative PCR analysis. In ROYT-/-mice, TCRα V regions were used at low frequency at the 5’ end while codon usage at the 3’ end increased in frequency by up to 3.88-fold in the thymus relative to wildtype (WT) littermates, indicative of a less mature genotype for TCRα. PCR analysis of a panel of thymic genes involved in ROYT-mediated lymphocyte development or implicated in tumorigenesis revealed marked changes in expression in ROYT-/-mice relative to WT littermates. These included decreases in Bcl-XL (0.03-0.10x WT), Rag1 (0.02-0.28x WT), Nab1 (0.04-0.12x WT), and Pten (0.04-0.43x WT), and an increase in Gzma (3.08-6.12x WT) in ROYT-/- mice, little to no change in expression of these genes was observed. These results indicate that the mouse thymus, lack of ROY function alters T-cell receptor codon usage and alters the normal balance of apoptosis and proliferation required for thymocyte differentiation. Additional studies are ongoing to determine whether these early changes are causally related to the development of thymic lymphomas.

2077 Biosynthesis of Estrogens in Mesothelioma Cancer Cells and Effect on Cell Growth


Malignant mesothelioma (MM) is a highly aggressive cancer with only about 10% patients surviving five years, but this period can be shorter depending on gender and subtype. The disease is associated with occupational and environmental exposure to asbestos, however, a higher prevalence of MM is seen in women due to environmental exposure. Inverse correlations between the levels of estradiol (E2), the most potent estrogenic hormone in women, and mesothelioma cancer patient survival post diagnosis suggests that decreased E2 levels inhibits mesothelioma cancer growth. Here we investigated whether MM cells could conduct denovo synthesis of the estrogenic metabolites estrone (E1) and E2 via the aromatase pathway using 4-androstene-3,17-dione (4-Adione) and testosterone (T) as precursors or via the sulfatase and 17beta-hydroxysteroid dehydrogenase pathway using E2 sulfate (E2S) as substrate. We also determined whether the estrogenic metabolite 5-androstene-3b,17b-diol (5-Adiol) could be formed from DHEA. All steroid measurements were made using stable isotope dilution liquid chromatography tandem mass spectrometry (LC-MS/MS). These metabolites were also tested to see if they affect MM cell growth. Our results showed that in M500-211H MM cells, E1 and E2S are converted to E2; by contrast the aromatase substrates 4-Adione and T were not converted to E1 or E2. In REN cells, only the conversion of E2S to E2 was observed. These data support the dominance of the sulfatase pathway over the aromatase pathway for estrogen synthesis in MM. We also showed that 5-Adiol is formed from the precursor DHEA in M500-211H cells. Our preliminary data showed that T and E2 modulated MM cell growth. This is the first documentation that MM can form estrogenic metabolites from precursors. This study will not only improve our understanding of the roles of estrogens and androgens in mesothelioma, but will also determine whether inhibiting steroid synthesis or using adjuvant hormonal therapy has a role in the treatment of mesothelioma.
The 2-year rodent carcinogenicity assays involving conventional rats and mice have been conducted for over 3 decades. As an alternative to the 2-year rodent carcinogenicity bioassays, 26-week short term carcinogenicity bioassays were approved using transgenic mouse strains, including Tg.rasH2. The Tg.rasH2 model, which can be used for both genotoxic and non-genotoxic compounds, has gained popularity and its use has increased over the years. Currently, more than 75% of all mouse carcinogenicity studies are being conducted in Tg.rasH2 mice. The Tg.rasH2 model predicts neoplastic findings relevant to human cancer risk assessment, produces fewer non-biologically significant neoplastic outcomes, and is thus often preferable to a 2-year rodent study. We published the largest historical control database for both neoplastic and non-neoplastic lesions in Tg.rasH2 mice. Our current historical control data base has grown sufficiently that we can now begin to look at the effects of vehicles or vehicle components on both non-neoplastic and neoplastic findings. We recently evaluated the incidence of tumors in male and female mice in 26-week Tg.rasH2 oral gavage carcinogenicity studies with methylcellulose as one of the ingredients of the vehicle versus various non-cellulose vehicles. Review of the data indicated that the total incidence of tumors in 475 mice administered with methylcellulose and 585 mice administered with non-methylcellulose vehicles was similar. Our analysis showed that the total incidence of tumors was 20.84% and 25.68% in males and females, respectively, with methylcellulose administered mice. The remainder of the historical control data base had a total tumor incidence of 24.1% and 21.37% in males and females, respectively and was considered similar to the studies where methylcellulose was a component of the vehicle. Additional comparisons by tumor location (lung, spleen and Harderian gland) is ongoing.

Arsenic is a naturally occurring and highly potent metalloid known to elicit serious public health concerns. Today, approximately 200 million people around the globe are exposed to arsenic-contaminated drinking water, and it is thus often preferable to a 2-year rodent study. We published the largest historical control database for both neoplastic and non-neoplastic lesions in Tg.rasH2 mice. Our current historical control data base has grown sufficiently that we can now begin to look at the effects of vehicles or vehicle components on both non-neoplastic and neoplastic findings. We recently evaluated the incidence of tumors in male and female mice in 26-week Tg.rasH2 oral gavage carcinogenicity studies with methylcellulose as one of the ingredients of the vehicle versus various non-cellulose vehicles. Review of the data indicated that the total incidence of tumors in 475 mice administered with methylcellulose and 585 mice administered with non-methylcellulose vehicles was similar. Our analysis showed that the total incidence of tumors was 20.84% and 25.68% in males and females, respectively, with methylcellulose administered mice. The remainder of the historical control data base had a total tumor incidence of 24.1% and 21.37% in males and females, respectively and was considered similar to the studies where methylcellulose was a component of the vehicle. Additional comparisons by tumor location (lung, spleen and Harderian gland) is ongoing.

DCE is a chlorinated hydrocarbon used as a chemical intermediate including in the synthesis of vinyl chloride which is used to make polyvinyl chloride. While DCE has induced tumors in both rats and mice, the overall weight of evidence for genotoxicity suggests that DCE is a non-genotoxic carcinogen. The present study was conducted to further assess the in vivo genotoxicity of DCE and explore a potential mode of action for tumor formation in rat mammary tissue. Fischer 344 rats were exposed to target concentrations of 0 or 200 ppm of DCE vapors (six hours/day, seven days/week) for 28 days. This concentration of DCE is approximately 20% higher than that reported to induce mammary tumors in rats. Endpoints examined included DNA damage (via Comet assay), GHS/GSSG levels, tissue adduct levels, cell proliferation and serum prolactin levels. One MoA hypothesis was that alterations in serum prolactin and cell proliferation were potential non-genotoxic key events leading to tumor formation. Exposure to DCE did not have an effect on serum prolactin levels or cell proliferation in mammary epithelial cells. DNA adducts were identified including the N7-guanylyl glutathione (GEG) cross-link adduct which was considered as a biomarker of exposure with higher adduct levels measured in the non-target tissue compared to mammary tissue isolated from the same rats. The results of the comet assay in mammary epithelial cells indicated that DCE did not result in increased DNA damage while the positive control N-Nitroso-N-methylurea resulted in a statistically significant increase relative to controls. While the results of this study do not identify a mechanism for DCE-induced mammary tumors in rats, the lack of any exposure-related genotoxic effects in the Comet assay or relevant target-tissue-specific DNA adducts further contributes to the weight of evidence for DCE as a non-genotoxic carcinogen.

Humans respond to an array of chemicals every day, including those that are endogenously produced like stress-induced hormones. However, the potential toxicological impacts of mixtures consisting of stress hormones and chemical exposures are rarely explored. To further investigate a mixture of induced stress and organophosphates, adult male C57BL/6J mice were exposed to corticosterone (Cort; 200μg/ml) in the drinking water for 7 days, and on the 8th day, were given a single intraperitoneal injection of diisopropyl fluorophosphate (DFP, a sarin surrogate; 400mg/kg). Mice were euthanized 30 minutes, 2 hours, and 24 hours post-exposure via a focused microwave radiation. To fully understand the effects of DFP and DFP+Cort on the brain, post-translationally modified proteins were isolated from the mice using a tris-EDTA buffered ELISA for the cortex. To optimize analysis of the specific data sets, a network parameter approach corresponding to radiality was used to assess the response of the phosphoprotein targets in relation to all other responses. This approach identified proteins that were substantially activated or inhibited within this network, and is informative with regard to the mechanisms of interactions that are occurring as a result of this mixture exposure.
**2082 Disruption of Thyroid Hormones by PBDE Mixtures: Potential for Co-Exposure Bias**

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PentaBDEs are ubiquitously found in human serum with highest average levels found in North America. Toxicological research in rodents generally shows that increased exposure leads to decreased thyroid hormone (TT4). Epidemiologic studies have been more inconsistent; one difficulty has been that PentaBDEs are typically highly correlated in human serum (r=0.4-0.97) and studies analyze one congener at a time. This can lead to confounding by co-exposure if only a subset of the mixtures is active; collinearity can make standard multiple linear regression on several exposures problematic. The current research therefore uses a recent mixtures statistical approach and discusses a newly discovered potential issue. Serum PBDEs and thyroid hormones were measured in a longitudinal cohort of Boston office workers sampled in 2010-2011; single congener results were previously published. Here, all major congeners were examined simultaneously using weighted quantile sum regression. For every quartile increase in weighted PBDEs, TT4 decreased by 0.4 µg/dL. The highest weighted congener was BDE-99 followed by BDE-47 and BDE-153. A newly discovered potential issue with mixtures study is co-exposure amplification bias; this problem is most stark when correlations between exposures are high and there is uncontrolled confounding for one or a subset of exposures. In multiple linear regression, this can lead to amplification of bias for some estimated coefficients and sign reversal for others. Uncontrolled confounding by physiology is a potential issue for studies of serum PBDEs and thyroid hormones. However, we showed that weighted quantile sum regression is not subject to co-exposure amplification bias. Further investigation of causal mechanisms vs. confounding for PBDE-TT4 associations are needed.

**2083 Binding Ratio of Highly-Bound Ligands to Albumin and Alpha-1-Acid Glycoprotein**

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Plasma protein binding is a key factor studied in pharmacokinetics/toxicokinetics of drugs. Many xenobiotics could extensively bind to two important plasma proteins, albumin (ALB) and alpha-1-acid glycoprotein (AGP). For the accurate determination of exposure, Perapamelan, a cell line, was used as a non-competitive antagonist of AMPA receptors is one of these xenobiotics. The main objective of this study is to determine for highly bound drugs if binding parameters (Bmax, KD and Cfree) to both proteins could be used to predict the binding ratio (RAGP/ALB). Thus, in vitro binding tests are made using rapid equilibrium dialysis. The test concentrations for PerapameLAN are 2.5, 7.5, 15, 20, 25, 35 µM. Four binding scenarios are suggested: 1) bovine ALB (40g/L), 2) bovine AGP (1g/L), 3) serum (Ser); containing the same concentrations of both plasma proteins injected in the same test chamber, and 4) with both proteins; each protein is injected in a different chamber of the insert. The Bmax values for are 84.3 µM, 57.52 µM and 7.60 µM with Ser, ALB and AGP, respectively. The KD values are 8.54 µM, 6.74 µM and 7.25 µM, respectively. Data from scenario 1 and 2 can accurately predict RAGP/ALB when Cfree < Bmax (AGP, 7.60 µM). Data from scenario 3 (Cfree) and 4 (Total) can accurately predict the bound concentrations of PerapameLAN to each of the proteins in serum. These preliminary binding kinetics (Bmax, KD and RAGP/ALB) would eventually guide us in suggesting and validating an in vitro-in vivo extrapolation model for hepatic clearance of highly bound ligands to both plasma proteins.

**2084 Effects of Low to Moderate Levels of Deoxynivalenol and Its Metabolites Combinations in HepG2**


The presence of mycotoxins in food matrix is not related with the presence of only one fungi strain in the matrix of analysis. The relation of mycotoxin/fungi strain is one-to-one, since one fungi can produce more than one mycotoxin. Fusarium mycotoxins (Fusarium moniliforme) after co-exposure to Fusarium deoxynivalenol (DON) and 3-acetyl-Deoxynivalenol (3-ADON) and 15-Acetyl-Deoxynivalenol (15-ADON). Both occur in significant amounts jointly deoxynivalenol (DON), in various cereal crops and processed grains. Mycotoxins' cytotoxic potential has been reported in several directions as generation of reactive oxygen species, lipid peroxidation, DNA damage and apoptosis-necrosis. The objectives of this study were to compare the cytotoxicity of DON, 3-ADON and 15-ADON alone or in combination in human hepatoma (HepG2) cells using the NR assay after 24 h of exposure; to evaluate the interactions of mycotoxins mixtures by the isobologram analysis and to compare the in vitro genotoxic effect by induction of micronucleus (MN) by flow cytometry of these mycotoxins alone and in combination. The HepG2 cells were exposed to DON, 3-ADON, 15-ADON combinations at concentrations from 0.6 to 4.5 µM DON, and from 0.1 to 1.5 µM for 3-ADON and 15-ADON. The results revealed that the IC50 values obtained for individual mycotoxins range from 4.3±0.4 to 10.2±0.4 µM, from 1.9±0.4 to 3.9±1.3 µM and from 2.0±0.3 to 6.0±1.0 µM for DON, 3-ADON and 15-ADON. Isobologram analysis provides a combination index (CI) value to determine the type of interaction that occurs. The interactions of DON, 3-ADON and 15-ADON became slightly synergism at low concentrations tested in the combinations (CI from 0.01±0.01 to 0.42±0.18) and turned into additive effect at higher concentrations (CI from 1.15±0.22 to 19.30±0.20). Moreover, all mycotoxins produce MN induction. MN test represents damage. Triple and the binary combination 3-ADON+15-ADON reached the highest MN score. In conclusion, mycotoxins combinations have higher cytotoxic effect than mycotoxins alone tested. Therefore, more assays on combinatorial effects are needed to understand the realistic exposure scenario of consumers. This work was supported by the Spanish Ministry of Economy and Competitiveness (AGL2016-77610-R).

**2085 Variations in Histopathological Responses within the Liver and Spleen to Various Acute Crude Oil Exposures**

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Observed toxic effects from crude oil exposure can vary based upon their constituent composition. We have determined values for several toxic effects of acute exposure to the following crude oils: LA Sweet, Nigerian ‘Qua Iboe’, Iraqi ‘Basra’, Venezuelan Merey and Leona, Ecuadorian Oriente, and Colombian Vasconia (all from ONTA, Inc., Toronto), and Deepwater Horizon (DWH) samples A0083Q “Mass Aug 15” and A001EP/EQ “SOB MAY 22” plus an oil mix prepared to mimic the composition of the DWH samples A010G4 “Surrogate” (AEOM, Inc., Fort Collins, CO). Female Sprague Dawley rats were treated with 2 daily doses of 0, 2.5, and 5 mL/kg, (p.o.) and whole blood and serum were collected 48 hours later for hematology and clinical chemistry analyses. Liver and spleen were also obtained 48 hour later upon necropsy, weighed and then immediately fixed in formalin. Rats treated with all crude oils had elevated relative liver weights and alkaline phosphatase, and decreased relative spleen weights. For histopathology, 5 µm sections were cut from formalin-fixed, paraffin-embedded livers and spleens, and stained with H and E. Hepatocytes showed treatment-related differences: centrilobular hypertrophy, vacuolization, sinusoidal disruption, and periportal necrosis. Within the periarterial lymphatic sheaths (PALS) of spleens were observed differing numbers of apoptotic lymphocytes following exposure to different crude oils in treated rats when compared to control. Spleens with crude oil induced-lymphoid apoptosis display an increase in macrophage infiltration giving the classical “starry night” pattern within the PALS. These apoptotic lymphocytes were observed with degeneration and necrosis, and seen being phagocytized by macrophages. These data suggest that variations of occurrence and severity in the observed toxic effects within the liver and spleens of acutely treated animals reflect the different composition of the crude oils.

**2086 Anti-BPDE-DNA Adducts after Exposure to Benzo[a]pyrene Are Increased in the Presence of Aromatic Amines in RT4 Cells**

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Exposures to both, aromatic amines and polycyclic aromatic hydrocarbons are associated with increased risk of bladder cancer. However, only little information is available on the cellular and molecular effects of co-exposures on the urothelium. The aim of the present study was to investigate the activity and induction of cytotoxicity P450 1A1 (CYP1A1) and the formation of specific DNA adducts of benzo[a]pyrene (BaP) in human bladder cell lines RT4 bladder papilloma cells to BaP and various concentrations of 2-naphthylamine (2-NA) and 4-amino-benzene.
phenyl (4-ABP). For this purpose, the cells were co-incubated for 24 h with 1 µM B[a]P + 10-100 µM 4-ABP and 1 µM B[a]P + 10-100 µM 2-Na. Compared to 1 µM B[a]P alone, co-treatment with 2-Na resulted in enhanced CYP1A1 activities (luminescent assay) and induction (immuno-noblots), whereas decreased activities and induction were observed for co-incubation with 4-ABP. In contrast, increased anti-BPDE-DNA adduct rates were observed for both co-exposures of B[a]P + 2-Na and B[a]P + 4-ABP and compared to 1 µM B[a]P alone. The effect was even more pronounced for co-exposures with 4-ABP than for 2-Na. We repeated the experiments in the presence of a specific arylhydrocarbon receptor (AhR) antagonist. Although, as expected, CYP1A1 activities and protein were no longer measurable, anti-BPDE-DNA adducts and adducts were still detectable. In case of co-exposures of B[a]P and 2-Na, the observed anti-BPDE adduct rates were even higher than in the absence of the AhR antagonist. Our results suggest that additional enzymes are responsible for the enhanced formation of anti-BPDE adducts in the presence of 2-Na and 4-ABP rather than CYP1A1 alone. Since the highest adduct rates were observed when CYP1A1 activities were low (4-ABP without AhR inhibitor) or completely inhibited (2-Na with AhR inhibitor), CYP1A1 appears to even have some detoxifying properties in the used cell model.

**2087** Coupling In Vitro and Computational Methods to Investigate the Transport of Herbicide/Insecticide Mixtures into Saliva

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Portable sensors and PBPK models have previously been developed to describe the relationship between salivary and plasma concentrations of commonly applied herbicide and insecticide metabolites, 2,4-Dichlorophenoxyacetic acid (2,4-D) and 3,5,6-trichloro-2-pyridinol (TCPy). These efforts not only offer a better understanding of potential adverse health effects elicited by common household chemicals but further provide justification for biomonitoring saliva to assess risks associated with occupational exposures. While understanding the pharmacokinetics of a single chemical is necessary, chemical mixtures are more reflective of the reality of everyday exposures. An in vitro system was employed to evaluate the chemical transport of a simultaneous dosing of 2,4-D and TCPy across a monolayer of serous-acinar cells cultured from the submaxillary saliva glands of Sprague-Dawley rats. The transport of chemicals across the cell layer (via passive diffusion) was influenced by the binding of proteins (i.e. unbound chemicals were able to pass through the cell layer). At a constant concentration of TCPy (200 µM), levels of protein bound to TCPy in cell culture media (Advanced DMEM:F12 supplemented with 2% fetal bovine serum) decreased much as 52% with an increasing co-exposure to 2,4-D (0-2300 µM), indicating that these chemicals compete for common binding sites on proteins. This suggests that exposure to chemical mixtures may influence measureable levels of herbicides/insecticides in saliva samples. Additional protein binding (at physiological conditions) and kinetic-cellular transport experiments quantifying chemical transport and binding rates were conducted to further explore implications of chemical mixture transport. Supported by Centers for Disease Control-National Institute for Occupational Safety and Health (CDC-NIOSH) grant R01 OH011023.

**2087a** Combined Cytotoxic Effects of Lead (Pb) and Disinfection Byproducts (DBPs) on Chinese Hamster Ovary (CHO) Cells and Heterogeneous Human Epithelial Colorectal Adenocarcinoma (Caco-2) Cells

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Disinfection aimed at inactivating pathogens is an essential process in drinking water treatment plants. Chlorine and chloramine are common disinfectants used in this process today. During disinfection, halogenated disinfection byproducts (DBPs) can be generated from the reactions of chlorine with natural organic matter (NOM) and bromide ions in surface water. As halogenated DBPs have been shown to induce adverse effects on cells in culture and rodent models. A certain amount of disinfectant residue is maintained in the water distribution system to prevent the regrowth of microorganisms. Lead (Pb)-containing plumbing materials are widely present in water distribution pipelines in the U.S. Disinfectant residues can react with the pipe materials and enable the release of lead (in the form of Pb2+) into water. Pb2+ is an abundant toxic metal ion that primarily affects the peripheral and central nervous systems, kidneys, red blood cells, and calcium metabolism after ingestion. In this study, we measured the toxicity of lead (Pb2+) and halogenated DBPs on Chinese hamster ovary (CHO) cells and heterogeneous human epithelial colorectal adenocarcinoma (Caco-2) cells separately as well as the synergistic toxic effects of Pb2+ and DBPs co-exposures. Data was collected over a dose-response and time-response experimental design and compared to series of controls. The results showed the combined toxic effect of lead and each DBP on the cell cultures were both dose and time dependent. Our data suggest that lead (in the form of Pb2+) affects the formation of DBPs and the overall quality of drinking water.

**2078b** Report of the 2017 Society of Environmental Toxicology and Chemistry (SETAC) Conference on Risk Assessment of Chemical Mixtures

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Exposure to complex mixtures, sequentially or simultaneously, has the inherent potential to impact our health and that of the environment. Presence does not mean risk, hence assessments are conducted to evaluate the degree of risk(s) and when necessary mitigate exposures. SETAC recently held a Focused Topic Meeting on mixtures to review the current state of the science for conducting and interpreting the risk of chemical mixtures using a “One Health” approach. International experts invited from Asia, Europe, and the United States presented their perspectives and opinions on various facets of the risk assessment paradigm for mixtures of pharmaceuticals, pesticides, industrial organic pollutants, and metals. Examples and case studies from the conference included reanalysis of the dose and adversity assessment, incidence of non-additive interactions, approaches for assessing undefined mixtures, use of adverse outcome pathway (AOP), and priority mixtures warranting specific attention. A general conclusion was that mixtures matter, and the dose addition approach was considered to be generally applicable for mixture toxicity assessments using a variety of human and ecological life cycle assays. Currently available models provide the necessary degree of flexibility, including the use of in vitro data as research continues to fill data gaps. However, additional empirical data are needed to prudently regulate mixtures in environmental as well as human health contexts. These findings and conclusions in this presentation have not been formally disseminated by the Centers for Disease Control and Prevention/the Agency for Toxic Substances and Disease Registry and should not be construed to represent any agency determination or policy.

**2088** Computational Association of Chronic Phosphate Consumption, Exafk Consumption, and Celiac Disease in the Saharawi People of Western Sahara


Celiac disease (CD) is a long-term autoimmune disease affecting 1% of people globally; whereas, the Saharawi people of Western Sahara have an unusually high incidence of 5.8%. The Saharawi people possess HLA-DQ2 isoforms making them genetically predisposed to CD. A change in dietary habits over the last few decades may also be responsible for the Saharawi’s high incidence of celiac disease. Prior to the introduction of the European diet, the Saharawi subsisted on mainly camel meat and milk. While it is possible that the increase consumption of gluten-heavy foods may play a role in elevated disease incidence, other populations have a much lower prevalence of CD with similar diet consumption and HLA-DQ2 frequencies. The aim of this study was to determine whether there is an additional environmental component that increases the susceptibility of the Saharawi. The Saharawi live on 75% of the world’s phosphate rock and consume a local plant Askaf in large amounts, primarily in camel milk. A systems approach was used to determine chemical–gene interactions for phosphate and relevant phytochemicals in Askaf. Research shows that phosphate can stimulate Akt signaling, leading to increased downstream T-cell activation, B-cell activation and antibody production, resulting in greater CD pathology. In addition, flavonoids in Askaf can increase the expression of CTLA-4, an inhibitory receptor on regulatory T-cells, thereby suppressing the immune system. Saponins in Askaf can decrease Akt signaling, thereby decreasing T-cell activation and B-cell antibody production. From these findings, it was concluded that chronic phosphate exposure may contribute to a high incidence of CD in the Saharawi, and the decreased
The conjugated linoleic acid (CLA) isomers, trans-10, cis-12 (CLA10c12) and cis-9, trans-11 (CLA9t11), have opposite effects on early collagen-induced arthritis (CA); however, initial studies comparing these two isomers had major differences in design. The individual isomer's effects on autoimmunity suggest that CLA10c12 may play a more harmful role during the initial stages of disease than CLA9t11. Currently mixed isomer CLA is used as a treatment for inflammation; a direct comparison of the primary CLA isomers was performed to determine the effects each has on arthritis prevention. DBA/1 mice were fed a semi-purified diet containing 0.5% CLA10c12, 0.5% CLA9t11, or 1% corn oil (CO) starting three weeks before CA induction. The effects on disease incidence and severity as well as, anti-collagen antibodies, cytokines, and hepatic fatty acids were measured. Maximum arthritis incidence was reduced at least 34% in mice fed either CLA isomer compared to those fed a CO diet (P = 0.06). In mice that did develop arthritis, CLA10c12 reduced arthritic severity to a greater extent than CLA9t11 and CO (P = 0.03). One week prior to arthritis onset CLA10c12 fed mice had decreased plasma anti-collagen IgG (P = 0.04). CLA10c12 increased the 16:0 to 16:1c9 ratio supporting delta-9 desaturase enzyme inhibition by this fatty acid (P = 0.004). This new data supports continued exploration into the utility of CLA isomers as preventive treatments for chronic inflammation.

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The ability of a chemical to bond covalently to protein has been identified as the first key event in the skin sensitization adverse outcome pathway and is the basis for the Direct Peptide Reactivity Assay (DPRA), a validated skin sensitization alternative test. While the DPRA is applicable to a wide variety of chemicals it lacks a metabolic component which is critical for detecting chemicals identified as pro-haptens, non-sensitizing chemicals that are transformed into protein reactive haptens while not compromising the assay's ability to detect pro-haptens.

The Peroxidase Peptide Reactivity Assay (PPRA) was developed. The PPRA includes a horseradish peroxidase-hydrogen peroxide (HRP/P) metabolic component for the enzymatic activation of pro-haptens sensitizers. In the PPRA chemicals are incubated with cysteine-containing (CYS) and lysine-containing (LYS) peptides in the absence of HRP/P (CYS-, LYS-) and with CYS in the presence of HRP/P (CYS+). Evaluation of an expanded set of 209 chemicals tested in the PPRA revealed false negative results for 12 aldehydes and 4 thiazolines. A subset of 8 aldehydes and 2 thiazolines was selected to evaluate protocol modifications aimed at improving detection of these chemical classes. Dithiothreitol (DTT), a strong reducing agent added to the CYS reactions to prevent formation of CYS dimers via oxidation, was identified as the primary cause for low CYS depletion which resulted in the false negative result. When tested in a modified PPRA the maximum percent CYS depletion (DPMax) was increased approximately 3- to 27-fold in the CYS- without DTT reactions compared to CYS- with DTT, with 9 of the 10 chemicals having DPMax values which exceeded the positive cut-off value of 25%. Use of DTT only in the CYS with HRP/P reaction improved detection of reactive aldehydes and thiazolines while not compromising the assay's ability to detect pro-haptens.
Exposure to trichloroethene (TCE) is associated with autoimmune hepatitis (AIH). However, mechanisms contributing to TCE-mediated AIH are not known. We hypothesized that increased apoptosis and delayed clearance of apoptotic bodies, due to compromised Kupffer cell (KC) function, will result in breakdown of self-tolerance, autoimmunity, and ultimately AIH. Earlier, we have shown that dichloroacetic acid (DCA), one of the metabolites of TCE with strong acylating capability, can elicit an autoimmune response at much lower dose than TCE in MRL/+ mice. Furthermore, KCs, the liver resident macrophages, are crucial for hepatic homeostasis, but can also participate in the immunopathogenesis of AIH. However, contribution of KCs in TCE-mediated AIH and the underlying mechanisms are not understood. Therefore, using an in vitro model of immortalized mouse KCs and a reactive metabolite of TCE, DCAC, we investigated the molecular mechanism of TCE-mediated AIH. KCs were pre-treated with different concentrations of DCAC for 24 hours, followed by addition of lipopolysaccharide (LPS) to the culture system for 4 hours before harvesting the cells. Markers of apoptosis (PD1, MFG8 and Caspase1) and inflammasome (NLRP1 and NLRP3) were analyzed by real-time PCR. Also, the phagocytic potential of KCs was evaluated by using fluorescent labeled beads. DCAC treatment resulted in the activation of inflammasome cascade (NLRP1, caspase1 and IL18) and apoptosis-related MFG8. Interestingly, DCAC treatment resulted in decreased phagocytic activity of KCs in a dose-dependent manner. These findings suggest that DCAC induced apoptosis and inflammasome activation, while inhibiting phagocytic function of KCs. Our data support that increased apoptosis and impaired KC function by DCAC could be contributory to TCE-mediated AIH. Supported by NIH ES026887.

**Comparison of the Acute Respiratory Response to Crystalline Silica and Multi-walled Carbon Nanotubes in Male and Female Mice**

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Systemic lupus erythematosus (SLE) is a complex chronic disease and patients often have a poor prognosis. As with many autoimmune diseases, the occurrence of SLE is dramatically higher in women than in men, with a ratio of 9:1. However, the reason behind women’s increased susceptibility is unknown. In addition to genetics, environmental factors are thought to contribute to the development of autoimmune disease. However, there remains a gap in our knowledge of how sex-based differences in response to environmental stimuli contribute to the etiology of autoimmune disease. Epidemiological evidence has linked the inhalation of crystalline silica (SiO2) with the development of SLE, however, sex-differences in inflammatory responses to SiO2 are not understood. Additionally, engineered nanomaterials such as multi-walled carbon nanotubes (MWNTs), are becoming an increasing concern in the etiology of lung disease, but the relatively few studies evaluating sex-differences are inconclusive. This study compared the acute respiratory responses of female and male C57BL/6 wild-type mice to SiO2 and MWNTs, in order to characterize differences in the immune response and susceptibility to lung injury. Most comparisons were similar between sexes. However, twenty-four hours after a single exposure to SiO2, males had significantly fewer alveolar macrophages in their lung lavage fluid, but a greater number of neutrophils than treatment-matched females. Furthermore, one week after a single exposure to SiO2, both sexes of mice had an equal number of AMs, but a higher percentage of these AMs were activated in male mice, as determined by MHCII expression. Our results demonstrate a differential macrophage response between sexes in response to SiO2, which may provide a basis for sex-based differences in antigen processing and presentation mediating the development of SLE. Funded by NIH grant R01ES023209.

**Oral Sensitization of Mice to a Cow's Milk Allergen Induces Increased Grooming Behavior and Epigenetic Changes in the Brain**

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Consumption of a certain food item, such as cow’s milk or wheat gluten, is reportedly associated with behavioral changes in susceptible individuals although causative evidence is rather limited. We hypothesize that such behavioral changes may be a type of allergic manifestation that affects the central nervous system (CNS) in individuals with sub-anaphylactic food hypersensitivity. To determine whether a food allergen would induce behavioral changes, we used a mouse model of milk allergy and investigated peripheral events that might contribute to alterations in CNS function and ultimately behavior. Four-week-old male and female C57BL/6 mice were subjected to weekly oral sensitization to either a vehicle or β-lactoglobulin (BLG), one of the major allergens in the whey protein fraction of cow’s milk. After a 5-week sensitization period, all animals were challenged with BLG during the 6th week and their behavior was assessed before sacrifice. We observed that BLG-sensitized males, but not females, exhibited increased grooming behavior. Associated with the behavioral deviation, immunoglobulin E (IgE) was elevated in the male BLG-sensitized mice when compared to sham mice using BLG-specific IgELISA. Quantitative RT-PCR analyses from intestinal tissue suggested that proinflammatory cytokines increased in the BLG mice. Interestingly, immunohistochemical staining of the brains from these mice showed distinct 5-hydroxymethylcytosine changes in the hippocampus and thalamus, and parts of the cerebral cortex. These results indicated a food allergen-induced epigenetic modification in specific areas of the brain, providing evidence for food allergen-mediated inflammatory responses and molecular alterations in the brain.
2098 Assessment of Dermal Sensitization Risk Potential of Cured Inks Utilizing a Novel Extraction Method in the Local Lymph Node Assay (LLNA)

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Humans come in contact with a variety of different materials that have been printed with cured inks. Currently, there are a number of different cured inks on the market that have little to no assessment of hazard or risk. It is known that during the curing process that not all the polymers are cured to the printed material, whether it is molskien or a number of different plastics. When humans use, little has been done to assess the sensitization (allergic response) to humans. The Local Lymph Node Assay (LLNA) has been used for more than two decades to assess a material’s potential to induce an allergic response. The normal convention of the LLNA is to solubilize the material in a number of different organic solvents and topically dose the mice with this material. We modified this process to determine a more risk-based assessment instead of a hazard assessment. If there is no exposure potential, then there is no hazard or risk associated with a material. We designed an extraction process to model a realistic human exposure. In this project, the LLNA was used to assess 1) the uncured ink that could prior to cause an allergic response; 2) if the cured ink could be extract from plastics with either 0.9% physiological saline or 100% ethanol. The saline was used as an extractant to determine if there is an exposure risk if a human holds either a printed plastic water bottle, ink printed molskien, and ink printed plastic cup. The extraction was conducted at 37°C for approximately 24 hours, which was modified from the ISO 10933-12. The LLNA determined that the uncured ink is definitively a dermal sensitizer. The uncured ink was tested at 5, 10, and 25%, which produced stimulation indices (SI) of 3.8, 5.8, and 7.1, respectively. However, when the extractions were conducted in saline, there was no sensitization seen in the cured ink printed on a plastic bottle or molskien. Topical application of the saline extractions of the ink applied to bottle and molskien at 10%, 25%, and 50% resulted in SI values less than 1.6. Therefore, these are not dermal sensitizers in the Local Lymph Node Assay.

2099 Organochlorine Insecticide Exposure Effect on Changing Spraying Farmers’ Thyroid Stimulating Hormone (TSH) Level in Kertasari District, Bandoeng Regency


The use of insecticides in agriculture is now often done by farmers to achieve sustainable agriculture against insect pests. One insecticide widely used in Indonesia is class of organochlorine insecticides. Organochlorine insecticides are persistent both in the environment and in the body of living things that are exposed. Long-term exposure to organochlorine insecticides can have an impact on the health changes on sprayer farmers, one of which is a thyroid disorder in the form of increasing level of thyroid-stimulating hormone (TSH). This research was conducted on spraying farmers in Kertasari district, Bandoeng regency. Sampling was taken when farmers do insecticide spraying activity using bad sampling to check dermal exposure. This report describes organochlorine concentration, which exposes spraying farmers and then convert to the accumulation of the intake to see the effect on TSH level in farmers’ blood. This paper also described the factors that can affect TSH levels in the farmers’ blood and the risk value between an exposed farmer and unexposed farmer. Organochlorine insecticides that were detected in the field, namely lindane, heptachlor, aldrin, endosulfan, DDT, dieldrin, and endrin, with each highest value of exposures, were 07818 mg/cm², 0.021 mg/cm², 0.5824 mg/cm², 0.0464 mg/cm², 0.0025 mg/cm², 0.0014mg/cm², and 0.1108 mg/cm². The results of statistical calculation there is a relationship between the intake and the increasing of TSH level which the factor has a strong relationship was lindane intake (Pearson correlation. r=0.656,p<0.000). The risk value also showed that exposed farmers have higher risk of getting hypothyroid disease than unexposed farmers.
in an effort to reduce animal use. In silico approaches present a rapid and cost-effective way to screen novel compounds for potential reactivity with biological nucleophiles. Currently, two in silico approaches are available to screen chemical: profilers (which identify reactive groups within chemicals) and quantitative structure-activity relationship (QSAR) models (including expert knowledge-based systems). To understand the utility of QSAR models in predicting in vivo outcomes, we curated a database of 581 compounds with local lymph node assay (LLNA) data from the public literature and internal sources. The local lymph node assay (LLNA) measures the lymph node proliferative responses of treated mice. Compounds that elicit a stimulation index (SI) $\geq 3$ are considered sensitizers. The EC3 (estimated concentration in % required for SI=3) values are used for comparison of sensitizing potential derived from local lymph node responses. The sensitization potential of the compounds in our database was assessed with three QSAR models (DEREK Nexus, TIMES-OASIS and MultiCase) with metabolism predicted using the skin metabolism simulator in the OECD Toolbox or the OASIS metabolizer module. When metabolites predicted by either simulator were included in the analysis, the three QSAR models predicted a positive LLNA result with reasonable sensitivity (>79%). Similar findings were seen for extreme and strong sensitizers (those with LLNA ECFIC $\geq 0.1$ - 1% and ECFIC $\geq 0.03$ - 0.1% respectively), with marginal sensitivities for less potent sensitizers. When the analysis was restricted to compounds curated from internal sources, the power of these models to predict the LLNA outcome of the parent compound or its metabolites was reduced (sensitivity range of 58.3% - 85.3%). Finally, we demonstrate the utility of a consensus approach wherein assessment of compounds with multiple models improves the sensitivity of the analysis (97.6% - 100% for compounds being flagged by one or more models, with metabolites predicted via the OECD Toolbox or OASIS metabolizer modules).

## 2102 Development of an Updated Carcinogenicity Potency Database and Analysis of Thresholds of Toxicological Concern

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The existing Carcinogenicity Potency Database (CPDB) has been curated and extended with new data to facilitate a re-evaluation of the Threshold of Toxicological Concern (TTC) for carcinogenicity. New data have been added from the National Toxicology Program (NTP) and other open sources. All data were subject to a thorough review to ensure accuracy of the chemical structure as well as toxicological information. The new CPDB now comprises data for more than 650 chemicals with acceptable studies, according to defined criteria, of which $\geq 570$ were carcinogenic with $\geq 450$ associated with reliable genotoxicity data. The CPDB was subjected to various analyses to determine points of departure, notably TD25 and TD50 as well as Benchmark Dose Levels. These analyses were compared and demonstrated the need for appropriate dose response data. A strategy was also implemented to identify mode of action with regard to genotoxicity. In vivo, in vitro and in silico data were applied through a defined strategy to identify genotoxic and non-genotoxic carcinogens. Specifically, where available, experimental genotoxicity (mutagenicity or clastogenicity) data or information from computational models (QSARs and structural alerts) for DNA reactivity, in vivo micronucleus effects and chromosomal aberration were utilized. Analysis of the new CPDB confirmed the conservative nature of the current TTC values for carcinogens. The new CPDB is publicly available as an Access file and searchable via COSMOS DB (cosmosdb.eu) and is intended to support further TTC evaluations for carcinogenicity. The funding of the CEFIC LRI-B18 Project is gratefully acknowledged.

## 2103 Identifying and Annotating Hallmark Genes Relevant to Toxicogenomics

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Fifty (50) Hallmark gene sets were derived from an integrated analysis of the Broad Institute’s Molecular Signatures Database (MSigDB). These gene sets are ideal for interpretation of toxicogenomic studies as their derivation considered a number important factors including gene coregulation, links to canonical pathways and biological processes, links to mechanistic and disease signatures, and limits on redundancy with other Hallmark gene sets. One significant challenge in using these gene sets is their limited coverage of gene space (~4500 genes). To extend the 50 Hallmark gene sets we have performed WGCNA on approximately 124,000 curated studies from the GEO database to identify co-regulated modules that do not overlap with the existing Hallmark gene sets. From this exercise, we have identified ~76 additional gene sets. Using NextBio’s ontology search database, the function and co-regulation features of these new hallmark gene sets were annotated at the same levels including canonical pathway association, biotest connectivity, cell/tissue expression and disease association. Once fully populated and curated, these Hallmark gene sets will be evaluated for utility to serve as a basis for interpretation of in vivo genotoxic dose-response studies performed by the NTP.

## 2104 Evaluation of the Applicability of Existing QSAR Models for Predicting the Genotoxicity of Pesticides

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The guidance document issued by the European Food Safety Authority (EFSA) Panel on Plant Protection Products and their Residues (doi: 10.2903/j.efsa.2016.4549) establishes a process for the definition of pesticide residues, including the evaluation of the potential risk based on toxicity potential for dietary exposure. While the toxicological dossier is developed for active substances, often none or only limited information about toxicological properties of their metabolites is available. Thus, the use of QSAR models and read across is proposed for the assessment of genotoxic potential of all identified metabolites as a first step in the residue identification procedure. Within an ongoing EFSA-fund project (OC/EFEA/PRAS/2016/01) the applicability and reliability of existing 52 commercial and freely-available QSAR models were critically evaluated for the prediction of genotoxicity of pesticides. The test set was provided by EFSA and it contains experimental genotoxicity data for 54 pesticide active substances and the comparison between the test set and a reference pesticides dataset showed the same range in physico-chemical properties, logP, pKa and solubility. Results from five endpoints were taken into consideration for the evaluation of models: bacterial reverse mutation, in vitro mammalian chromosome aberration, in vitro mammalian cell gene mutation, mammalian bone marrow chromosome aberration, mammalian erythrocyte micronucleus. The models were evaluated from the following perspectives: a) fraction of pesticides compounds of the test battery falling within the applicability domain of the model; b) number of false positives, false negatives, true positives, and true negatives. The overall comparisons of the sets of predictions showed that, depending on algorithms, assumptions, training sets and applicability domains, the QSARs have different strengths and weaknesses. Disclaimer: The results shown here have been produced under a contract with EFSA. The opinions expressed are those of the contractor only and do not represent EFSA’s official position.

## 2105 Gene Signatures Reveal Differences Between Preclinical Rat Liver Testing Systems

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Toxicogenomics (TGx) combines toxicology with genomics or other high-throughput molecular profiling technologies to gain an enhanced understanding of the molecular mechanism of toxicity. In the molecular toxicogenomics area, we developed a pair ranking (PRank) method to assess in vivo to in vitro extrapolation (IVIVE) using toxicogenomic datasets from the Open Toxicogenomics Project—Genomics Assisted Toxicity Evaluation System (TG-GATEs) database. Using this approach, we demonstrated that toxicogenomic data generated in hepatocytes in vitro can predict the ranking of a drug’s potential to cause hepatotoxicity in vivo. To further understand the capabilities of preclinical models of hepatoxicity, we applied PRank methodology to toxicogenomic data in TG-GATEs from three different assay systems: primary rat hepatocytes at 24 hours, in vivo single dose rat, and in vivo 28-day rat studies. A high similarity between the two in vivo assay systems was noted (PRank score = 0.90), indicating the potential utility of shorter-term in vivo studies to predict outcome in longer-term and more expensive in vivo model systems. There was a moderate similarity between rat primary hepatocytes and in vivo repeat-dose studies (PRank score = 0.77) but a low similarity (PRank score = 0.56) between rat primary hepatocytes and in vivo single-dose studies. When the comparison was limited to gene sets relevant to specific toxicogenomic pathways, we found pathways such as lipid metabolism were consistently over-represented in all three assay systems. Similarly, all three assay systems could distinguish compounds from different therapeutic categories. This suggests that any
noted differences in assay systems was biological process-dependent and, furthermore, that all three systems have utility in assessing drug responses within a certain drug class. In conclusion, this comparison of three commonly used rat TGx systems provides useful information in utility and application of TGx assays.

2106 A Case Study to Establish a Standardized Read-Across Process for Pesticide Active Substances and Their Metabolites for Assessment of Genotoxicity

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The European Food Safety Authority (EFSA) Panel on Plant Protection Products and their Residues issued a guidance document for identifying pesticide residues and evaluating their potential risk based on their toxicity and dietary exposure potential. Although a comprehensive toxicological dossier is developed for each active substance, toxicity data of metabolites are limited. While the possibility of applying in silico approaches to fill the data gap is currently being evaluated, EFSA recently released a new public genetic toxicity database file (EU OPEN Data Portal) containing 293 unique test substances and 766 related components to create 819 chemical species comprised of parents and related components (i.e., metabolites, mixtures or formulations). Data for 33 genetic toxicity endpoints were made available in 17,927 studies.

In vitro toxicokinetics data of blood partitioning and metabolic clear-

2109 In Silico Mutagenesis and Molecular Docking of Mouse and Rat Estrogen Receptor α with Paraben Analogs

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Numerous structures of human estrogen receptor α (ERα) ligand binding domain (LBD) in complex with agonists or antagonists have been reported and used to identify and characterize endocrine disruption (EDs) that mimic the LBD interactions exhibited by 17β-estradiol (E2). However, although rats and mice are used for in vivo studies and as tissue sources for in vitro studies of EDs, extrapolations to humans are hampered because crystal structures for rodent LBDs are lacking. Whereas the sequences of rodent and human LBDs are >96% identical, subtle differences in 3D structure could create differences in ligand binding. To address this knowledge gap, we used the human ERα LBD structure (PDB 3UJD) as a template to create structures of rat and mouse LBDs by in silico mutagenesis followed by energy minimization and molecular dynamics refinement with the YASARA molecular modeling suite. Model quality was assessed using MolProbity, and the best mouse and rat ERα LBDs were employed to compare molecular dockings of 21 paraben analogs using Autodock VINA with YASARA as the graphical front-end. All 21 paraben analogs displayed a general preference to localize to the known LBD of human ERα. We further compared all ERα LDB amino acid identified using YASARA for each ligand docked either to the mouse or rat receptor relative to the human ERα LBD docked structure. Sorensen similarity coefficients for receptor contacting residues with each ligand (mean ± SEM) were 93.1 ± 7.5% and 92.5 ± 7.1% between human and mouse or rat receptors, respectively. The number of receptor contacting residues between human and mouse or rat ERα among all ligands tested was found to have Pearson correlation of 0.913 and 0.914, respectively. The number of receptor atom contacts between human and mouse or rat ERα among all ligands tested was found to have a Pearson correlation of 0.976 and 0.979, respectively. The results indicate that our constructed mouse and rat ERα LBD receptors interact with ligands in like manner to the human receptor, thus providing a high level of confidence in extrapolations of rodent to human ligand-receptor interactions. Supported in part by NIEHS T32ES07062.

2017 Withdrawn by Author

2018 Application of In Vitro Toxicokinetics for Dose-Exposure Estimates of Substituted Phenols and Alkyl Glycols


Advances in toxicity testing have led to a rise in the application of in vitro and high throughput approaches to predict potential adverse effects following chemical exposures. As a result of this progress, new exposure and in silico methods were developed to help interpret and relate these measures with animal and human dose exposure estimates. The objective of this research applied new in vitro toxicokinetic data and models to predict doses where potential toxicological effects would be anticipated in vivo based on measures from the US Environmental Protection Agency ToxCast™ high throughput in vitro assay toxicity database. In vitro toxicokinetics data of blood partitioning and metabolic clearance were generated for Health Canada Chemical Management Plan grouping of substituted phenols (dimethylated alkyl benzenedioxils, methylated alkyl phenols, and methylated alkyl ester benzenepropanoic acids) and alkyl glycols (ethylene glycols and glycol esters). Using in vitro estimates of the parameters, toxicokinetic models were able to convert the toxicity assays concentrations into oral equivalent human doses. The estimated doses for liver cell activity were greater than 50 mg/kg/day for both chemical grouping. Responses range from cytotoxicity, cell cycle perturbation, DNA binding or even deregulation of steroidal and non-steroidal receptors. The data was collected using Dream.TK (Dose-Response Extrapolation & ASSay Modelling with Toxicokinetics) a new R package designed to work with the raw ToxCast™ database. The package features several table outputs used to retrieve the results of various assays, including effective concentrations and assay receptor targets, and incorporates various data analysis tools (e.g. clustering and principle component analysis). Advances in exposure characterization such as in vitro toxicokinetics and in silico tools can conservatively help translate in vitro toxicity screening information into biologically relevant point of interest for chemical prioritization and risk assessment.

2110 Comparative Toxicogenomics Database (CTD): An Integrated Resource for Chemical, Gene, Phenotype, and Exposure Data

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The Comparative Toxicogenomics Database (CTD; http://ctdbase.org) is a freely available resource that advances understanding about the mechanisms by which environmental exposures impact human health. Since its public release in 2004, the content of CTD has evolved to meet the evolving needs of the environmental health research community. Today CTD provides manually curated content describing chemical-gene interactions, chemical- and gene-diseases relationships, chemical-phenotype (non-disease) and population-based exposure data. CTD data are updated manually and currently comprise over 1.6 million chemical-gene interactions, curated information on approximately 15,000 chemicals, 43,000 (cross-species) genes, and 7,000 diseases. In addition, over 1,000 population-based exposure studies have been curated, including data for 975 chemicals, 326 genes, 371 diseases, and 282 phenotypic outcomes in populations from over 150 countries. All CTD curation is captured using community-accepted ontologies and controlled vocabularies, allowing integration across curated content as well as external data sets to make novel inferences. These data can be explored with user-friendly query and analytical tools to inform hypothesis development connecting chemicals/drugs, genes/proteins, diseases/phenotypes, taxa, functional annotations, pathways, and exposure events. These data also provide unique opportunities to generate predictive adverse outcome pathways, connecting molecular
initiating events to population-level health outcomes. CTD has become an invaluable research tool for the toxicology community that aims to advance understanding about environmental influences on human disease and underlying mechanisms from model systems to population-based studies.

### 2111 Hepatic Transcriptome Analysis to Assess the Effects of Prenatal Bisphenol A Exposure on Developing Chicken Embryos

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There is a growing concern about the toxic effects of bisphenol A (BPA) in developing organisms because the early life stage is a critical window to chemical exposure. However, there is less information regarding the effects of BPA on avian embryos. We thus performed in ovo BPA injection test of chicken eggs and investigated the effects on the developing embryos by analyzing their hepatic transcriptome with next generation sequencing (NGS). Fertilized chicken eggs were divided into five groups: control (corn oil), BPA-L (BPA 10 ng/g egg), BPA-M (BPA 1,000 ng/g egg), BPA-H (BPA 100,000 ng/g egg), and E2 (17β-estradiol 10 ng/g egg). On the 2nd incubation day, test chemicals were injected into the air sac. On the 21st incubation day, RNA solutions were extracted from liver samples and used for NGS. In order to detect embryonic death, eggs were observed on the incubation day 7, 14, and 21. To predict the high-risk diseases and its mechanisms, phenotype, gene ontology (GO), and transcription factor enrichment analyses of the genes differentially expressed by BPA- or E2-treatment were carried out by R, Comparative Toxicogenomics Database (CTD), TFaction, and Enrichr. Measurement of phenotypes showed that survival rates were significantly decreased in the BPA-H. This result suggested that BPA could affect the development of avian embryos. Data analyses of NGS indicated that miRNA levels of 204, 4078, 1766, and 3006 genes were significantly changed in BPA-L, BPA-M, BPA-H, and E2, respectively. This demonstrates that more pronounced effects on the transcriptome were induced in BPA-M, BPA-H, and E2 than in BPA-L. Phenotype enrichment analysis suggested that liver cirrhosis and reperfusion injury may be induced by BPA treatment in developing chicken embryos. Considering that reperfusion injury is one of the cardiovascular diseases, the abnormal development of blood circulation system induced by BPA exposure may contribute to an increase in the embryo mortality. GO enrichment analysis revealed effects on cell cycle in the BPA-treated groups, suggesting a higher risk of liver cirrhosis triggered by disrupted cell cycle in the later growth stage. Transcription factor enrichment analyses identified that activation of MYC, TP53, SP1, and E2F by BPA were important molecular initiating events in the adverse outcome pathway of BPA.

### 2112 Evaluation of Bioinformatics Approaches for Next-Generation Sequencing Analysis of microRNAs with a Toxicogenomics Study Design

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MicroRNAs (miRNAs) are key post-transcriptional regulators that affect the protein translation by targeting miRNAs. Its role in disease etiology and toxicity is well recognized. Given the rapid advancement of next-generation sequencing techniques, miRNAs profiling has been increasingly conducted with RNA-seq, namely miRNA-seq. Analysis of miRNA-seq data requires several steps: (1) mapping the reads to miRBase, (2) considering mismatches during the hairpin alignment, and (3) counting the reads. The choice made in each step with respect to the parameter setting could affect the results. Computational simulations including miRNAs bioinformatic analysis would significantly enhance miRNA detection and novel miRNA identification. Furthermore, these parameters do not act in isolation and their joint effects impact miRNA-seq results and interpretation. In toxicogenomics, the variation associated with the parameter setting should not overpower the treatment effect such as dose/time-dependent effect. In this study, four commonly used miRNA-seq analysis tools were comparatively evaluated with a standard toxicogenomics study design. We tested 30 different parameter settings on miRNA-seq data generated from thioacetamide-treated rat liver samples for 3 dose levels across 4 time points, followed with four normalization options. Because both miRDeep and miRNAkey yielded the larger variation across different parameter settings than that resulted from the treatment effects, our analyses were mainly focused on the side-by-side comparison between miRDeep2 and stRNAbench. While the number of miRNAs detected by miRDeep2 was almost the subset of these by stRNAbench, the number of DEMs identified by both tools was comparable under the same parameter settings and normalization method. Change in the number of nucleotides out of the mature sequence in the hairpin alignment (window option) yielded the largest variation for miRNA quantification and DEMs detection. However, such a variation is relatively small compared to the treatment effect when the study is focused on DEMs that is more critical to interpret the toxicological significance. These results indicated that a larger variation across different toxicogenomics databases, such as TOX21, TOXG, and TOXSeq, may also exist in the literature. Therefore, it is recommended to use multiple miRNA-seq tools for microRNA analysis in toxicogenomics studies.

### 2113 Facilitating Exposure Data Analysis in the Comparative Toxicogenomics Database: A Case Study of Heavy Metals and Metabolic Syndrome

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Exposure science aims to connect environmental stressors, such as chemicals, to health outcomes in human populations. Exposure science data is now a prominent component of the Comparative Toxicogenomics Database (CTD; http://ctdbase.org), a freely available resource that promotes understanding about the role of the environment on human health by providing manually curated information on chemical, gene, phenotype, and disease relationships. Over 50 exposure-related details, including stressor sources, population demographics, detection levels, geographic information, and health outcomes are curated using the exposure ontology (ExO) and other controlled vocabularies. CTD provides a centralized, searchable repository of exposure data that aims to facilitate meta-analyses and inform study design by allowing comparisons across studies and experimental parameters. Summary and detailed views of exposure data can be retrieved and downloaded using customized query sets and user-refined displays of data. Currently, over 1800 exposure articles have been curated identifying relationships among 975 chemicals, 326 genes, 371 diseases, and 282 phenotypic outcomes in populations from over 150 countries. We demonstrate the value and scope of exposure data integration in CTD through a case study assessing the potential connection between heavy metal (cadmium, lead, and mercury) exposure and metabolic syndrome. CTD tools and curated content provide immediate perspective on the state of the data, differences in study designs and foci as well as research gaps. Centralization and integration with mechanism-based experimental data also uniquely enable comparisons between exposure-related responses in human populations and model systems. This presentation will outline the current exposure data content and analysis capabilities with a focus on heavy metals and metabolic syndrome.

### 2114 Impact of Deleterious Single Nucleotide Polymorphisms in Catechol O-Methyltransferase Conferring Risk to Post-Traumatic Stress Disorder

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One of the prevalent neurological disorders includes post-traumatic stress disorder (PTSD) which is drawing attention over the past few decades. It occurs as a consequence of a life-threatening event such as physical or sexual assault, combat, car accident, or natural disaster. Some of the risk factors for PTSD include environmental and genetic factors. Among the genetic risk factors, polymorphisms in the catechol-O-methyltransferase (COMT) gene have been associated with risk for PTSD. In the present study, we aim to analyse the impact of deleterious single nucleotide polymorphisms (SNPs) in COMT gene associated with PTSD using computational-based screening and molecular dynamics simulations. The data on the COMT gene associated with PTSD were collected from Online Mendelian Inheritance in Man (OMIM) database and PubMed search. The SNP datasets were downloaded from the dbSNP database. The amino acid sequence of the COMT protein was retrieved from the Uniprot database, and its three-dimensional structure was downloaded from protein databank. To study the structural and dynamic effects of COMT, wild-type, and mutant forms we have retrieved from the Uniprot database, and its three-dimensional structure was downloaded from protein databank. To study the structural and dynamic effects of COMT, wild-type, and mutant forms we have performed molecular dynamics simulations (MDS) at a time scale of 300 ns. Results from computational screening using the computational tools SIFT and Polyphen-2 showed that the SNP rs4680 (V158M) in COMT conferring deleterious with a phenotype in PTSD. Results from MDS showed some major fluctuations in the structural features, such as root mean square deviation (RMSD), radius of gyration (Rg), root mean square fluctuation (RMSF) and secondary structural elements including α-helices, sheets,
and turns between wild-type and mutant forms of COMT protein. In conclusion, our study provides new insights into the deleterious effects of the V158M mutation on the COMT structure. Supported by NIH grants R01MH094755, RO1AI23947, RO1 AI129788, P01 AT003961, P20 GM103641, and RO1 AT006888.

2115 Predicting Risk of Cardiac Liabilities in Drug Discovery Using Venn-Abers Predictors

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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 CV risk using in vitro assays of selected ion channels that regulate heart function, hERG (Kv 11.1), NaV1.5, CaV1.2, Kv4.3, and Kv7.1. Inhibition of these ion channels is an early indicator of CV 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 have been preferred since such predictions generally lack a good measure of the risk for the individual compounds. In 2005 Vovk, Gammerman and Shafer introduced the concept of Venn predictors. Venn predictors offers a way to assign a calibrated probability to predictions and are a part of the conformal prediction framework that uses past experience to determine precise levels of confidence in new predictions. Venn-Abers is a special case of Venn predictors that can be applied on top of a scoring classifier under standard assumptions regarding data generation. In essence this means that the obtained confidence capabilities can be trusted as a probability. Given the data, it is the best estimate of the probability. Assessing potential safety risk for a compound is commonly done in terms of bounds above expected Cmax. To mimic that, we propose to compute the probability that the IC50 of an assay is below n-folds of Cmax and thus create an individualized prediction for the compound rather than a fixed limit. In this work, we show how Venn-Abers predictors can be applied to a panel of assays, how it can be used together with Cmax to assess cardiac risk of potential drugs, and be an effective way to deliver enhanced decision-making capabilities to projects.

2116 Differential Gene Expression and Concentration-Response Modeling Workflow for High-Throughput Transcriptomic (HTTr) Data: Results from MCF7 Cells

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Increasing efficiency and declining cost of generating whole-transcriptome profiles has made high-throughput transcriptomics a practical option for chemical bioactivity screening. The resulting data output provides information on the expression of thousands of genes and is amenable to a variety of downstream applications. However, HTTr chemical space coverage and routes to submission challenges that are not inherent with traditional cell-based screening assays, which produce univariate outputs. We present a microfluidics-based laboratory workflow for HTTr screening of MCF7 cells in 384-well format and a HTTr analysis pipeline for data quality control, differential gene expression, and concentration-response modeling. MCF7 cells were plated in either DMEM+10% HI-FBS or phenol red-free DMEM+10% CS-HI-FBS and allowed a 24 h recovery period prior to exposure. A total of 44 chemicals were screened in 8-point concentration-response (0.03-100 µM) in each media at three exposure durations (6, 12, 24 h) in three independent cultures (n=1 /treatment/culture). Chemicals were tested using an acute assay, which was uniquely randomized with respect to treatment positioning. Cell lysates were analyzed using a TempO-Seq human whole-transcriptome assay to a target read depth of 3M. Cell viability and apoptosis assays were run in parallel to exclude conditions causing cytotoxicity. For count data, total and differential mapped reads were used with no unique reads used for performance. Data were subset by chemical x media x time with matching controls. Probes were filtered using a median raw read count > 5. Count data were scaled and transformed, and differentially expressed genes (DEGs) were determined using DESeq2. Reproducibility of read counts in technical replicas was high, with pairwise correlations >0.85 and median CVs of between 20 and 40%. The correlation of log2FC values for DEGs among biological replicates was also high (median > 0.75) within each media x time condition. ANOVA results demonstrated a broad range (10s to 1,000s) in the number of concentration-respon-
sive genes across chemicals. Concentration-response modeling demonstrated a broad range of probe and pathway level BMDs across chemicals for each media x time combination, and facilitated identification of no observable transcriptional response levels. This abstract does not necessarily reflect US EPA policy.

2117 Authoritative Listings of Carcinogenic Compounds, Identification, and Comparison to QSAR Modeling Tools’ Results

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Carcinogenic compounds have been identified by the National Toxicology Program (NTP), International Agency on Research for Cancer (IARC), and listing through California Proposition 65. These are considered authoritative sources for hazard assessment and Restricted Substance Lists (RSLS). Often such resources and RSLS identify groups of compounds without full lists of applicable Chemical Abstract Services Registry Numbers (CASRNs) or substance names. Scivera, through SciveraLens® and Rapid Screen has developed comprehensive versions of these lists. For example, IARC lists 851 CASRNs in monographs volumes 1-111 while Scivera has identified over 6000 CASRNs by including those associated with subgroups. For the current project, the Scivera Toxicology Team compared carcinogenicity modeling tool alert results with the IARC Group 1 compounds. From the comprehensive Scivera IARC Group 1 list, 1,068 CASRNs had a SMILES notation which allowed for modeling: 446 CASRNs returned a structural alert for carcinogenicity from these tools, while 622 had no alert found. Among these IARC Group 1 compounds that did not return a structural alert were polychlorinated biphenyls (PCBs) and nickel and cadmium compounds. This highlights and demonstrates the need for methods to fill data gaps for carcinogenicity by either ensuring all compounds in sublists are appropriately identified and/or using additional structures to trigger alerts. The Scivera Team is currently working on further comparisons to identify opportunities to support and improve commonly used modeling tools including using other carcinogenicity lists and other hazard endpoint models and lists.

2118 In Silico Prediction of DILI: Extraction of Histopathology Data from Preclinical Toxicity Studies of the etOX Database for New In Silico Models of Hepatotoxicity

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The etOX consortium extracted in vivo data from unpublished preclinical toxicity studies of 13 industry partners. This new database contains high-quality toxicity results in high detail level from 1,947 drug candidates (8,196 studies) supplemented with 1,286 chemicals from the RepDose database (2,695 studies). Different compilation steps were applied to transform these data into usable in silico model training datasets. Initially, all toxicology findings were extracted from study reports (paper/PDF). Then the verbatom terms for all treatment-related hepatotoxicity findings were harmonized using special ontologies. Finally, to receive model training sets with sufficient compound numbers and chemical space coverage, all primary histopathology terms were combined and grouped to different first- and then second-level clusters of similar toxicity mechanisms: e.g., primary necrosis terms such as “centrilobular,” “periportal,” etc. were grouped to first-level cluster “necrosis,” then clusters such as “necrosis,” “vacuolization,” etc., were grouped to second-level cluster “degenerative lesions.” With this approach, various training datasets were compiled depending on the species (rat, dog, and monkey), treatment durations (2 weeks-2 years) and administration routes. Then, different modeling approaches were applied on these datasets, including structural alerts, fragment-based and molecular descriptor-based machine learning approaches (e.g., random forest, decision tree, k-nearest neighbor). Models were validated and externalized, first by internal validation (test set) and then through external validation using Sanofi’s confidential data. For example, best external validation results (n=66) were achieved for the first-cluster rat necrosis models (229 positives, 198 negatives) using fragment-based (Sensitivity: 0.80, Specificity: 0.77) and a molecular descriptor-based decision tree approach (Sensitivity: 0.81, Specificity: 0.68). These validation results show that by reasonable clustering histopathology data from etOX, it is possible to develop highly predictive in silico models for drug-induced liver injury (DILI).
Breast cancer is a leading cause of cancer death among women. The human epidermal growth factor receptor 2 (ERBB2/HER2-Neu) has expanded expression in about 15% of breast cancer samples. The presence of such HER2-enriched samples is generally associated with poor prognoses. Resistance to therapy with the HER2-targeting monoclonal antibody, Trastuzumab, remains an issue in treatment of HER2-enriched breast cancers. In this study, we explored the transnational regulation of HER2 expression across a variety of intrinsic subtypes of breast cancer to help identify targets for adjunctive therapy. The PAM50 algorithm was applied to data from breast invasive carcinomas (BRCA) RNAseq version 2 samples from the Cancer Genomics (ATCCGA). Using the mutual information-based Algorithm for the Reconstruction of Accurate Cellular Networks (ARACNe), transcriptional regulatory network-wrks were constructed using basal-like samples only, HER2-enriched samples only, and all samples. A trees-based learning algorithm, Gene Network Inference with Ensembl of Trees (GENIE3), was also used for that same purpose. In the aggressive basal-like subtype, in which there is practically no expression of HER2, the gene’s expression is uniquely dependent on the tumor-suppressing gene, BATF2 (which has increased expression in basal-like samples relative to HER2-enriched samples), predicted a direct and indirect role of TOX3. In HER2-enriched samples, HER2 is uniquely dependent on the regulatory network of relationships, of which the progesterone receptor (which is absent in both basal-like and HER2-enriched samples) plays both a direct and indirect role. Also in this pan-subtype network, FXBP1, RARG, FOXM1, FOXA1, NFE2L1, POU2F3, ZNF500, and ZNF213 play direct regulatory roles in HER2 expression. The results indicate differences in regulating genes for HER2 across subtypes. The activities/expressions of E2F2, HOXAS, and CRY2 are candidates for targeting in potential adjuvant therapy in the treatment of Trastuzumab-resistant HER2-enriched breast cancers.

For pesticides registered in the United States, cumulative risk is assessed for chemicals that share a common mechanism of toxicity. Recently, the Environmental Protection Agency’s (EPA) Office of Pesticide Programs developed a screening framework to help address its statutory obligation to assess potential cumulative risks. As part of this evaluation, all available toxicological information for a pesticide group is evaluated to determine if the information supports a testable hypothesis of a common mechanism of toxicity. High-throughput screening data from the EPA ToxCast program offer potential mechanistic insight for many pesticides. The utility of such data to assess common mechanism groups has not yet been evaluated. In this study, a workflow was developed to categorize the chemical activity of 615 ToxCast substances using the Alan Wood Compendium of Pesticide Common Names. Computational analyses were conducted using the half-maximal activity values (AC50) and chemical specificity metrics (IZ-score) for 1,900 in vitro assays. A case study was conducted with anilopyrimidine pesticides (cyprodinil, pyrimethanil, mepanipyrim) to evaluate how ToxCast can be used with chemical structure, apical outcome and pesticidal mode of action information to evaluate potential common mechanisms of mammalian toxicity. Hierarchical clustering indicated greater similarity between the bioactivity profiles of cyprodinil and pyrimethanil which were also the most structurally similar. Analyses of the genes and pathways perturbed upon exposure to cyprodinil and pyrimethanil revealed potent (AC50 < 10 µM) and specific (Z-score > 2) activities for key molecular targets involved in neurotoxicity and liver function. In vitro data suggests observed following acute (decreased motor activity, hypothermia) and repeated-dose (liver necrosis/hypertrophy, spongiosis hepatis) exposure to pyrimethanil and cyprodinil. In contrast, mepanipyrim showed liver effects (fatty liver) and no evidence of neurotoxicity. These results may be used to support a testable hypothesis for a common mechanism of toxicity in mammals for pyrimethanil and cyprodinil. Overall, this research demonstrates how ToxCast data can facilitate the identification of common mechanisms which are pertinent to sensitive endpoints obtained from animal studies.
The underlying principle of read-across is that the biological activity of a compound is inherent in its molecular structure. Analogues are typically identified by structural similarity then evaluated on the basis of their bioavailability, reactivity, and metabolic similarity. While structural similarity is the most common read-across, a critical consideration is whether structural differences impact biological activity. This source of uncertainty can potentially be addressed with toxicokinetic (TK) information. We report progress on a case study to investigate the feasibility of using in vitro high-throughput metabolism experiments in concert with in silico metabolism predictions to substantiate biological similarity to enable quantitative read-across. Parent chemicals were incubated with a suspension of primary rat hepatocytes. Possible metabolites were predicted in silico using expert systems (Meteor Nexus & TIMES). Suspect screening analysis was performed using liquid chromatography-mass spectrometry (LC-MS/MS) in silico and the in silico predicted chemicals representing different read-across scenarios were identified: two proof-of-concept examples and two test cases. Candidate source analogues were identified based on structural similarity and information availability for the two proof-of-concept chemicals (similar metabolism [methyl estrogenic metabolite] and different metabolism [2-nitrotoluene vs 4-nitrotoluene]), whereas for the two test cases, the experimental and in silico results will be integrated to substantiate the validity of the source analogues to inform selection of the most appropriate analogue. To date, in silico metabolism predictions have been generated for the proof-of-concept chemicals and compared to the in vivo metabolic profiles reported in the literature to assess their agreement. The metabolism predictions from the different tools complemented each other in capturing the primary pathways and, in particular, the pathway that has been associated with the genotoxicity of the nitrotoluennes, but no tool correctly captured all the metabolites observed. This abstract does not necessarily represent US EPA policy.

Heavy metals are a prominent environmental concern due to the association between the rate of disease primarily from environmental exposures and occupational hazards. Heavy metals present in sediment or water can easily enter into the food chain and affect humans, animals, and ecosystem. Metatranscriptomics has been used to assess microbial community activities and functions, as well as seawater communities. Here we propose to develop transcriptomic predictive benchmarks for ecosystem recovery and remediation success of heavy metals in river water as well as discover key regulatory elements. We propose to apply transcriptomic technology to test heavy metal remediation because of its sensitivity and because it allows for comprehensive examination of genomic and epigenomic molecular changes that hyperaccumulation of low levels of Zn, Cd and Ni may produce and that are not detectable by monitoring and surveillance of water surface or sediment levels of these chemicals. Hazard identification based on experimental and ecologic studies suggest that exposure to metalloestrogens Cd, As, and Ni produced health deficits in aquatic organisms, including in bottom- and surface-dweller species of sport fish. Gene set enrichment analysis of ESTs and RNA-Seq data revealed that nuclear respiratory factor (NRF1) regulatory genomic and epigenomic networks were highly sensitive to environmental heavy metal stressors. Further analysis of our metatranscriptomic findings of heavy metals should provide insight into health of fish communities for ecosystem recovery and remediation success of heavy metals.
Molecular Determinants of Brain Vascular Disorders from Exposure to Polychlorinated Biphenyls
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Autism is a brain-based disorder resulting in a wide spectrum of abnormal behaviors and difficulties with social communication and interaction. Genome-wide expression analyses on human autopsies show a significant increase in plasticity markers CD34 and nestin, and suggest that autistic brains undergo a constant state of angiogenesis. The constant fluctuation in the cellular structure of blood vessels may impact the blood delivery system to the brain and ultimately be neurologically limiting. Environmental risk factors associated with the development of autism are largely unknown. Population studies have recently demonstrated an association between autism spectrum disorder in children with maternal pregnancy serum levels of polychlorinated biphenyl 153 (PCB153). PCB153 is one of the largest contributors to total PCB body burden in humans that has been shown to accumulate specifically in the brain in vivo. We have previously shown that PCB153 activates the transcription regulator ID3 in exposed human blood-brain barrier endothelial cells. ID3 is biologically relevant to neurological and behavioral research because of its involvement in the stress response, neural plasticity, and neural circuitry. Here we propose to apply transcriptomic technology to the question by testing for ID3 regulated animal models that may explain brain vascular toxicity and allow for comprehensive examination of genomic and epigenomic molecular changes. Using transcriptomic studies of PCB exposed children, we identified an ID3 gene regulatory network that can be used to predict molecular risk factors of autism in the population. Gene network enrichment analysis using genome wide ChiP-seq data identified ID3 target genes associated with PCB exposure and brain vascular processes. Bayesian algorithm BANJO suggest that modified expression of several “key Markov genes” may be required for the development of autism, and its application may be useful in identifying individuals who are susceptible to brain vascular toxicity from exposure to PCBs.

Revealing DMSO-Induced Bias in ‘Omics Analysis In Vitro
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landscape, suggesting that DMSO can interfere with regulatory systems (FDR <0.05) found in both tissues indicate changes in the epigenetic state and altered genes, processes of transcriptional regulation were investigated in untreated microtissues). Pathway analysis of these DEGs identified hundreds (DEGs; FDR >0.05) in both cardiac and hepatic tissue (compared to untreated microtissues). Since affected pathways displayed a majority of downregulated genes, processes of transcriptional regulation were investigated in detail, focusing on DNA methylation. Differentially methylated regions (FDR <0.05) found in both tissues indicate changes in the epigenetic landscape, suggesting that DMSO can interfere with regulatory systems in cardiac and hepatic tissues. While the field is evolving towards more sophisticated in vitro models, using 3D conformation and/or physiologic-consistent low dose concentrations, omics technologies clearly demonstrate that the effect of DMSO on cell regulation has to be kept in mind when designing in vitro studies and interpreting the data. Consequently, the lowest possible dose of DMSO should be used as even low incubation concentrations may induce solvent-induced bias.

Vector Mapping Technology (VMT) for High-Resolution Tracking of Gene Therapies in Live Animals

New molecular tools, such as CRISPR/Cas9, have revolutionized the field of gene therapy. Nonetheless, significant challenges remain for directing genetic modifications to diseased sites with high specificity. Viral vectors engineered for organ-specific transgene expression could yield safe and efficacious delivery routes for gene therapy. However, the development of such vectors is hampered by conventional methods for tracking biodistribution (i.e. quantitative PCR (qPCR) and immunohistochemistry (IHC)); which are inaccurate, invasive, laborious and were historically prone to sampling error. These assays also typically require a large number of experimental animals, thus raising important ethical concerns and increasing overall costs. Noninvasive reporter gene imaging offers an alternative to traditional assays with the potential to streamline biodistribution studies while reducing animal use. The sodium iodide symporter (NIS) is a self-protein expressed endogenously in the thyroid and stomach. Beyond its physiological role in trapping iodide within cells, NIS can function as a reporter gene to enable noninvasive, tomographic SPECT or PET imaging via uptake of radiotracers. Unlike luciferase, NIS reporter gene imaging is high-resolution and can be used in store organs and to monitor the skeletal muscle. In contrast, background signal in control mice was restricted to sites of endogenous NIS expression (e.g. thyroid and stomach). Endpoint qPCR and IHC analyses confirmed AAV transduction and NIS expression in the heart, liver, and muscle. Combined, these data establish NIS reporter gene imaging as a powerful, noninvasive alternative to traditional biodistribution assays.
Advantages of combining BLI and SPECT or PET imaging of NIS for tumor tracking in pre-clinical studies. BLI can be used for sensitive early detection and relatively easy monitoring of tumor growth, while NIS imaging at later times can be used to achieve superior resolution, deep tissue imaging, and precise anatomical localization.

2133 Evaluation of Targeted Sequencing for Transcriptional Analysis of Archival Formalin-Fixed Paraffin-Embedded (FFPE) Samples


Next-generation sequencing provides unprecedented access to genomic information in archival FFPE tissue samples. However, costs and technical challenges related to RNA isolation and enriched use of whole-genome RNA sequencing for large-scale studies of FFPE specimens. Recently, a targeted sequencing platform called TempO-Seq was developed for gene expression profiling without the need for RNA isolation or pre-amplification. In this study, we compared genomic responses between the new TempO-Seq mouse array (containing 3,045 probes targeting 2,756 genes) and the Illumina-based whole-genome platform (RNA-Seq) in FFPE and paired frozen (FROZ) samples. Archived liver specimens were analyzed from control mice (CON) and mice treated with 600 ppm of phenobarbital (PB) for 7 days (n=6/group). Portions of each liver were (a) snap frozen and (b) fixed in 10% buffered formalin for 18 hours before processing to FFPE blocks. Quality sequencing metrics and expression of the preselected PB response genes Cyp2b10 and Cyp3a11 were evaluated. RNA-Seq used a higher read depth (68.5 ± 1.4 million/sample) than TempO-Seq (2.4 ± 0.1 million/sample) but provided similar alignment patterns to the mouse genome (86% and 81%, respectively). Total mapped reads were 52% lower for FFPE vs. FROZ samples but similar for TempO-Seq (2% higher for FFPE). On RNA-Seq, total reads mapped to Cyp2b10 were ~2,374-fold higher for PB (23,739 ± 1,647 vs. controls ≤10 in FROZ and ~629-fold higher in FFPE ≤10 in CON vs. 8,292 ± 547 in PB), indicating a signal loss of ~65%. For Cyp3a11, total reads were 78% lower in PB-treated FFPE vs. FROZ samples, but fold-change values were comparable: 58-fold higher in FROZ (1,754 ± 441 in CON vs. 102,297 ± 13,747 in PB) and 68-fold higher in FFPE (327 ± 77 in CON vs. 102,297 ± 13,747 in PB). Using TempO-Seq, Cyp2b10 reads were ~2,736-fold higher in PB (17,272 ± 1,567) vs. controls ≤10 in FROZ and ~2,201-fold higher in FFPE ≤10 in CON vs. 22,008 ± 2,197 in PB), while Cyp3a11 reads were 49-fold higher in FROZ (29 ± 3 in CON vs. 1,409 ± 86 in PB) and 50-fold higher in FFPE (67 ± 11 in CON vs. 3,347 ± 503 in PB). In FFPE samples, counts for Cyp2b10 and Cyp3a11 correlated strongly between TempO-Seq and RNA-Seq platforms (R² > 0.96, P < 0.05 for both markers). These findings support the idea that targeted sequencing can be used as a streamlined alternative method for analysis of RNA expression in FFPE samples. This abstract does not reflect US EPA policy.

2134 Development of pH-Targeting Fluorescent Probes to Map Inflammatory Response

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Elevated extracellular acidity due to increased glycolytic flux is a hallmark of many pathological states. Exploiting acidity to develop probes that monitor and track inflammation is a viable approach with the flexibility to range across a spectrum of toxicologically-relevant endpoints, from bioenergetic measurements to tumor diagnosis. pH-Low-Insertion-Peptide (pHLP) is a pH-sensitive peptide that is capable of cellular transmembrane insertion through a helix formation that is reliant on the protonation of aspartic acid residues under localized acidic conditions. Upon insertion, the C-terminus translocates inside the cell while the N-terminus remains extracellular. By tethering a fluorophore to the N-terminus, researchers may visualize inflammatory response, following xenobiotic exposure, in real time, which provides an orthogonal view to complement the findings of biomarker profiling in vivo. In vitro studies using pHLP-1 allow for the targeting and sorting of metabolically compromised cells for further study. Our laboratory has been optimizing pHLP variants clustered to fluorescein isothiocyanate to target transmembrane successful targeting of L6 rat skeletal myoblasts post-exposure to mitochondrial electron transport chain inhibitors. Our techniques have achieved 100% localization to acidic cells in under one minute. Furthermore, our in vitro work has investigated the binding constants associated with pHLP to pinpoint optimal concentrations for further in vivo work, as well as establishing a dose-response relationship between amount of pHLP and extent of cellular injury.

Noninvasive bioluminescent imaging (BLI) of tumor cells expressing luciferase can be used to track tumor growth and metastasis in living animals. High sensitivity and relative ease of use have made BLI common in pre-clinical studies. Nonetheless, BLI is limited by poor signal penetration, making it unusable for deep tissue or large animal studies. In vitro, Fluc and NIS models expressing firefly luciferase (Fluc) and the sodium iodide symporter (NIS). To this end, we generated tumor microtissues and the Visikol HISTO-M clearing agent to illustrate the pound are greatest. It was sought to solve the problem of 3D cell culture opacity by employing an optical clearing agent designed specifically for these tissues. Here, we combine 3D InSight™ Primary Human Liver Microtissues and the Visikol HISTO-M clearing agent to illustrate the effect of tissue clearing on high content confocal screening of steatosis and cytotoxicity measured by several known anchors was demonstrated that the addition of tissue clearing allowed for a 3-fold increase in cells detected, the ability to characterize dose response as a function of tissue depth and an increase in dose response sensitivity.

Imaging of testis revealed that the parent drug was mostly detected in the subcapsular region at both time points: 2 and 8h after dosing. The parent drug was observed in all histological regions of the testes; the strongest signal was in the vascular interstitial tissue, whereas the signal in the germ cell layer and lumen was weaker. The latter findings are related to the lack of blood vessels in these regions. A weak signal was evident in these compartments; it cannot be excluded that parent drug penetrates the blood-testes barrier to some extent. The combination of QMSI and histology can be used to study the location of a test substance and its metabolites in a PKPD study without the use of radio-labeled compounds.


The use of in vitro three-dimensional (3D) cell cultures has increased dramatically for drug screening as 3D culture models more accurately mimic the in vivo environment compared to traditional monolayer cultures. However, unlike for traditional cell culture, imaging analysis of 3D cultures is limited due to the thickness of 3D cell cultures, typically >100 µm, which causes light scattering, limiting imaging of the surface-layer cells of the 3D culture. This limitation prevents complete characterization of the cell population within whole-mount 3D cultures. Furthermore, this technical limitation introduces an unavoidable sampling bias in imaging analysis, since only the exterior cells can be imaged where concentrations of nutrients, oxygen, and drug compound are greatest. It was sought to solve the problem of 3D cell culture opacity by employing an optical clearing agent designed specifically for these tissues. Here, we combine 3D InSight™ Primary Human Liver Microtissues and the Visikol HISTO-M clearing agent to illustrate the effect of tissue clearing on high content confocal screening of steatosis and cytotoxicity measured in several known anchors was demonstrated that the addition of tissue clearing allowed for a 3-fold increase in cells detected, the ability to characterize dose response as a function of tissue depth and an increase in dose response sensitivity.

2131 Improved Characterization of Compound Toxicity through the Application of a Tissue-Clearing Technique to 3D In Vitro Model Screening


2132 Precise Anatomical Localization and Deep Tissue Imaging of Tumors Expressing Luciferase and the Sodium Iodide Symporter (NIS)

I. P. 2018 SOT Annual Meeting
Mitochondrial toxicity has been implicated in several high profile clinical trial failures and withdrawals. Standard approaches to monitoring metabolic perturbations are limited to endpoint assays that provide population-based measurements and limited kinetic information. We developed a genetically encoded ATP sensor to enable direct, automated live cell analysis of cellular ATP levels using IncuCyte® S3. In this study, our live cell imaging approach to categorizing compounds as nontoxic, cytotoxic, or mitotoxic using the glucose/galactose switch model was evaluated. Substituting galactose for glucose in growth media blocks the ability of cells to generate ATP via glycolysis, conferring reliance on mitochondrial oxidative phosphorylation to generate ATP and enhancing sensitivity to mitochondrial-driven toxicity. Cell lines stably expressing a genetically encoded, fluorescent ATP sensor or a control (non-ATP binding) sensor were adapted to and plated in glucose or galactose media. ATP levels were monitored using an IncuCyte® S3 equipped with a specialized filter set and data acquisition module. Cellular ATP levels were measured over the course of 24 hours following treatment with a panel of compounds previously classified as nontoxic, cytotoxic, or mitotoxic. Little to no change in ATP was observed following administration of nontoxic compounds. Cytotoxic compounds induced similar reductions in ATP in both glucose- and galactose-grown cells. In contrast, mitotoxic compounds displayed a leftward shift in potency and more substantial and/or prolonged decreases in ATP under galactose grown conditions. Reductions in ATP levels could be observed within minutes of compound treatment, highlighting the sensitivity and specificity of the IncuCyte® S3 sensor assay. Transient reductions in ATP followed by recovery, which would have been missed by typical endpoint assays, were also observed using our live cell analysis approach. Visualization of morphology and quantification of phase confluency in tandem with automated ATP sensor analysis provided by IncuCyte® S3 adds additional insight into the heterogeneity of cellular responses to compound addition. Overall, our data demonstrate that direct measurement of ATP by live cell imaging provides a robust, predictive tool for mitochondrial toxicity screening.
Evaluation of biological activities of compounds has been greatly advanced by transcriptionomics. However, gene signatures provide only indirect information about the underlying biological phenomena. Here, we present an alternative approach, wherein cell response is characterized by changes in the network of signal transduction pathways that regulate gene expression. Using a multiplexed reporter assay, we assessed the activity of transcription factors that lie at the apexes of these pathways, generating a quantitative signature (TF activity profile [TFAP]). We show that perturbations of human cell systems and biological pathways produce distinct TFAPs. Strikingly, perturbagens of a given system, regardless of structural and mechanistic dissimilarities, have produced an invariant TFAP, representing an "archetypal signature" of bioactivity. We found archetypal signatures for multiple bioactivities, including mitochondrial, HDAC, and ubiquitin/proteasome pathway inhibitors; cytoskeleton disruptors; and DNA-damaging agents. These TFAPs enabled correct identification of compounds with given bioactivity among uncharacterized chemicals. Furthermore, we show that observing changes of a compound's TFAP with concentration enables the identification of multiple target systems and reveals the on-target and off-target activities of drugs. Thus, TFAPs represent a new kind of descriptors that enable a straightforward, mechanism-based evaluation of compound polypharmacology. Support for the NIH grants R44GM125469. The views expressed in this presentation are those of the authors and do not necessarily reflect the views or policies of the US EPA. Mention of trade names does not constitute an endorsement by the US EPA.

Intestinal microbiota play a role in a variety of host physiological aspects. Thus, when assessing the toxicological profile of a substance its effect on the microbiome cannot be ignored. In this study, an antibiotic alteration of the gut microbiome was carried out. During 28 days, clindamycin or lincomycin were administered orally to Wistar rats. With the aim of identifying metabolites derived from the gut microbiome, metabolic profiles of fecal and plasma were assessed. As these antibiotics have an almost negligible systemic toxicity, the metabolomics findings in the plasma of treated animals is, most likely, exclusively related to changes in the gut microbiome. An increase of taurocholic acid and 2-hydroxybutyrate together with a decrease of glycochenodeoxycholic acid was found as common changes in plasma and feces profiles of antibiotic treated rats. Taurocholic acid and cholic acid were both highly increased in feces. However, cholic acid was decreased in plasma profiles whereas taurocholic acid was increased. Latest findings show that there is an evident accumulation of primary bile acids like cholic acid and taurocholic acid in the gut. This is likely owed to the disruption of microbial communities and an increase of bile production via the liver derived from a lack of secondary bile acids. Additionally, 7a-dehydroxylation bacteria can convert the small amount of secondary bile acids to yield cholic acid and taurocholic acid and are favored after antibiotic treatment. However, the significant lowering of cholic acid in plasma indicate that this metabolite is not being reabsorbed from the gut into the portal vein presumably because of its limited aqueous solubility and precipitation in the protonated form in the intestinal tract. The results of these metabolomic investigations show that antibiotic treatment has a great impact on the microbiome and that they may be greatly affected by microbial community structure and function. The finding suggests that through metabolic analysis it is possible to assess the bile acid pool composition shifts derived from microbiome perturbations. Since bile acids and the composition of the bile acid pool have been hypothesized to be related with several disease states, the investigations of these metabolites is of high significance in the field of toxicology.
Trivorex® is a polyvalent neutralizer and absorbent for chemical spills in industries and hospitals. It is a yellow powder including colored pH indicators. Trivorex® can neutralize both acids and bases and absorb both aqueous and lipophilic solutions. It is nonflammable and does not contain VOCs. None of its components are toxic or suspected carcinogens, and it does not contain any crystalline silica. In vitro tests have been performed in order to show its efficiency. It has been tested on 20 ml of standard corrosives and irritants at different concentrations. Capacity of Trivorex® was recorded based on the amount needed for absorption and neutralization. According to the European waste legislation (commission regulation EU N° 1357/2014 and commission decision 2014/935/EU), for a waste to be classified as nonhazardous, the producer has to prove the waste does not have any hazardous properties. This proof needs to be provided based on experimental tests or a study of the potential, or real danger of the mixture has to be provided. We report here a way to transform a chemical waste into a nonhazardous waste with Trivorex® neutralizing absorbent. This decancellation process has been, up until now, only possible for some corrosive chemicals (i.e., hydrochloric acid or sodium hydroxide). The decancellation process creates a safe waste that can be manipulated easily and safely by any operator. Laboratoire Prevor has developed a method to evaluate if a chemical can be neutralized by Trivorex® and the hazards of the created waste. It is then possible to ensure that the neutralized chemical can be evacuated as a nonhazardous waste with the waste code 15 02 03. This evaluation method has been validated by INERIS (French National Institute for Industrial Environment and Risks). The list of accredited chemicals is now available online. Based on these results, Trivorex® appears to be a good way to deal with dispersal of chemicals in industries and legally transform these wastes into nonhazardous waste due to the neutralization process.

**2146 Thrombogenicity Testing for Blood-Contacting Medical Devices in a Human Blood Loop: Continuing Model Validation**

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ISO 10993-4 thrombogenicity testing is widely used for meeting regulatory requirements for approval of blood-contacting medical devices. We have successfully developed an in vitro model using minimally heparinized ovine blood that has been successfully used in lieu of the NAVI model in recent submissions. This study describes the translation of the methods from the ovine assay to a human blood assay to enhance the predictability of clinical outcomes. Fresh human blood was collected from a healthy donor and heparin added to a final concentration range from 0.7 to 1.4 IU per mL of blood. Blood was introduced into the loop (total volume ~100 ml) no more than 4 hours after the completion of the draw. Activated clotting time (ACT) of the blood was measured prior to addition to the loops and after the exposure period. Up to 3 positive or negative controls (polyurethane medical grade tubing, ~10 cm in length) simulating catheter type devices were inserted into the loop. Each loop was filled with blood and air was removed and the loop was closed. The blood was circulated using a peristaltic pump at a flow rate ~0.5 L/min. Up to four loops were prepared for testing with positive or negative controls. After 4 hours, the loop sections containing the positive and negative controls were opened, photographed in situ and evaluated for the presence of thrombus in accordance with standard in vivo thrombogenicity scoring for acutely implanted devices. The percent of the total surface of the control articles covered by thrombus was determined (the primary scoring parameter). A thrombogenicity score was then assigned to each individual article based on % surface area using a scale of 0-5, where: 0 = thrombus present on <2% total surface area up to a score of 5 = thrombus present on >75% total surface area; more consistent with the ISO 10993-4 guidance. Typical scores using standardized positive and negative control materials were reproducible over several months of testing resulting in scores of 0-2 (0 to 25% surface area) for the negative controls and scores of 4-5 (51-100% of the article surface area covered with thrombus) for the positive control. A head-to-head comparison of the performance of the ovine and human blood assays using similar anticoagulation (minimal heparin) and identical test and control articles will allow a higher confidence in translatability of in vitro assays using animal blood (ovine or porcine) to predict clinical safety.
Chemical characterization (described in ISO 10993-18) refers to the determination of chemical identities and quantities of substances contained in a medical device. The results of these determinations are used to conduct toxicological risk assessments wherein the potential for patient harm due exposure to these substances are evaluated. Extractables studies, which are often chemically untargeted, are used to produce comprehensive and accurate information regarding potential leachable substances of a spectrum of chemical properties. The untargeted and chemically diverse nature of extractables studies and the need to achieve low levels of detection creates a challenge for the analyst. The current work is intended to establish improved methodology for untargeted chemical characterization. Selected materials include silicone tubing, polyvinyl chloride (PVC) tubing, ethylene propylene diene monomer (EPDM) rubber. These materials were extracted in hexane, iso-propanol, and water at a sample mass-to-extraction volume ratio of 45 mg/mL. Extractions were performed in a shaking incubator at 37 and 50 ºC in sealed scintillation vials at 200 rpm for 24 h and analyzed by direct injection on an Agilent 7890B-5977B GC/MS equipped with a 30 m DB-5MSUI column. Extractions were compared to matrix matched controls. Agilent Unknown Analysis software with the NIST 2014 library was used to perform chemical identification and scoring using the internal algorithm. Various compounds were detected in all extracts of the materials. Generally, the number of extractable compounds was greatest in hexane (40), followed by iso-propanol (3 to 16), and then water (1 to 6). While the number of compounds in water and iso-propanol appeared equivocal between 37 and 50 ºC, a consistent increase (range 140-218%) in the number of compounds in hexane at 37 degrees versus 50. Examples of individual identifications include various phthalates, pyrene, and benzothiazole. Investigation of semi-quantitative measurement of identified species is ongoing. The current results indicate the successful GC/MS work-up of three materials from extraction to data analysis. The work has highlighted the need to focus on method development in areas including extraction conditions, analytical methodology, identification assignment, semi-quantification of analytes, and statistical analysis.

Hydrogen peroxide (H2O2) is a chemical of toxicological concern because it elicits clinically relevant adverse health effects: embolism, hemo-lysis, and methemoglobinemia (Bek et al., 2009). To minimize risk from infections, hospitals have been reported to add silver-stabilized H2O2 to the facility water system, resulting in unintentional exposure of patients undergoing hemodialysis to H2O2, which caused toxicity (Davidovits et al., 2003). Maintenance of heater-cooler units (HCU) with 3% H2O2 for preservation against bacterial contamination within the HCU water circuit is also reported (Sax et al., 2015). HCU play a critical role in cardiovascular surgery by supplying temperature-controlled water to the cardiopulmonary bypass and cardioplegia heat exchangers in order to control patient temperature. Many HCU contain polymeric heat exchange fibers rather than stainless steel, and because of its small size, H2O2 may cross the semi-permeable membrane and adversely impact patients’ blood (Hoenich, 2009). To address this safety concern, a dose-response assessment was conducted to derive a parenteral provisional tolerable intake (pPTI) value below which additional risk control measures would be unwarranted to prevent toxicity. Embolism is identified to be the critical adverse health effect of H2O2 because the point of departure (POD) is lower as compared to other adverse effects. The critical adverse health effect study is by Lorincz et al. (1948), who administered H2O2 into rabbit blood up to 15 minutes, and reported the NOAEL for embolism to be 6 mg H2O2/L blood/min. To derive the pPTI value, uncertainty factors were applied that accounted for interspecies differences (i.e., 10) and reliability of the critical adverse health effect study (i.e., 100). Additional uncertainty factors (e.g., interspecies difference in potency) were not applied because (a) the mechanism by which H2O2 elicits embolism is thought to be the release of undissolved oxygen, and (b) H2O2 is rapidly converted within blood into undissolved oxygen; the latter is the toxicant for embolism. Based on the identified POD and uncertainties described, the TI for unintentional H2O2 exposure to blood circulation from HCU is calculated to be 6 µg/L blood/min, which is 1,000x lower than the NOAEL reported in Lorincz et al. study. Because the critical adverse health effect study does not meet current reliability criteria (e.g. ToxRTool), the pPTI should be verified.
Implantable medical devices can release metal ions that accumulate in peri-implant tissues. Although risk assessment approaches have been developed to assess the potential for adverse health effects to occur following the release of compounds from devices into the systemic circulation, it is difficult to predict the likelihood for toxic effects to occur in tissues adjacent to an implanted device. To overcome this problem, it may be possible to use in vitro cytotoxicity data as a surrogate for effects occurring in local tissues in vivo. The goal of this study was to compare the concentration of nickel (Ni) that results in decreased cell viability in vitro to the concentration of nickel that results in necrosis and inflammation in tissues surrounding an implanted Ni alloy wire. NOAEC values ranging from 3 to 15 µg Ni/mL were obtained following 24-hr treatment of L929 fibroblasts with concentrations of Ni chloride and Ni sulfate from 0.005 to 50 mM. Cell viability was assessed using MTT and Alamar Blue assays; morphology was evaluated using fluorescent microscopy. Previously published data have shown that Ni concentrations of 5-10 µg/g are associated with a mild inflammatory response in tissues 7 days after subcutaneous implantation of a Ni-alloy wire in rats; higher Ni concentrations in tissue closer to the implant produced severe inflammatory response and necrosis. The in vitro NOAEC for these effects was less than 5 µg Ni/g tissue. Similar values were obtained using a biofilm model to predict tissue concentrations of nickel in tissue adjacent to a nickel-containing implant. The concentration of Ni required to produce an inflammatory/necrotic response in tissues in vivo (5-10 µg/g) corresponds well to the concentration of Ni that results in decreased cell viability in vitro (greater than 3-15 µg/mL). As a result, in vitro cytotoxicity studies may serve as a promising alternative to predict the concentration of Ni that produces adverse effects in tissues adjacent to an implanted nickel-containing device.

Cytotoxicity of a Lithium Phenyl(2,4,6-trimethylbenzyl)Phosphinate (LAP) and Gelatin Methacryloyl (GelMA) Hydrogel in Human Kidney Primary Proximal Tubule Cells (hRPTEC)


Bioplotting is a type of 3D printing that uses photopolymers to encapsulate cells, and exposes cells to shear stress, UV light, and chemicals whose toxicity can be fully understood in vitro. Combining the photoinitiator LAP produces a photopolymer commonly used in bioplotting; however, unrelated monomer and/or the presence of photoinitiator can result in cytotoxicity. In this study, hRPTEC were exposed to extracts of GelMA hydrogels that were photoinitiated with the LAP concentration and UV exposure time. Rheological properties, cell viability, and cell proliferation were assessed to gauge the mechanical properties and cytocompatibility of the formulations. Samples of 10% w/v GelMA with 1, 0.5, 0.25, or 0.1% w/v LAP were exposed to 86, 342, or 855 mJ/cm2 of 365 nm light in a UV curing oven. Shear storage (G′) and loss moduli (G″) were measured for 20 mm diameter x 1 mm thick gels between 0.02 and 2% shear strain at 5 Hz and 37 °C. In addition, each sample was weighed and extracted in 10x the mass of cell medium at 37 °C for 24 h; extracts were frozen at -20 °C until use. The prototypic neuropathicostasins cisplatin (CisPt) and cadmium chloride (CdC12) served as positive controls at 10, 25, and 50 µM concentrations. hRPTEC were exposed to the extracts and positive controls for 24 h. Viability and proliferation were assessed using Alamar Blue and the CyQuant Direct Cell assay, respectively. Below 0.1% shear strain, G′ values ranged from 1385 to 19.5 Pa and G″ values ranged from 33.7 to 13.0 Pa for GelMA with 1% LAP at 855 mJ/cm2 and 0.1% LAP at 86 mJ/cm2 exposure, respectively. Both CisPt and CdC12 significantly reduced viability at 25 and 50 µM and reduced proliferation in a concentration-dependent manner. No significant effect on cell viability was found in extracts under the tested LAP concentrations or UV exposures; however, proliferation was reduced by 30% at the highest LAP concentration. No statistically significant difference in cell proliferation or viability was found among the UV exposures. The common GelMA-LAP combination was found to be cytocompatible albeit with a reduction in proliferation. Furthermore, gels were fabricated over a wide range of mechanical properties with minimal to no cytotoxicity. Therefore, GelMA gels photoinitiated with LAP show promise for use in acellular tissue engineering scaffolds.

An Assessment of Gender-Specific Risk of Implant Revision following Primary Total Hip Arthroplasty: A Systematic Review and Meta-Analysis

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Total hip arthroplasty (THA) has been a successful reconstructive procedure to mitigate pain associated with diseases of the hip joint. However, THA procedures require revision due to mechanical or biological failure. The purpose of this study was to synthesize and examine the evidence on the relative risk of revision in men and women following primary THA procedures. We conducted a systematic literature review of cohort studies reporting THA revision risk estimate by gender. Study quality scoring and a random effects meta-analysis were performed to estimate the meta-relative risk (meta-RR) and corresponding 95% confidence interval (95% CI) of revision, comparing men to women. Males had a statistically significant increased risk of revision following primary THA (meta-RR=1.33 (95% CI: 1.13-1.57)), when compared to females. When stratified by cause of revision, males had a statistically significant increased risk of revision due to any-cause (meta-RR=1.16 (95% CI: 1.01-1.33)), aseptic loosening (meta-RR=1.54 (95% CI: 1.05-2.25)), and infection (meta-RR=1.55 (95% CI: 1.11-2.15)). For primary THA operations performed during the 2000s, males in Europe had a statistically significant increased risk of revision (meta-RR=1.42 (95% CI: 1.25-1.61)) while males in the United States had a statistically significant decreased risk of revision (meta-RR 0.80 (95% CI: 0.72-0.89)). These results support evidence of gender-specific risks would help reduce post-surgery complications.
The four-hour canine non-anticoagulated venous implant (NAVI) model is a standard test procedure used to assess acute thrombogenicity of blood-contacting medical devices. The FDA final guidance (2016) on ISO 10993-1 recommends that thrombogenicity be assessed as part of a safety or functional study conducted in a relevant animal model, if such a study is generally conducted for a particular device type. This abstract describes using a porcine model to assess a percutaneous transluminal angioplasty (PTA) balloon catheter with a balloon coated with paclitaxel (drug) and a citrate ester (excipient). Acute thrombogenicity was first evaluated in a non-anticoagulated model using three swine. Test and control (commercially marketed comparator) devices were placed in the L and R femoral veins and exposed to blood for 6 hours. The animals were euthanized, and the 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 (ISO 10993-4 2017). Thrombogenicity scores were 3-4 (moderate to extensive thrombus) for the test articles and 2-3 (mild to moderate thrombus) for the control articles. In a subsequent study with clinically relevant levels of heparin as performed per the FDA guidance, results showed non-existent to minimal thrombus for both test and control articles. No thromboembolism was observed in the heart or lungs of any animal. These results support the use of the porcine model for NAVI and AVI testing and demonstrate the hemocompatibility of this device. In addition, these studies conducted on smaller balloon size were successfully used to support a device modification with longer balloon sizes (up to 150% longer). Future considerations for thrombogenicity testing of drug-device combination products should incorporate the topography of drug surfaces and geometrical features (folding patterns) in selecting the appropriate model (NAVI vs. AVI) for favorable outcomes.
that generate nano-sized chromium oxide particles are unlikely to result in cytotoxic or inflammatory responses in periprosthetic tissues, and 2) adverse biological responses likely occur only under suboptimal wear conditions that generate a sufficient dose of larger particles with greater amounts of cobalt.

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Shortly after 2010, some cohorts with well-fixed MoM THA devices started to experience elevated revision rates (above 1% per year). These reports coincided with a major shift towards the use of non-traditional revision criteria that consisted of blood metal measurements and non-radiographic soft tissue imaging. The purpose of this analysis was to evaluate the impact of the non-traditional diagnostic testing on the revision rate trends. We reviewed the available published studies that reported revision rates for MoM THA devices and stratified the results by 1) recalled or discontinued MoM THA devices with mechanical challenges, 2) well-fixed MoM THA devices that received traditional patient management (hip scores and radiographs), and 3) well-fixed MoM THA devices that received non-traditional patient management (blood metal testing and soft tissue imaging). Discontinued or recalled devices with design issues had high revision rates, with 75% of studies (21/28) reporting greater than 1% revisions per year. Revision rates for well-fixed MoM THA devices that received traditional patient management consistently showed lower revision rates: 100% of the studies (44/44) reported estimated revision rates of 1% or less per year. In contrast, patients with well-fixed MoM THA devices that were evaluated with blood metal testing and soft tissue imaging consistently had high revision rates, with 79% of studies (15/19) reporting greater than 1% revisions per year. Our review found that some patients with well-fixed implants underwent revision operations even when symptoms (e.g., pain, discomfort, instability) were mild or absent. We conclude that the recent increase in revision rates for well-fixed MoM implants is due to a divergence in revision criteria and does not appear to be related to any actual changes in MoM implant performance (e.g., higher rates of implant loosening due to high wear). The implications of these results are discussed with regards to patient management as well as specificity and sensitivity of diagnostic testing for local tissue responses.

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Characterizing Cytotoxic and Inflammatory Responses to Metal-on-Metal Wear Debris from Normal Versus Edge-Loading Conditions

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The majority of previous studies that evaluated inflammatory and cytotoxic responses to wear debris from metal-on-metal implants utilized particles with physical and chemical characteristics that differed from clinically relevant particles released in vivo from MoM implants under normal wear conditions. Additionally, no previous study has attempted to understand the potential difference in biological responses to clinically relevant MoM wear debris generated under normal versus abnormal (e.g., edge-loading) conditions. The goal of this study was to understand the potential cytotoxic and inflammatory response to clinically relevant MoM wear debris generated during normal versus edge-loading conditions. Wear debris particles from each condition were isolated from Pinnacle MoM simulator fluid and sterilized prior to biological testing in a murine macrophage cell line (RAW 264.7). Particles generated from edge-loading conditions were larger and contained more cobalt (Co) compared to particles from normal wear conditions. Particles and ions from normal implant conditions up to 1021 cycles per mL (1000 ppb total Co and chromium (Cr)) did not induce significant toxicity or inflammation as detected by MTT assay and TNF-a cytokine release, respectively. In contrast, particles and ions released from edge-loading conditions induced significant cytotoxicity and TNF-a cytokine release when RAW 264.7 cells were exposed to 1021 cycles per mL (61124 ppb total Co and Cr) for 24 hours. The 25% inhibitory concentration (IC50) was approximately 26,000 ppb (Co and Cr) was calculated for the metal concentration which resulted in a 25% decrease in cell viability. The IC50 value for edge loading particles and ions was over 150-fold greater than metal levels observed in synovial fluid in well-functioning implants. These results indicate that 1) normal wear conditions...
antioxidant potential in BEAS-2B cells, pretreatment with a "non-toxic" HEMA dose for 24 hours did not reduce the toxic response to a subsequent HEMA exposure. In summary, the results indicate that HEMA induced the Nrf2 activity in BEAS-2B cells, while the Nrf2 pathway was continuously active in A549 cells. However, the results do not support a hypothesis suggesting that increased Nrf2 activity protects cells against HEMA-induced toxicity. This may point to mechanisms independent of oxidative stress to explain HEMA toxicity.

2163 Extractable Characterization of Critical Components Used in Cartridges of Electronic Nicotine Delivery Systems
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Electronic Nicotine Delivery Systems (ENDS) contain liquid formulations (e-liquids) that are heated to generate aerosols. The potential of chemicals to leach from the device materials has been an area of interest for this type of consumer product. ENDS cartridges are commonly composed of polymers, elastomers, metals, glass, and electronic components. Characterization of extractables from such materials can be used to establish a repository of potential analytes for leachable testing. In this study, an extractables analysis was conducted on critical components from a "cig-a-like" ENDS product. Critical components are defined as components that are in contact with the e-liquid, aerosol, or surrounding critical components were solvent extracted under exaggerated temperatures and conditions with consideration of the e-liquid formulation in selection of the solvents. Extraction methods included elevated temperature sealed vessel, reflux, and Soxhlet extraction. Extraction solvents included low-pH water (pH = 2.5), high-pH water (pH = 9.5), isopropanol, and methylene chloride for organic extractables. Nitric acid (5%) was used as a solvent for metals/elements. Additionally, the components were placed in sealed vials for headspace analysis of volatile organic substances. The resulting extracts were chemically characterized via spectroscopic and chromatographic methods to establish the metal/elemental and organic extractable profiles. These analyses provided semi-quantitative extractable profiles of the ENDS critical components. Characterization of the extractables from materials of ENDS supports verification of materials selection, manufacturing processes, as well as development of a repository for potential future leachable assessments.

2164 Presence of Particulates and Color in Device Extracts: Do They Really Pose Any Safety Risk to Patients?

For in vitro test systems. The device extract is often found to be colored and/or particulates leading to uncertainties in assessing their biological significance. A polyurethane catheter upon extraction at 50°C for 72h showed visible green color in the sesame oil but not in the saline extract. The colorant was identified by UV-Vis method and not by the conventional GC-MS or FTIR. Since this is a urethral stent, the extractions were repeated in a physiologically relevant solvent—synthetic urine. Results showed no visible color leaching in synthetic urine, indicating that the pigment is not bioavailable and thereby supporting the safety of the device. Wire guides are stainless steel metallic structures, which facilitate the placement of catheters used in cardiology and angiographic procedures usually for a period of 30 minutes. During biocompatibility testing devices that were cut into sections for extraction in saline (70°C for 24h) were found to contain particulates. Chemical analysis by ICP-MS showed the presence of zinc, iron, or silicone, and their amount per device was found to be below the permissible daily exposure levels. It is pertinent to note that during normal clinical use, the device is not cut. Therefore, studies were repeated with intact devices for extraction at 37°C for 6h. The results indicated no visible particulates with passing biocompatibility test results. Further, when the wire guides were analyzed using an in vitro model with flow in a mock blood vessel, the size and number of particulates were within the limits of USP <788>, supporting the safety of the product. Finally, the saline extract (50°C for 72h) of a blood-contacting catheter was found to be orange-brown in color due to the presence of minocycline and rifampicin. In the in vitro assay, the extract was non-hemolytic after the absorbance values were corrected for background interference. In summary, the visible particulates and color in test extracts sometimes come from undue manipulation of test article and/or overly extreme extraction conditions.

2165 In Vitro Assessment of Medical Device Extracts’ Potential to Produce Skin Sensitization
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The ISO norm 10993 part 10 describes the procedure for the assessment of medical devices and their constituent materials with regard to their potential to produce irritation and skin sensitization. The success of the recent Round Robin Study to assess in vitro skin irritation of medical device extracts, with Reconstructed Human Epidermis (RHE) models, opened the way to in vitro approaches for this endpoint. For skin sensitization of medical devices, polar (physiological saline) and nonpolar (sesame oil), were spiked with different concentrations of sensitizer and successfully detected with SENS-IS assay in the Episkin model and the SkinEthic RHE model. Then, samples of polymers used in medical device industry, MED-2000 silicone, were loaded with 6 known sensizers (10% final concentration): 1-phenyl-1,2-propanedione, 1-Chroro-2,4-dinitrobenzene, Diethyl maleate, p-Benzoquinone, Propyl gallate and Phenyl Benzoate and extracted in polar and nonpolar solvents in accordance with ISO 10993-12:2012. After optimization of the original protocol used for near chemical methods, the SENS-IS method was able to correctly classify the 6 polymers. These preliminary results show that the Episkin model and the SkinEthic®-RHE model could be used in the SENS-IS assay to assess the sensitizing potential of medical devices extracts. Further studies are engaged with a more comprehensive set of molecules. If confirmed, these results would allow toxicologists, familiar with either the Episkin or the SkinEthic-RHE model to assess skin sensitization with the SENS-IS assay.

2166 Fluoropolymers: Biomaterials That Satisfy Global Polymer of Low Concern Criteria

Fluoropolymers, common biomaterials used in medical devices, are high-molecular-weight polymers within the per- and polyfluoroalkyl substances (PFAS) group of fluorinated substances. PFAS are currently in the focus of researchers and regulators due to widespread presence of per- and polyfluorinated sulfonate (PFOS) and perfluorooctanoic acid (PFOA) in the environment and biota, including humans. Fluoropolymers, such as polytetrafluoroethylene (PTFE), have unique properties that constitute a distinct class within the PFAS group. Fluoropolymers have thermal, chemical, phototoxic, hydrolytic, oxidative, and biological stability. They have negligible residual monomer and oligomer content and low to no leachables. Fluoropolymers are practically insoluble in water and not subject to long-range transport. With a molecular weight well over 100,000 Da, fluoropolymers cannot cross the cell membrane. They are not bioavailable or bioaccumulative, as evidenced by biocompatibility studies on PTFE: acute and subchronic systemic toxicity, irritation, sensitization, local toxicity on implantation, cytotoxicity, in vitro and in vivo genotoxicity, hemolysis, complement activation, and thrombogenicity. Clinical studies of patients receiving permanently implanted PTFE cardiovascular medical devices demonstrate no chronic toxicity or carcinogenicity, or reproductive or developmental or endocrine toxicity. This poster includes fluoropolymer biocompatibility, human clinical and physical-chemical-thermal-biological data to show that fluoropolymers satisfy globally recognized assessment criteria to be considered as “polymers of low concern”. Fluoropolymers are distinctly different from other polymeric and non-polymeric PFAS and should be separated from them for hazard assessment or regulatory purposes. Grouping fluoropolymers with all classes of PFAS for read-across or structure-activity relationship assessment is not scientifically appropriate.
As part of a toxicological risk assessment, an analysis of leachable substances was conducted for a ventricular catheter, which is intended for use in patients to drain CNS fluid that contributes to excess intracranial fluid pressure. To capture leachables that may be released during long-term implantation, a chemical characterization was performed using prolonged extractions, along with non-volatile residue (NVR) as a surrogate to monitor the endpoint of exhaustive release of organic chemicals. Separated extractions were conducted with solvents of three different polarities: isopropanol and hexane, which exhibit somewhat nonpolar characteristics, yielded much higher extractable masses of organic analytes—chiefly silicones—than purified water. Based on the results of NVR analysis, concentrations of individual chemical analytes were measured following a single, prolonged solvent extraction of the device. With isopropanol, successive extractions demonstrated exhaustive conditions were achieved (based on NVR levels <10% of the initial extracted amount) after three 72-hour extractions at 50 degrees C, with solvent removal and analysis of NVR after each step. The NVR data exhibited an asymptotic depletion curve, consistent with previous GC/MS analysis results from consecutive extractions. To evaluate whether the prolonged, continuous extraction was exhaustive, the leachable NVR from 3 consecutive 72-hour extractions was compared to NVR measured from a single, prolonged extraction covering the same time frame (216 hrs). A statistical comparison found that leachable NVR was significantly higher with three successive extractions as compared to that observed following a single prolonged extraction, and suggests that solvent replenishment with successive extractions is necessary to overcome the influence of the polymer/liquid partition coefficient. These results will be discussed in the context of the physical-chemical properties that can limit leachability, e.g., analyte solubility, polymer swelling, diffusion rate, and equilibrium partitioning. Based on these findings, the total leachable analyte mass as determined with a single, prolonged extraction was adjusted by a factor of 1.24, equal to leachable NVR measured with multiple, successive extractions divided by that measured with a single prolonged extraction. Use of this adjustment assured that leachability was not underestimated when evaluating risks based on prolonged extraction data.
sure to arsenic. However, the long-term exposure resulted in increased growth of HK-2 cells as compared to control HK-2 cells. Additionally, the arsenic exposed cells also acquired morphological changes of EMT and stemness. These changes were further confirmed by analysis of gene expression in arsenic exposed HK-2 cells. Most importantly, the arsenic exposed cells acquired the pathogenic features of fibrosis as supported by fibrotic morphological changes as well as the changes in expression of pro-fibrotic genes. These changes in growth pattern and EMT properties of arsenic exposed cells were reversed by the treatment with epigenetic reagent, 5-Aza-2’-deoxycytidine. Therefore, the findings of this study suggest that acute exposure to arsenic can cause loss of kidney epithelial cells with potential significance to acute kidney injury (AKI), whereas the chronic exposure to arsenic can increase the risk of chronic kidney diseases specifically the kidney fibrosis. Additionally, our data suggests that the adverse effect of arsenic in kidney epithelial cells is mediated by epigenetic mechanism and therefore potentially can be reversed by epigenetic therapeutics.

**2172 Arsenic Disrupts Muscle Stem Cell Determination through Fibroblast Mitochondrial Maladaptation that Directs a Dysfunctional Extracellular Matrix Memory**

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Chronic arsenic (As) exposure increases risk of adverse health outcomes that include skeletal muscle dysfunction and mobility decline. We reported that As impairs muscle maintenance and regeneration by inducing maladaptive mitochondrial phenotypes in muscle stem cells (MuSC), connective tissue fibroblasts (CTF), and fibers. We also found that As impairs muscle functional memory in extracellular matrix (ECM) that disrupts the MuSC niche and misdirects differentiation of muscle progenitor cells from myogenesis to fibrogenesis. We asked whether dysfunctional mitochondria in As-exposed CTF direct ECM alteration, and whether restoring CTF mitochondrial function reverts ECM memory. CTF were isolated from mice exposed to 0 or 100 μg/L As(III) in drinking water for 5 weeks. The CTF were cultured to elaborate ECM for 2 days before decellularization. Human muscle progenitor cells (HMPC) were seeded on the decellularized ECM and differentiated for 2 days. Myogenesis was impaired when HMPC were seeded on ECM derived from As-exposed CTF, relative to control CTF. In addition to this, desmin, indicators of myogenic differentiation, were downregulated in As-CTF plated on As-CTF ECM, while PDGFRα and CD34, indicators of fibroblast/adipocyte progenitors, were increased. To demonstrate that As-impaired mitochondrial function in CTF is responsible for a pathological ECM, the MuSC were treated with 500 μM As(III) for 7 days. To verify that As-impaired muscle differentiation and muscle regeneration in vivo. Our data indicate that As impairs muscle maintenance and regenerative capacity by targeting CTF mitochondria, and that therapies restoring muscle mitochondria may effectively treat muscle dysfunction in As-exposed individuals. Supported by NIH grants R01ES023696, R01ES023696.S1, and R01ES022529.

**2173 Selene Restores the Pax6 Gene Expression in Arsenic-Exposed Neuronal Cells of Golden Syrian Hamster, Mesocricetus auratus**

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Arsenic is an environmental pollutant distributed worldwide that generates public health concern. Various types of cancers and other diseases, including neurologic disorders, have been associated with arsenic consumption in drinking water in human populations. At a molecular level, arsenic and their metabolites have the capacity to provoke genome instability, causing an altered expression of genes. Pax6 gene, which encodes a transcription factor, is a target of arsenic in neuronal cells. The aim of this study was to evaluate the effect of antioxidants, as α-tocopherol succinate (alfa-TOS) and sodium selenite, on gene expression levels of Pax6 in the brain and cerebellus of Golden Syrian hamsters exposed chronically to arsenic in drinking water. Reverse-transcription coupled to quantitative PCR confirmed that arsenic downregulates the Pax6 expression in nervous tissues. Treatment with α-TOS did not modify Pax6 expression in presence of arsenic; however, the combined treatment of sodium selenite with arsenic restored the Pax6 expression in brain and cerebellus of arsenic-exposed hamsters. Although the chronic exposure to arsenic can increase the risk of chronic kidney diseases specifically the kidney fibrosis. Additionally, our data suggests that the adverse effect of arsenic in kidney epithelial cells is mediated by epigenetic mechanism and therefore potentially can be reversed by epigenetic therapeutics.

**2175 Suppression of Erythroid Progenitor Cell Development by Environmentally Relevant Levels of Arsenic**

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A high prevalence of anemia has been reported in human populations chronically exposed to arsenic (As) in drinking water. However, the mechanistic basis for this association has not been fully elucidated. Our recent findings showed that 60 day drinking water exposure of adult male C57BL/6J mice to environmentally relevant levels (500 ppb) of arsenite (As3+) suppressed the development of early bone marrow erythroid progenitor cells and resulted in anemia. Therefore, the purpose of this study was to evaluate the in vitro effects of As3+ in isolated cell culture in suppressing cell proliferation, viability, differentiation and maturation of primary mouse bone marrow erythroblasts. A significant dose-dependent suppression in the colony-forming ability of the earliest stage of committed erythroid differentiation, burst-forming unit erythroid cells (BFU-E), was found at concentrations as low as 100 nM As3+ and monomethyarsonous acid (MMA3+). Additionally, using primary mouse bone marrow cells, we developed an in vitro differentiation model to monitor erythroid maturation based on progressive changes in CD71 and Ter119 surface marker expression utilizing flow cytometry. After 24 h, a significant accumulation of the earliest stages of erythroid differentiation (i.e., BFU-E/colony-forming unit erythroid cells) as well as a subsequent decrease of the immediate down-
Arsenic (+3 Oxidation State) Myelotransferase (AS3MT) Genotype Is Associated with Metabolites That Are Linked to Diabetes Susceptibility in Individuals Exposed to Arsenic in Chihuahua, Mexico

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The enzyme Arsenic-3-Methyltransferase (AS3MT) is responsible for the metabolism of inorganic arsenic (iAs). Polymorphic variants of AS3MT are associated with increased risk of iAs-associated diabetes mellitus (DM). At present, it is unclear what metabolic processes are influenced by AS3MT impacting the risk of DM development in the context of iAs exposure. To investigate the role of AS3MT single nucleotide polymorphisms (SNPs) as a susceptibility factor in the development of DM, we integrated genotype and metabolomics data from DM and non-DM individuals exposed to iAs. The 123 profiled individuals come from the Chihuahua Cohort in Mexico. To characterize metabolite profiles in relation to six SNPs in AS3MT linear regression modeling was used. The results demonstrate that four SNPs, rs17881215, rs3740393, rs3740390 and rs1928353, were associated with 12 urinary and plasma metabolites. Among the identified metabolites, an enrichment for, amino acid metabolism was identified. Interestingly, the enriched metabolites included phenylalanine, arginine and methyl-L-histidine which have been previously linked to blood sugar control in humans and delay of suppression of liver gluconeogenesis. This novel integration elucidates a potential mechanistic basis for the findings that AS3MT polymorphisms are associated with risk of DM development in iAs exposed populations.

2178 Requirement of NADPH Oxidase Activity for Arsenic Inhibition of PARP-1

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Human epidemiological studies show a correlation between arsenic and ultraviolet radiation (UVR) exposure with respect to the development of skin cancer. Previous studies have shown that exposure to environmentally relevant arsenic levels, particularly in conjunction with UVR exposure, results in oxidative damage to DNA and proteins via the generation of reactive oxygen species (ROS). However, arsenic and UVR generate ROS by different mechanisms, with evidence supporting arsenic induced ROS via stimulation of NADPH Oxidase (NOX). Additionally, it has been shown that exposure to arsenic can result in the inhibition of poly(ADP-ribose) polymerase-1 (PARP-1) activity, resulting in retention of DNA damage. Together, the evidence supports the hypothesis that arsenic hinders the function of specific DNA repair proteins, and consequently sustains DNA damage induced by UVR. To further elucidate the mechanism of arsenic co-carcinogenesis, it is necessary to determine the requirements for PARP inhibition and arsenic augmentation of UV-induced DNA damage via NOX. In this study, we used a selective inhibitor of NOX, apocynin, and a ROS scavenger, MnTMPyP, to determine the relative contribution of arsenic exposure on normal human neonatal epidermal keratinocytes (HEK). The PrestoBlue viability assay was performed on HEKn cells to confirm non-cytotoxic concentrations. We found that treatment of HEKn cells with apocynin or MnTMPyP ablated arsenic-stimulated ROS measured by DCF staining, decreased PARP oxidation, and partially restored PARP activity. It is predicted that the partial restoration of PARP activity will decrease retention of UV-induced DNA damage augmented by arsenic with studies underway. Current analysis includes in vivo studies of DNA damage retention in arsenic exposed p91phox/- mice. Due to the prevalence and severity of skin cancer, it is important to elucidate the mechanisms by which arsenic acts as a co-carcinogen in humans.

2179 Low Arsenic Concentrations Are Pro-Atherogenic in the Apolipoprotein E Knockout Mouse Model

D. Plourde, C. Chiavatti, M. Lemaire, C. Lemarie, S. Lehoux, and K. K. Mann. Lady Davis Institute, Montreal, QC, Canada.

In humans, arsenic exposure increases the risk of atherosclerosis, the gradual occlusion of the large arteries with fibro-fatty plaque. Epidemiologic data indicate that this may occur at low arsenic concentrations, near the maximum contaminant level of 10 ppb. We have previously shown that 200 ppb sodium arsenite increased the atherosclerosis in apolipoprotein E knockout (apoE-) mice after 13 weeks, but the effects of lower concentrations was unknown. Therefore, we analyzed the effects of oral exposure to sodium arsenite from 10-200 ppb after 13 weeks. Importantly, we found that even at the lowest concentration of arsenite, there was a significant increase in atherosclerotic plaque size in the aortic arch and in the aortic sinus. In our previous studies, we found that arsenite exposure resulted in decreased smooth muscle cells and collagen within the plaque. This change is indicative of a less stable phenotype that could increase the risk of rupture and subsequently, myocardial infarct or stroke. In addition, we observed that lipid increased within the plaque without concomitant increase in macrophage content, suggesting the macrophages were retaining more lipid intracellularly. We also assessed these plaque components in apoE-/- mice exposed to 10-200 ppb arsenite. Interestingly, we observed the macrophage lipid accumulation occurred at lower concentrations than the decreased smooth muscle cell/collagen content. Together these data suggest that in the apoE-/- model, low concentrations are pro-atherogenic and that macrophage lipid homeostasis is more sensitive to arsenite-induced perturbation than the smooth muscle cells.

2176 Requirement of NADPH Oxidase Activity for Arsenic Inhibition of PARP-1

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2180 Arsenic Inhibits Autophagic Flux by Disrupting STX17-SNAP29-VAMP8 Complex Formation

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Chronic exposure to arsenic has been linked to an increased risk of a number of diseases, including cardiovascular disease, diabetes, and cancer. While the adverse health effects associated with arsenic exposure are well known, the mechanisms underlying arsenic-associated disease pathogenesis remain unclear. As such, a better understanding of the pathways targeted by arsenic and its derivatives is much needed. Previous work in our lab has shown that environmentally relevant doses of arsenic can inhibit the autophagy-lysosome pathway, resulting in protein aggregation and the non-canonical activation of the antioxidant transcription factor Nrf2. Both the prolonged activation of Nrf2, as well as chronic autophagic dysfunction, can have pronounced effects on overall cellular function and metabolism, and thus may be key contributors to the progression of arsenic-linked pathologies. Here, we demonstrate that arsenic inhibits autophagy by disrupting formation of the STX17-SNAP29-VAMP8 SNARE complex, where SNAP29 mediates the fusion of STX17-containing autophagosomes with VAMP8-bearing lysosomes. Mechanistically, arsenic disrupts SNARE complex formation by increasing OGlcnAcylation of SNAP29, which has been shown to prevent the interaction between STX17 and VAMP8. Mutation of four key OGlcnAcylation sites in SNAP29, knockdown of OGT, the enzyme responsible for protein OGlcnAcylation, or pretreatment with rapamycin, a known activator of autophagy, can all rescue arsenic-induced autophagic dysfunction. Interestingly, arsenic can also disrupt autophagosome/lysosome and lysosome trafficking, inferring there may be multiple mechanisms by which arsenic affects the autophagy pathway. These findings reveal two distinct mechanisms by which environmentally relevant concentrations of arsenic can perturb proteostasis and alter cellular metabolism, providing a novel subset of therapeutic targets for disease intervention in people exposed to unsafe levels of arsenic.

2181 Effects of Embryonic Exposure to Arsenic on Hepatic IGF-1 Signaling

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Arsenic (As) is a naturally occurring mineral that often exists as a contaminant of food and drinking water. While the World Health Organization (WHO) has set a global drinking water standard of 10 ppb, there are areas of the world where water concentrations are much higher. Arsenic exposure has been shown to decrease birth weight and reduce weight gain during childhood, and impair skeletal muscle differentiation. One potential mechanism for the reduced growth is through interference of the insulin-like growth factor (IGF) pathway. Killifish (Fundulus heteroclitus) were exposed from fertilization until hatching to 0, 10, 50, and 200 ppb arsenite. After hatching, they were transferred to clean water and grown out to 8, 16, 28, and 40 weeks to assess changes in growth, arsenic metabolism, and transcript levels of hepatic IGF-1 and IGFBP-1. As they age, hepatic IGF-1 and IGFBP-1 expression greatly increased, allowing the exposed fish to compensate and catch up with their control counterparts. As they age, hepatic IGF-1 and IGFBP-1 expression greatly increased, allowing the exposed fish to compensate and catch up with their control counterparts. However, IGF-1 and IGFBP-1 mRNA levels remain significantly elevated, even 40 weeks after the exposure has ended.

2182 SPARC in a Cell Culture Model of Arsenic and Cadmium-Induced Bladder Transitional Cell Carcinoma

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Bladder cells that have been exposed to long-term, low doses of either arsenic (As^3+) or cadmium (Cd^2+) resulted in significant repression of SPARC (Secreted Protein Acidic and Rich in Cysteine). This metal exposure gave rise to 13 independent transformed cell populations with SPARC levels at or below detectable limits. SPARC was the most repressed gene in the microarray analysis across all of these cell lines. This expression change indicates a possible fundamental role for SPARC in order for cell transformation to occur. SPARC has been shown to have tissue-specific roles and acts as either an oncogene or a tumor suppressor dependent upon the tissue being studied. Its role in bladder and bladder cancer is unknown. SPARC is known to interact with collagen and inhibit integrin receptor signaling pathways affecting cell adhesion, proliferation, and survival. Thus, SPARC’s role in cell attachment and spreading of the As^3+ or Cd^2+ transformed cell lines was assessed. Cells were seeded on collagen, and cell attachment and spreading was observed every 15 minutes then analyzed using Fiji and LASX software from NIH and Leica. Preliminary results show that cell attachment appears to be delayed or deterred in cells expressing SPARC compared to metal transformed cells not expressing SPARC. Results also show a tendency for increased spreading in SPARC expressing cells when compared to the non-SPARC expressing counterparts. These results suggest that SPARC is binding to collagen and delaying or deterring cells from attaching; however, once attached SPARC appears to promote cell spreading. This indicates that SPARC may play a critical role in the metastatic ability of bladder tumor cells. Ultimately, SPARC may be initially acting as a tumor suppressor in bladder cancer induced by exposure to As^3+ or Cd^2+ suggesting its need to be repressed.

2183 Arsenic Enhances Influenza Virus Infection in MDCK Cells

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Arsenic is a ubiquitous environmental toxicant that has been associated with both infectious and non-infectious human respiratory diseases. Published studies in mice revealed that exposure to arsenic through drinking water alters the antiviral immune response, increasing susceptibility to influenza infection. Given the well-known impact of arsenic on epithelial cell biology, we posited that arsenic exposure could increase the infectivity of respiratory epithelial cells to viral infection. In this study, we have characterized influenza virus A/H1N1 infectivity in arsenite-exposed (29 weeks) and unexposed MDCK cells. We assessed the production of viral proteins in MDCK using western blotting and quantified viral mRNA levels using qRT-PCR. We observed a 9.5-fold increase in viral matrix (M2) protein and a 0.35-fold increase in viral mRNA levels in arsenite-exposed MDCK compared to unexposed MDCK (48h post-infection). Using plaque assay, we tested infectivity of influenza A virus in arsenite-exposed and unexposed MDCK. Arsenite exposure resulted in 66.18% increase in number of plaques, 125.53% increase in plaque area, and 40.55% increase in plaque size in arsenite-exposed MDCK compared to unexposed MDCK (48h post-infection). Using plaque assay, we tested infectivity of influenza A virus in arsenite-exposed and unexposed MDCK. Arsenite exposure resulted in 66.18% increase in number of plaques, 125.53% increase in plaque area, and 40.55% increase in plaque size in arsenite-exposed MDCK compared to unexposed MDCK (48h post-infection). Additionally, chronic exposure to arsenic reduced the efficacy of anti-viral drug Oseltamivir. Arsenite-exposed MDCK treated with Oseltamivir also showed increase in plaque number, plaque area, and plaque size (48h p.i.). Taken together, we demonstrated that MDCK cells chronically exposed to 1 μM of sodium arsenite for 29 weeks showed increased susceptibility to influenza A virus infection. This study could help reveal new potential mechanisms through which arsenic increases the risk of influenza virus infection in exposed human populations.
Exposure to inorganic arsenic (iAs) is a persistent global public health problem. An individual’s iAs metabolism is a critical factor in determining susceptibility to iAs-induced diseases. Recent population-based studies have shown that obesity may influence iAs metabolism, with higher body mass index (BMI) associated with a greater proportion of dimethylated iAs in urine (%DMAs). This study aimed to characterize the association of obesity with urinary iAs metabolites, namely inorganic arsenic (i-As), urinary monomethylated arsenic (U-MMAs) and U-DMAs. We analyzed cross-sectional associations between BMI and each urinary metabolite using a cohort of 1,166 iAs-exposed adults recruited from 2008-2013 in Chihuahua, Mexico. Potential covariates were sex, age, smoking, alcohol consumption, education, and drinking water iAs. All analyses were done using multivariable linear regression models. In the overall cohort, the median (IQR) BMI was 28.4 kg/m² (7.4). The median (IQR) proportion of metabolites was 8.9% (5.8), 14.1% (6.8) and 76.6% (10.8) for U-As, U-MMAs, and U-DMAs, respectively. In unadjusted models, the change in the proportion of urinary iAs metabolites was -1.08% (95% CI: -1.42, -0.75) for %U-iAs, -1.53% (95% CI: -1.83, -1.22) for %U-MMAs, and 2.61% (95% CI: 2.12, 3.10) for %U-DMAs per 1-SD increase in BMI. After adjusting for confounders, the estimated change in the proportion of urinary metabolites associated with a 1-SD increase in BMI was -0.95% (95% CI: -1.30, -0.59), -1.31% (95% CI: -1.64, -0.98) and 2.26% (95% CI: 1.74, 2.79) for %U-As, %U-MMAs, and %U-DMAs, respectively. In support of previous studies, these findings indicate that obesity is associated with a decrease in %U-iAs, %U-MMAs, and %U-DMAs and higher excretion of %DMAs. This suggests that obesity may alter iAs metabolism, and therefore, may alter susceptibility to iAs-induced disease.

Inorganic arsenic (As) exposure is a global public health problem. Chronic exposure to iAs, either through drinking water or food, has been associated with increased risk of disease. Metabolism of iAs occurs through sequential methylation steps catalyzed by arsenic (+3 oxidation state) methyltransferase (As3mt), S-adenosylmethionine (SAM) provides methyl groups for this reaction. Differences in iAs metabolism are associated with differing risks of diabetes and efficiency of iAs metabolism has been linked to dietary intake of folate, an essential substrate for SAM synthesis. The goal of this project was to determine if modifying iAs metabolism using genetic (As3mt-knockout) or nutritional intervention (folate intake) would modify the diabetogenic effects of iAs exposure. We also assessed the combined effects of iAs exposure and a high-fat diet (HFD). 7-week old male and female C57BL6 (WT) and As3mt-KO mice were exposed to 100 ppb As in drinking water for 37 weeks. Mice were fed a purified low-fat diet (<50 ppb As) with either low (0.2 mg/kg) or high (10 mg/kg) levels of folate for 24 weeks, followed by a HFD with the same low/high folate levels for 8 weeks. Metabolic phenotype and body composition were examined after 24 weeks of iAs exposure on the low-fat diet and after 8 weeks of iAs exposure on HFD. After 24 weeks, As3mt-KO mice gained more fat and had higher fasting plasma insulin compared to WT mice, regardless of exposure. Interestingly, male KO mice were more insulin resistant than female KO mice though there were no differences in %fat mass. At 24 weeks, iAs exposure had minimal effect on metabolic parameters in WT mice. High fat intake had little effect on iAs metabolism and metabolic phenotype. HFD for 8 weeks increased %fat mass in all mice and increased the difference in insulin resistance between WT and KO mice and between males and females. Notably, with HFD, iAs-exposed male KO and WT mice with low folate had higher %fat mass and insulin resistance compared to iAs-exposed unexposed controls. Our data suggest that folate intake and low, chronic iAs exposure have little effect on metabolic parameters in WT mice. However, iAs exposure may elicit adverse effects when combined with a HFD, highlighting potential toxicant-nutrient interactions. Male As3mt-KO mice are more prone than females to develop an adverse metabolic phenotype, possibly due to iAs exposure through diet or drinking water.
Inorganic arsenic (iAs) is a naturally occurring element that contaminates the groundwater in multiple countries worldwide. This is of public health concern because chronic ingestion of arsenic-contaminated drinking water increases the risk of multiple cancers, cardiovascular and respiratory disease, diabetes, and adverse developmental and neurological effects. Disruption of the endocrine system provides a possible mechanism linking iAs exposure to a vast array of disease processes. Early-life iAs exposure alters plasma glucocorticoid (GC) levels in adult mice. GCS are steroid hormones that have widespread effects on the metabolic, cardiovascular, immune, reproductive, and central nervous systems. Consequently, arsenic-related health effects may result in part from the effect of iAs on GC homeostasis. This is the first study to investigate the long-term effect of early-life iAs exposure on GC homeostasis in humans. Lifetime iAs exposure estimates and plasma samples were collected in 2013 from 101 subjects born during 1958-1970 and currently living in Antofagasta, a city in northern Chile historically exposed to drinking water containing an average iAs concentration of 860 μg/L over this 13-year period. Differences in plasma GC levels between individuals with high (N=54) versus low (N=47) early-life iAs exposure were measured using a novel cell-based bioassay. Individuals in the highest tertile of cumulative iAs exposure had lower plasma GC levels than those in the lowest tertile of exposure. A flatter diurnal GC slope was also observed among the highest cumulative exposure group. These results demonstrate that early-life iAs exposure may have long-term effects on GC homeostasis and highlights the potential endocrine disrupting effects of iAs in humans. Future studies should evaluate the role of GCS in the etiology of arsenic-related disease in order to inform interventions and policy aimed at reducing iAs exposure, particularly during early-life.

Manganese (Mn) is an essential metal and nutrient yet toxic in excess. Individuals at risk of Mn toxicity include patients receiving total parenteral nutrition supplemented with Mn, patients with liver cirrhosis, and miners and welders exposed to Mn rich fumes and particulates. In 2012, the first known inherited disease of Mn excess was reported in patients with mutations in SLC30A10, a Mn efflux transporter that is highly expressed in the liver, brain, and duodenum and hypothesized to be essential for Mn excretion. Characterized by increased blood Mn levels, dystonia, polycthemia (increased red blood cell counts), and liver cirrhosis, SLC30A10 deficiency is a novel disease that offers a unique opportunity to investigate systemic Mn regulation. The overall goal of this project is to establish the role of SLC30A10 in mammalian Mn homeostasis by generating and characterizing mouse models of SLC30A10 deficiency. Our studies indicate that mice globally deficient in Slc30a10 develop tissue Mn excess and polycthemia, similar to patients with SLC30A10 deficiency. As Mn is eliminated predominantly in the feces via hepatobiliary excretion, we hypothesized that hepatocyte-specific Slc30a10 deficiency would lead to a phenotype similar to that of global Slc30a10 deficiency. Surprisingly, mice with hepatocyte Slc30a10 deficiency have minimal tissue Mn excess. To explore the role of Slc30a10 in hepatobiliary Mn excretion, we employed a surgical approach in which we ligate the bile duct, cannulate the gallbladder, and inject 54Mn into the portal vein. From this surgery, we can determine the rate of 54Mn excretion into bile. Results indicate that global and hepatic Slc30a10 deficient mice have impaired hepatobiliary Mn excretion, suggesting that hepatic Slc30a10 is required for hepatobiliary Mn excretion. However, systemic 54Mn excretion studies show no impairment in fecal excretion. Instead, 54Mn absorption studies reveal increased Mn absorption in global Slc30a10 deficient mice, which may reflect a direct role for intestinal Slc30a10 in regulating Mn levels. Characterization of intestinal Slc30a10 deficient mice is underway. Understanding these mechanisms of Mn homeostasis is important for developing pharmacological treatment for both inherited and acquired Mn toxicity.

Manganese is an essential metal, but elevated levels are toxic. Loss-of-function mutations in SLC30A10, a manganese efflux transporter, cause a heritable disorder of manganese metabolism resulting in elevated manganese levels and motor deficits. To understand the consequences of loss of SLC30A10 function at the organism level, we generated Scl30a10 knock-out mice. During early development, knockouts were indistinguishable from controls. Surprisingly, after weaning and compared with controls, knock-out mice failed to gain weight, were smaller, and died prematurely (by 6-8 weeks of age). At 6 weeks, manganese levels in the brain, blood, and liver of the knock-outs were 20-60-fold higher than controls. Unexpectedly, histological analyses revealed that the brain and liver of the knock-outs were largely unaffected, but their thyroid exhibited extensive alterations. Since hypothyroidism leads to growth defects and premature death in mice, we assayed for changes in thyroid and pituitary hormones. At 6 weeks and compared with controls, the knockouts had markedly reduced thyroxine levels (50-80%) and increased thyroid-stimulating hormone levels (800-1000-fold), indicating that Scl30a10 knock-out mice develop hypothyroidism. A low-manganese diet produced lower tissue manganese levels in the knock-outs and rescued the phenotype, indicating that manganese toxicity was the cause. Our unanticipated discovery highlights the importance of determining the role of thyroid dysfunction in the onset and progression of manganese-induced disease and identifies Scl30a10 knock-out mice as a new model for studying thyroid biology.
Pb in soil can be a significant source of exposure to this toxic metal, particularly for young children with high levels of hand-to-mouth activity that increase soil ingestion. Among approaches used to reduce exposure to Pb in soil are remediation strategies that alter physical and chemical properties of Pb in soil. Remediation is undertaken with the goal of reduced bioavailability of Pb in ingested soil, although effects of remediation on Pb bioavailability are not commonly quantified. Here, we determined effects of several remediation strategies on Pb RBA using an untreated soil from a Pb-contaminated site in Joplin, Missouri, and the same soil that underwent *in situ* treatment with trisodium phosphate (TSP), with Iron Rich (Fe) and TSP (Fe/TSP), with compost (C) and TSP (C/TSP), or with rototilling (R) and phosphoric acid (PA) (P/RPA). Diets used in the mouse assay were prepared by addition of test soils to powdered purifiedAIN-93G rodent diet at 0.15, 0.3, and 0.6% (w/w) basis, yielding diets with ~ 3 to 25 ppm Pb. For these assays, young adult female C57BL/6 mice had free access to soil-amended diet for 9 days during which cumulative consumption of diet was monitored. At termination, blood, bone, and kidney were collected for determination of Pb contents. Using dietary soil level as the basis for comparison, remediation of the soil with TSP, Fe/TSP, and C/TSP significantly reduced Pb levels in blood, bone, and kidney. Analyses of relations between cumulative ingestion dose of Pb and levels of Pb in blood, bone, and kidney in mice receiving soil-amended diets provided tissue-specific estimates of Pb RBA for remediated soils relative to bioavailability of Pb from untreated soil. These tissue-specific RBA estimates for remediated soils were used to define a range of values for each treatment tested. Ranges of estimated fractional RBA were 0.35-0.77 for TSP, 0.2-0.5 for Fe/TSP, 0.33 to 0.65 for C/TSP, and 0.23-0.43 for R/P/PA treatment. Thus, remediation procedures applied to this Pb-contaminated soil reduced Pb RBA compared to that found in mice that received diets amended with untreated soil. The mouse assay should be a useful tool for evaluation of the efficacy of various soil remediation strategies and for assessing the performance and duration of treatment effects under field conditions. Disclaimer: The views expressed do not represent the views or policies of the US EPA.

**2193 Dynamics of Community Lead Dust and Children’s Blood Lead 10 Years after Hurricane Katrina in Metropolitan New Orleans**

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The sustainability of urban communities is associated with environmental quality. One factor negatively affecting environmental quality is lead pollution. A safe threshold for lead exposure in humans has not been identified and this is accompanied by the lack of an effective primary prevention of lead exposure. Previous research focused on 176 Census Tracts (communities) located in the city of New Orleans4. These were matched for soil lead and children’s blood lead in pre- and ten years post-Hurricane Katrina. This report extends the study to 268 communities in New Orleans. GIS tools were used to describe, and map the pre- and post-Katrina data. Comparing pre- and post-Katrina results, decreases in both soil lead and children’s blood lead response were observed. Disparities in lead exposure continue to exist between children living in the interior compared to children in outer communities of New Orleans. This investigation demonstrates that reduction of soil lead is beneficial in urban communities and an effective method for primary prevention of lead exposure.

**2194 Lead Levels in Eggs in Kabwe, Zambia: Implication of Human Exposure**

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Lead (Pb) is one of the earliest metals used by humans and the toxicity causes neurological symptoms and even death in the worst cases. Kabwe, Zambia is one of the World’s Most Polluted Places” reported in 2016. Our research group has been investigating heavy metal pollution in Kabwe and found high Pb accumulation in environmental and biota samples. The present study was undertaken to assess the levels of Pb in eggs and to investigate the human exposure through the consumption of eggs in Kabwe, Zambia. Eggs and soils around houses, where chickens are kept at, were collected in Kabwe, Zambia. Commercial eggs sold at local shops were also collected. The metals were extracted from shells, contents (yolk and white protein were mixed) of eggs and soils using microwave digestion system and the concentrations were analyzed by ICPMS. The mean Pb concentrations in local egg shells (n = 40) and commercial egg shells (n = 13) were 550 μg/kg and 0.7 μg/kg dry weight, respectively. The mean Pb concentrations in local egg contents (n = 37) and commercial egg contents (n = 13) were 310 μg/kg and 33 μg/kg dry weight, respectively. There was a significant correlation between Pb concentrations in shells and egg contents (p = 0.01, Spearman’s p = 0.9). Pb concentration in egg shells was significantly negatively correlated with thickness of egg shells (p < 0.01, r = -0.7) and whole weight of eggs (p = 0.01, r = -0.6). The mean Pb concentration in local eggs was 678 mg/kg dry weight. There were significant correlations between Pb concentrations in egg shells and soils (p < 0.01, r = 0.6) as well as egg contents and soils (p < 0.01, r = 0.8). The mean amount of Pb in the local egg contents was 0.01 mg per egg. Thus, current Pb levels of local eggs were lower than the level of tolerable weekly intake (2 μg/P). High Pb exposure in chickens could affect the reproductive function, the hatching rate and the survival rate of chicks negatively.

**2195 Hypoxia Signaling and Hepcidin Deficiency in Inherited Manganese Excess Due to slc30a10 Mutation**

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Manganese (Mn) is an essential metal, yet toxic in excess. The first disease of inherited Mn excess was reported in 2012 due to mutations in SLC30A10, a novel Mn transport protein. Such mutation in patients was presented with hypermanganesemia, dystonia, polycythemia (increased red blood cell counts) and cirrhosis. These phenotypes are attributed to Mn excess, however the exact molecular mechanisms have yet to be elucidated. To address this issue, the lab has generated a Slc30a10 knock-out mouse model that recapitulates several aspects of the human disease, including Mn excess and increased red blood cell counts. These mice also show increased Epo levels and decreased hepcidin levels, both known hypoxia-regulated genes. Gene expression analysis of liver tissues revealed up-regulation of 55% of hypoxia-regulated genes suggesting activation of hypoxia signaling. Mice also exhibited increased radiolabeled Fe absorption and tissue iron levels, consistent with hepcidin deficiency. Results from these experiments suggest that Mn excess can lead to a hypoxia mimetic state, in absence of true tissue hypoxia, leading to aberrant Epo levels, polycythemia, hepcidin deficiency, increased radiolabeled Fe absorption, and tissue overload of Fe.

**2196 Lead-Induced Cholesterologenesis and Alterations in 3-Hydroxy-3-Methylglutaryl Coenzyme A Reductase Activity in Rat**

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Lead poisoning is currently one of the most common disease of environmental origin and is increasing in developing countries. Exposure to lead induces a variety of biochemical responses. To investigate the effect of lead on cholesterol metabolism, male albino rats (n=34) were exposed to distilled water for the same periods. Total, HDL, VLDL, hepatic (200, 300 and 400ppm) for 4, 8 and 12 weeks. Control animals (n=18) received distilled water for the same periods. Total, HDL, VLDL, hepatic and hepatic micromolar, brain and brain micromolar cholesterol levels were determined spectrophotometrically. Lead accumulated to various levels in the tissues of the rats. Hypercholesterolemia was generally observed in the dose groups ranging from 5% (the 200ppm groups at 4 weeks) to 10% (in the 200ppm group at 8 weeks). Lead inhibited reverse cholesterol transport in a time-dependent manner as evidenced by decrease in HDL cholesterol (17% in 4-week 400ppm, 35%, 43% and 49% in 200ppm, 300ppm and 400ppm groups respectively at 8 weeks). Increased hepatic and brain cholesterologenesis was observed with highest increase occurring at 8 weeks; 41%, 39% and 94% for the liver and 57%, 54% and 25% for the brain. Hepatic and brain 3-Hydroxy-3-Methylglutaryl Coenzyme A (HMG-CoA) reductase activities were up-regulated in most of the dosage groups with highest increase (35%)
2197 Role of RE1-Silencing Transcription Factor (REST) in Manganese-Induced Repression of Excitatory Amino Acid Transporter 2 (EAAT2) in Astrocytes

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Chronic overexposure to manganese (Mn) induces a neurological disorder referred to as manganism, sharing pathological features with Parkinson’s disease (PD). Mn-induced excitotoxic neuronal death is considered one of the critical molecular mechanisms of Mn-induced neurodegeneration. The astrocytic excitatory amino acid transporter 2 (EAAT2) plays a pivotal role in preventing excitotoxic neuronal death by eliminating excess glutamate from the synapse. Previously, we have shown that Mn decreased the EAAT2 expression at the transcriptional level via the activation of Mn-dependent yin yang 1 (YY1) transcription factor. The repressor element 1-silencing transcription factor (REST) contains multiple YY1 binding sites in its promoter region and is known to be neuroprotective in Alzheimer’s disease (AD). In the present study, therefore, we tested if REST is involved in Mn-induced repression of EAAT2 in human astrocytes. We have found that Mn repressed the REST gene by decreasing its promoter activity and mRNA levels, suggesting that YY1-repressed REST mediate Mn-induced decrease in EAAT2 expression and function. We also found that chemical activation of epigenetic modifiers histone deacetylases (HDAC) 1 and 2 abolished the effects of REST on EAAT2 promoter activity, indicating epigenetic histone modification can influence on REST activity on EAAT2 gene regulation. Taken together, our findings suggest that REST is involved in Mn-induced repression of EAAT2 via activation of YY1. This result could contribute to finding molecular targets for the development of therapeutics to treat neurological disorders associated with excitotoxicity including AD, PD, and manganism.

2198 Blood Lead Levels Are Associated with Hemoglobin, Nitric Oxide Levels, and Proximity to Mining in Adults Residing in the Peruvian Highlands

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A significant positive association was observed between blood lead and hemoglobin (β = 0.10, p = 0.025). We also found a significant negative association observed between blood lead and hemoglobin (β = 0.47, p < 0.001). An inverse association was observed between blood lead and HDL cholesterol (r = −0.191, p = 0.01), while an inverse association was observed between blood lead and LDL cholesterol (r = 0.122, p = 0.001). These findings indicate that up-regulation of HMG-CoA reductase activity may mediate lead-induced hepatic and brain cholesterologenesis.

2199 Effects of N-Acetyl Cysteine on Mitochondrial Biogenesis in Lead-Mediated Neurotoxicity

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Exposure to lead (Pb) can lead to neurological pathologies such as cognitive impairment and Alzheimer’s disease. Increasing evidence supports the theory that brain injury due to high-level lead exposure is mediated, in part, by mitochondria dysfunction. However, the molecular mechanisms underlying neurotoxic effects following lower level lead exposure remain largely undefined. In these studies, we hypothesized that lead exposure to low doses of lead contribute to brain injury through mitochondrial malfunction. To identify genes with altered expression in response to lead exposure and important in mitochondrial functioning we examined changes in transcript levels of genes regulating mitochondrial biogenesis and repair in neuronal PC12 cells. In these studies we evaluated genes known to be causal to mitochondrial dysfunction using a PCR array platform; we analyzed expression of mitochondrial biogenesis genes, expression of their relevant transcriptional regulators and also employed a micro RNA (miR, miRNA) PCR array platform to analyze alterations to the expression of miRNA species. We found that Pb-exposure resulted in a >10-fold increase in specific mRNA species encoding solute carrier family 25 species, inner mitochondrial membrane peptidase-like (Immp1) protein, uncoupling protein 3 (mitochondrial, protein carrier) and superoxide dismutase 2 (MnSOD). We also found >10-fold decrease in expression encoding mitochoendrial biogenesis complex components including a distinct subset of solute carrier family 25 species as well as decreases in SOD1 gene expression. Using the miR PCR array platform we observed that four microRNA species miR-22-3p, miR-25-3p, miR-125b-5p, and miR-32-5p levels were increased more than 2-fold in lead treated PC12 cells. Interestingly, alteration of expression of these mammalian levels were decreased in the presence of the anti-oxidant drug N-acetyl cysteine (NAC). It was noteworthy that we also found a different pattern of microRNA expression in the presence of NAC. Taken together these results, indicate that NAC may mitigate neuronal changes following exposure to low levels of lead. We speculate that these results may provide important clues to approaches for preventing mental health consequences following lead exposure. Supported by NIH AR055073 (DEH).

2199a Exposure to MMA III Modiﬁes the Expression of Genes Involved in mRNA Methylation of 6 Adenosine (m6a) and Those Related to Cancer Stem Cells in UROtsa Cells


Among the mechanisms of gene expression regulation involving the post-transcriptional modification of mRNA, the N6-methylation at the adenosine (m6a) is the most prevalent. In mammals, such modification is catalyzed by a methyltransferase complex, which is composed by the methyltransferase-like 3 (MTTL3), methyltransferase-like 14 (MTTL14), and the Wilms’-tumor 1-associated protein (WTAP); the proteins involved in the removal of this mark are the fat-mass associated protein (Fuin) and the Alkb homolog 5 (ALKBHS). The m6a mark in mRNAs influences a variety of RNA processing steps including splicing, mRNA stability, and translation. Studies have shown that the tumor initiation, cancer progression, relapse, and resistance to therapy in many cancers are due to the existence of a population of abnormal stem cells, known as cancer stem cells (CSC). Interestingly, genes associated with pluripotency and lineage-specific differentiation are controlled by m6a levels; therefore, reduced m6a levels can lead to a mis-regulation of these genes and the acquisition of stem cell characteristics; hence all these enzymes’ expression is frequently deregulated in cancer. Human exposure to inorganic arsenic (iAs) is associated with bladder cancer development; UROtsa cells have been used as a model to reveal the involved molecular mechanisms. To evaluate the role of m6a mRNA in iAs-induced bladder cancer, in this work we evaluated the methyltrans-
Manganese (Mn) exposure from welding fume continues to be an occupational hazard for welders in the United States. Currently, the recommended threshold limit value (TLV) for respirable Mn in the United States is set at 0.02 mg/m³. While epidemiological studies suggest that this limit is not adequate, the risk of Mn exposure for welders is substantial, as evidenced by increased SOX2 and NANOG mRNA demethylation by FTO.

Manganese (Mn) is an essential bioactive metal that serves as a cofactor for numerous enzymes and kinases, such as glutamine synthetase and ATM. To study mechanisms of cellular Mn homeostasis, a practical and accurate method of intracellular Mn measurement (MRS), an observable difference in thalamic GABA (a major inhibitory neurotransmitter) was only seen between high-exposed (average 0.22 mg/m³) and low-exposed (avg. 0.13 mg/m³) welders (p<0.01), but not between low-exposed welders and controls. Trends were similar, but not significant, for R1 (an imaging parameter proportional to Mn accumulation in the region) between low-exposed welders, high-exposed welders, and controls in the substantia nigra (SN). We utilized a segmental regression analysis to assess whether a threshold for exposure to Mn over the past year prior to scanning may exist for these imaging markers. A Bayesian model was used to incorporate prior knowledge from past study results and the information in the current data set. The model shows that there exists a probable exposure threshold of approximately 0.075 mg/m³ (95% CI: 0.03 - 0.23) for changes of thalamic GABA to occur. Additionally, there exists a probable exposure threshold of 0.16 mg/m³ (95% CI: 0.075 - 0.225) when assessing the changes of R1 in the SN across all exposures in the cohort. This preliminary analysis shows that the relationship between exposure to Mn and its effects in humans is non-linear at these occupational exposure levels, and that a probable threshold for changes in imaging markers is around 0.16 mg/m³. However, the experience of the exposed welders generally suggests that Mn toxicity occurs in the brain.

Restless Legs Syndrome (RLS) is a common neurological disorders seen in ~10% of the US population. RLS-associated sleep deprivation can seriously impact life quality, causing anxiety, depression and attention-deficit/hyperactivity disorder (ADHD) symptoms. Moreover, RLS may portend hypertension, heart disease and stroke. RLS exhibits both familial and non-familial (idiopathic) forms, with ~60% of cases seen in ~10% of the US population. RLS-associated sleep deprivation can seriously impact life quality, causing anxiety, depression and attention-deficit/hyperactivity disorder (ADHD) symptoms. Moreover, RLS may portend hypertension, heart disease and stroke. RLS exhibits both familial and non-familial (idiopathic) forms, with ~60% of cases being the result of Fe deficiency or elevated concentrations of another metal that opportunistically increases when Fe levels are low. Here we present novel data that BTBD9 functions to regulate Mn homeostasis in Caenorhabditis elegans. A blast search identified hpo-9 as the BTBD9 homolog in C. elegans, with ~75% sequence similarity. A mutant strain tm3719 (hpo-9⁻⁰⁻⁻) carrying 761 bp deletion of hpo-9 was obtained. We found that hpo-9⁻⁻ worms were more sensitive to Mn exposure. Upon Mn treatment, hpo-9⁻⁻ worms showed a significantly lower survival rate and more severe DAergic neurodegeneration compared with wild type worms. Interestingly, no difference was seen when worms were exposed to Fe. However, a low level of Fe (0.1 μM) pretreat-
ment was able to protect Mn-induced lethality. To better characterize HPO-9 protein, a transcripntional fusion construct was created with green fluorescent protein (GFP) driven under hpo-9 promoter. We found that GFP was present high in the head and pharynx and low in the intestine and seam cells. Using a confocal microscopy, we found that hpo-9 was expressed in dopaminergic neurons, indicating that HPO-9 might play a role in dopamine signaling. To confirm that, we over-expressed HPO-9 in DAergic neurons of hpo-9/- worms and found that it rescued Mn-induced DAergic neurodegeneration. Together, our results suggest a novel role for hpo-9/BBT9 in regulating Mn homeostasis and possibly dopamine signaling in C. elegans. Supported by NIEHS ES010563.

2204 Expression of Matricellular Proteins following Neurotoxic Injury


The CNS extracellular matrix (ECM) influences molecular events critical for CNS development, synaptic plasticity, and injury repair. Many ECM elements (e.g. laminin, collagen) found in other tissues are transiently expressed during CNS development and are nearly absent in the adult brain. Matricellular components of the ECM support long term neuronal survival and also play a role within the adult neural stem cell niche by modulating the behavior of newly-generated cells, but their response to injury is poorly understood. Matricellular proteins including fibronectin, members of the SPARC superfamily (SPARC, hevin), tenascin-c, and Fibronectin were examined by immunofluorescence microscopy and qPCR in a toxicant model of hippocampal injury. Perineuronal nets (PNNs) and associated proteins were also examined. 8-week old C57BL/6 mice were given trimethyltin (TMT, 2.8 mg/kg, i.p.) then euthanized up to 30 days post-treatment. Immunofluorescence showed weak hippocampal expression of SPARC and hevin, which was unchanged by TMT injury across all time points. Tenascin-c was highly expressed in the rostral migratory stream of control and treated mice with weak deficits, but hippocampal markers showed no detectable alteration in expression after TMT treatment. Increased expression of thrombospondin-1 was detected in the dentate gyrus and molecular layer between 4 to 10 days post-TMT in a subpopulation of activated astrocytes co-expressing nestin. Wisteria floribunda agglutinin (WFA) and anti-aggregan antibody, markers of perineuronal nets, showed prominent labeling in the injured dentate gyrus at 96h post-treatment and remained elevated at 30 days. Staining with microglial markers (Iba1, Isoleucin B44) indicated co-localization with WFA+ cells in the dentate gyrus, in addition to increased WFA labeling by granule cell neurons. qPCR of hippocampal tissue showed a 2-fold decrease in transcription activity of tenascin-c between 12 and 24h post-TMT with return to baseline at later time points. SPARC mRNA was increased transiently between 48 and 96h post-TMT, while mRNA for other matricellular proteins showed no significant changes with injury. TMT induced a limited repertoire of ECMs in the CNS, which may help explain the modest repair of CNS tissues after chemical injury. Supported by ES-005022.

2205 Manganese Exposure Modulates AKT/mTOR Signaling by Acting Directly on Insulin-Like Growth Factor Receptors: Implications for Huntington’s Disease

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Manganese is essential to cellular function but toxic in excess. While studies have observed potent activation of critical cell signaling pathways by Mn exposure (AKT, mTOR, JNK, p38) how Mn activates these pathways is still unknown. Insulin-like growth factor 1 (IGF1) is a potent growth factor, similar to insulin, which can activate AKT/mTOR pathways. Interestingly, prior studies have shown that Mn and IGF1 may be coregulated in cell and mouse models. Furthermore, IGF1 may be coregulated in cell and mouse models. Additionally, models of HD exhibit decreased Mn uptake and Mn-induced signaling. In this new study we aimed to elucidate the relationship between Mn and IGF1 signaling in HD and determine whether Mn acts through IGF1 and its receptor, IGFR, to signal to AKT/mTOR. Using multiple cell models of HD (SThdh, PC12, human inducible neuronal cell lines), we have observed reduced Mn-induced phosphorylation of AKT and S6 (mTOR) in HD cells after 24hr exposures. Of a panel of seven other metals tested at similar concentrations in the SThdh model, this genotype-treatment interaction was specific to Mn. However, we found that the addition of Mn (50-200uM) to IGF1 (1-10nM) exposures results in additive responses to p-AKT(Ser473) and p-S6(Ser235/236) in all lines. Treatment with IGF1 and Mn also restores HD cell signaling. While Mn did not increase phosphorylation of IGFR, itself, the addition of Mn and IGF1 caused an additive increase in p-IGFR which suggest Mn increases the kinase activity of IGFR in the presence of saturating concentrations of its ligand, IGF, allowing increased autophosphorylation and downstream signaling. Using an siRNA knockdown (e.g. IRS-1) and pharmacological signaling. Using a BM3S63024 IuM approach, we isolated the molecular target of Mn to the IGFR receptor (IGFR) itself. Further, HD cells displayed basal impairments in Mn-induced IGFR signaling which mirrors the effects of pharmacological/genetic inhibition in WT cells. Together, our data substantiate a hypothesis in which Mn dyshomeostasis in HD may contribute to inadequate AKT/mTOR signaling via interactions at the IGFR.

2206 The Sidechain of Asn-43 of Human SLC30A10 Is Not Required for Manganese Efflux Activity in Hepatic and Neuronal Cell Lines

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Homozygous mutations in SLC30A10 lead to the development of familial manganese-induced parkinsonism. SLC30A10 functions as the cell’s primary manganese efflux transporter, and parkinsonism-causing mutations block its efflux activity. Interestingly, other transporters in the SLC30 family mediate zinc efflux. Thus, determining the mechanisms that confer manganese transport capability to SLC30A10 is essential to understanding its role in parkinsonism. We previously generated a predicted structure of SLC30A10, based on the crystal structure of the bacterial zinc transporter YiiP, and performed functional studies. In this study we established that residues corresponding to Zn binding residues in YiiP, were not all required for Mn efflux activity. Although alanine substitution of Asp-248 abolished manganese efflux, that of Asn-43 and Asp-47 did not, suggesting the mechanism of ion coordination in the transmembrane domain of SLC30A10 may be substantially different from that of YiiP/other SLC30 proteins. Furthermore, Nishido et al. published differing findings on the manganese efflux activity of SLC30A10. The authors expressed human SLC30A10WT or mutants in a chicken cell line and assessed transport activity of SLC30A10 mutants indirectly via cell viability. In this system, Asn-43 was shown to be required for manganese efflux activity, while when mutated to histidine, to mimic other Zn-transporting SLC30 members, SLC30A10Asn43His gains Zn efflux activity. So in order to discern between these divergent results, the requirement of the Asn-43 residue of SLC30A10 was rigorously tested in neuronal and hepatic cellular systems, which most closely resemble the physiological site of action of the brain and the liver, respectively. SLC30A10WT or Asn-43-Ala was stably expressed in HepG2 hepatic and AF-S neuronal cell lines. We then assayed the cells using three rigorous analyses. SLC30A10 subcellular expression was monitored using confocal microscopy, viability against manganese toxicity was performed using the MTT assay, and lastly intracellular metal content was quantitatively measured using ICP-MS. Our findings demonstrate that overexpression of the Asn-43-Ala mutant was not statistically different from cells expressing SLC30A10WT in both of these physiologically relevant systems. This corroborates our findings in the HeLa cell system and suggests that Asn-43 is, in fact, not required for manganese efflux activity.

2207 Differential Copper-Induced Death and Regeneration of Olfactory Sensory Neuron Populations in Zebrafish

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Fish rely heavily on olfaction to maintain behaviors essential for survival, including predator detection and avoidance, prey selection, social behavior, imprinting, and homing. Environmental exposures to copper (Cu) ions are known to cause olfactory toxicity. In this study, zebrafish were used as a model system to study olfactory death and regeneration in teleost fish following Cu exposure. Confocal imaging of double-transgenic zebrafish larvae with differentially labeled ciliated and microvillus olfactory sensory neurons (OSNs) was used to image and analyze OSN changes following Cu-induced death and recovery. Following a 3-hour or 24-hour exposure to Cu at 5 days postfertilization, both ciliated and microvillus OSN populations exhibited cell injury and death in a dose-dependent manner. Differential recovery and regeneration between the two OSN populations were observed following exposure, with more extensive damage and recovery observed in microvillus than ciliated OSN populations after 24 h at higher Cu concentrations. Cell
2208 High-Throughput and Sensitive Quantification of α-Synuclein Protein Aggregation in Welder’s Serum: Relevance to Development of Circulating Biomarkers for Manganese Neurotoxicity

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Chronic exposure to Mn is known to affect the extrapramidal motor control system resulting in Parkinsonian like neurological symptoms. Welders exposed to Mn-rich welding fumes are particularly prone to Mn neurotoxicity. Recently, we demonstrated that Mn interacts with α-synuclein (αsyn) protein and promotes its aggregation in cell culture and animal models of Mn neurotoxicity. Although brain Mn is traditionally used to diagnosis Mn neurotoxicity in humans, no reliable blood-based biomarker is available. In this study, we developed a sensitive quantification method that detects the effect of Mn exposure on αsyn aggregation by a real-time quaking-induced conversion (RT-QuIC) assay. First, we generated a highly 4-phenyl-1-butylamino human wild-type αsyn substrate and synthesized the αsyn filaments, or pre-formed fibrils (PFFs), from this substrate. The PFFs generated were tested vigorously for internal quality control using defined criteria set by the Michael J. Fox Foundation. The seeding ability of these PFFs were also tested in the RT-QuIC assay with αsyn as a substrate. Next, we optimized RT-QuIC assay conditions to further quantify αsyn aggregation in cell cultures and slice culture models of αsynucleinopathies. We also determined the amyloid formation rate for aggregated αsyn. Importantly, we also examined the utility of RT-QuIC assay in detecting αsyn aggregation in welder serum samples. Serum exosomes from 25 welders exposed to welding fumes and 16 welder-matched control subjects were tested for αsyn seeding activity in a blinded RT-QuIC assay. We could differentiate the welders from controls with >95% sensitivity and specificity using this assay, suggesting that exosomal αsyn aggregates can act as a novel biomarker for Mn neurotoxicity. Comparative analysis of RT-QuIC analysis and brain MRI data showed a positive correlation between amyloid seeding activity and years of Mn-containing welding fume exposures. Collectively, our findings demonstrate the efficacy of using a highly sensitive and rapid, high-throughput RT-QuIC diagnostic assay for detecting Mn-induced αsyn aggregation in occupationally exposed individuals. Acknowledgements: NIH grants N01888206 and ES026892, Eugene and Linda Lloyd chair.

2209 Putrescine Accumulation and Associated Polyamine Metabolism Is Responsive to Physiological and Toxicological Cellular Manganese in Human SH-SYSY Neuroblastoma Cells

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Manganese is an essential nutrient and is required for normal cellular processes. Manganese influences polyamine uptake and stimulates arginase activity, an enzyme upstream of the polyamine, putrescine. While increased manganese (Mn) exposure leads to parkinsonian symptoms and neurological disorders like depression, increased polyamines have also been observed in rapid progression of Parkinsonism in humans and in suicidal cohorts with major depression. Despite the observed similarities, the effect of Mn on polyamine metabolism has not yet been studied. Our aim was to test the hypothesis that dose-dependent increase in Mn causes increased polyamine accumulation and altered polyamine metabolism in a human neuroblastoma cell line. SH-SYSY cells were treated with a series of MnCl2 concentration (0 to 100μM) that resulted in cellular Mn representative of normal to pathological range (4 to 49 μg/mg protein) in human brain. Polyamines and other metabolites were analyzed by liquid chromatography-ultra high-resolution mass spectrometry. Among the polyamines detected, putrescine showed the most significant dose-dependent increase with cellular Mn over the normal to pathologic range (P < 0.0014). Metabolome wide association study of putrescine showed the highest positive correlation with the polyamine metabolite, N-acetylserpine (r= 0.79, P< 0.001, FDR<0.01). Putrescine was also positively correlated with other polyamines, methionine-related precursors, and arginine-associated urea cycle metabolites (rP>0.4, P<0.001, FDR<0.01). γ-Aminobutyric acid (GABA)-related and succinate-related metabolites were negatively correlated with putrescine (rP<0.4, P< 0.001, FDR<0.01). Pathway enrichment analysis showed that methionine and cysteine metabolism was the top altered metabolic pathway, while 29% of the total metabolites correlated with putrescine were involved in amino acid metabolism. In summary, high cellular Mn putrescine was positively associated with other polyamine metabolites in human SH-SYSY neuroblastoma cells suggestive of disrupted neurotransmitter, energy and oxidative homeostasis. Studies are warranted to investigate whether disruption of putrescine related metabolism could be a potential target of Mn toxicity leading to neurological disorders in humans.

2210 Comparing Parkinsonian Toxicants Effects on Dopa-Neurons Reveals Pathways and Biomarker

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Parkinson’s disease (PD) has a small genetic component of ~5-10%, indicating environmental causes. Pesticides such as rotenone, paraquat, and ziram only moderately increase risk for PD, suggesting that chemical(s) causing PD remain to be identified. First, we compared the toxic mechanisms of two chemicals that cause parkinsonism, 6-hydroxydopamine and 1-methyl-4-phenylpyridinium (MPP+), in killing three human neuronal cell models: SH-SYSY, neural stem cells, and LHUMES-immortalized dopaminergic neurons (Tong et al. 2016, Pubmed: 27143523). By screening for chemical interactions, we found that all three cell lines were sensitized to MPP+ by Sirintu activator SRT1720. Since SRT1720 increases substrate flow to the mitochondrial electron transport chain (ETC), wherein MPP+ blocks Complex I, we hypothesize that this interaction increased toxic radical formation from the ETC. All three models were also sensitized to 6-hydroxydopamine toxicity by buthionine sulfoximine, which blocks glutathione synthesis, and protected by 2,3-dimethoxy-1-naphalene (DMN), which chelates Fe2+. This study identified different pathways for response to oxidative stress caused by the two toxicants. Next, we used RNAseq transcriptional profiling to study how differentiated LHUMES dopa-neurons respond to neurodegenerative toxicants including the five parkinsonian chemicals: 6-hydroxydopamine, MPP+, rotenone, paraquat, and ziram. These five chemicals are induced overlapping sets of responsive genes in LHUMES-immortalized dopa-neurons, indicating commonalities in response pathways. Common responses included CNN2, FIBIN, ELF2N, PDK4, CMKR1, SLC30A2, and several metallothionein genes. For one biomarker gene that responded dynamically to all five toxicants, a promoter-reporter gene construct is being engineered in LHUMES cells using the CRISPR technique. This engineered cell line will enable us to screen chemical libraries for neurodegenerative potential using NCATS quantitative high-throughput screening format. Thus, we plan to use the dynamic response of the biomarker gene(s) in LHUMES neurons, and in human stem cells, to screen for environmental chemicals with the potential to cause PD or neurodegeneration.

2211 Roles of Microglia in Diesel Exhaust Particulate Extract-Induced Neuron Loss and Clearance of α-Synuclein Aggregates in the Larval Zebrafish Brain

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Parkinson’s Disease (PD) is the second most common neurodegenerative disease and is caused by both genetic and environmental factors. The main hallmarks of PD include aggregation of the protein alpha-synuclein leading to the formation of Lewy bodies, neuroinflammation, and loss of dopaminergic neurons in the substantia nigra. Epidemiological studies have reported an association between exposure to air pollutant (AP) and incidence of PD. One major component of AP is diesel exhaust (DE). We hypothesize that AP activates microglia, interferes with alpha-synuclein processing and contributes to neuronal injury. Transgenic zebrafish (ZF) embryos were utilized to determine the effect of a major component of AP, diesel exhaust particulate extract (DEPe), on microglia’s interactions with alpha-synuclein and their role in neurotoxicity. DEPe exposure led to a significant loss of dopaminergic neurons in the ZF brain and activation of microglia. Reduction of microglia using morpholino oligonucleotides did not alter DEPe-induced neuron loss. Microglia were found to engulf GFP-tagged human α-synuclein expressed in the ZF brain suggesting that they play a role in synuclein homeostasis. These data suggest that microglia do not play a significant role in DEPe-induced dopamine loss in our ZF model but do play a role in clearing aggregated α-synuclein.
alpha-synuclein. Current studies are focusing on the effects of DEPe-induced microglial activation on alpha-synuclein kinetics since both of these processes have been implicated to play a pathological role in the pathogenesis of PD. Ultimately, these studies will help elucidate the mechanisms by which AP contributes to neurodegeneration in PD.

2212 Neuroprotective Effect of Glycyrhiza glabra and Mucuna pruriens Extract on Rotenone-Induced Parkinsonism in the Wistar Rat Model

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The objective of this research was to evaluate the effect of combined Glycyrhiza Glabra root extract (GG) and Mucuna Pruriens seed extract (MP) on Rotenone-induced Parkinsonism in the Wistar rat model following daily administration through oral gavage for 28 consecutive days. Rotenone (2.5 mg/kg) was administered intraperitoneally to 6 groups of 10 male rats each (G2 to G7) to induce Parkinson-like symptoms. GG at 600 mg/kg b.wt and MP at 800 mg/kg b.wt were administered orally for 28 days to groups G4 and G5, respectively. A concurrent vehicle control group (G1) of male rats was dosed with 1% CMC alone. Group G2 served as a rotenone control. Group G3 served as a positive control group and was dosed with Levodopa (20 mg/kg). A combination of GG and MP was given orally to group G6 while GG and Bromocriptine (DOPA agonist) were given in combination orally for 28 days to group G7. A Functional Observational Battery (FOB) and bar test for catalepsy were performed as pre-treatment and on Days 8, 15, 21, and 28 of treatment to evaluate differences in the neurotoxic and behavioral findings. At termination, all animals were sacrificed and subjected to gross and neuropathological examination. Lipid peroxidation, SOD, and reduced glutathione were measured from brain tissue homogenate. GG + MP treatment (G6) revealed weight loss and higher grip strength compared to vehicle control and rotenone treated groups. The virally-mediated approach, group G6 was the only group in which significantly reduced motor activity was not seen. Lipid peroxidation, SOD, and reduced glutathione of GG + MP treated group (G6) were comparable to vehicle control group while significantly reduced (SOD and Reduced Glutathione) or increased (Lipid peroxidation) in rotenone treated groups. Histopathological evaluation of brain tissue revealed degeneration of dopaminergic neurons in animals treated with individual herbal extract (G4 and G5) which disappeared when animals were treated with herbal extract in combination (GG + MP, G6). Rotenone produced neurotoxicity at a dose level of 2.5 mg/kg b.wt./day when injected intraperitoneally. Combined treatment of GG + MP extracts revealed potential neuroprotective effects which can be useful in reducing the symptoms of Parkinson's disease.

2213 Cytochrome C as a Peroxidase Plays Role in α-Synuclein Radial Formation: Implications of α-Synuclein in Alterations of Biological Pathways and Neuronal Death II Maneb- and Parquat-Induced Model of Parkinson's Disease

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The pathological features of Parkinson's disease (PD) include accumulation of the protein α-synuclein in the surviving dopaminergic neurons. Though PD is multifactorial, several reports show an increased incidence of PD with coexposure to pesticides such as Maneb and parquat (MP). In pesticide-related PD, mitochondrial dysfunction and α-synuclein oligomers have been strongly implicated, but the link between the two has not yet been understood. Similarly, the biological effects of α-synuclein or its radical chemistry in PD is largely unknown. Mitochondrial dysfunction or α-synuclein inclusions leads to loss of cytochrome c in the cytosol. Once in the cytosol, cytochrome c has one of two fates: It either binds to apaf1 and initiates apoptosis or can act as a peroxidase. We hypothesized that as a peroxidase, cytochrome c leaked out from mitochondria can form radicals on proteins in proximity such as α-synuclein. We investigated cytochrome c release in response to MP with α-synuclein in dopaminergic neurons of MP co-exposed (Maneb: 30 mg/kg, ip and parquat:10 mg/kg, ip for 6 weeks) mice. Based on co-localization of cytochrome c and α-synuclein in vivo we used an in vitro system to study the reaction of cytochrome c and α-synuclein and used immuno-spin trapping (IST) to investigate the role of cytochrome c as a peroxidase in α-synuclein radial formation. IST experiments of samples from a reaction mixture consisting of cytochrome c, α-synuclein, and hydrogen peroxide indicated that cytochrome c plays a role in α-synuclein radial formation and oligomerization. Experiments with MP co-exposed α-synuclein knock out mice in which cytochrome c-α-synuclein co-localization can’t occur, mice showed diminished protein radical formation, and diminished neuronal death in compare to wild type MP co-exposed mice. Microarray data also showed that the absence of α-synuclein per se or its co-localization with cytochrome c confers protection from MP co-exposure as several pathways were unaffected in α-synuclein knock out mice. Taken together, these results show for the first time that peroxidase activity of cytochrome c contributes to α-synuclein radical formation and oligomerization, and α-synuclein through its co-localization with cytochrome c or at its own, affects several pathways which contribute to increased neuronal death in MP-induced model of PD.

2214 Viral-Mediated Dysregulation of Vesicular Monoamine Packaging Is Toxic to Dopaminergic Neurons


Dopaminergic (DAergic) neurotransmission underlies reward-motivated behavior and motor output, is the target of many drugs of abuse, and has been implicated in several diseases. DA handling is tightly regulated, and the sequestration of DA into synaptic vesicles is required to maintain neuronal health. The vesicular monoamine transporter 2 (VMAT2) loads DA into synaptic vesicles from the cytosol. Cytosolic DA is susceptible to metabolism and oxidation, which can generate reactive oxygen species and the toxic DA-quinone. DA sequestration is critical to maintaining neuronal health as evidenced by neurodegeneration occurring in a mouse model expressing 5% of the normal levels of VMAT2 (Caudle et al. 2017). Furthermore, VMAT2 overexpression attempts to target toxicity induced by MPTP (Lohr et al. 2014) and methamphetamine exposure (Lohr et al. 2015). To understand the mechanisms underlying neurotoxicity induced by dysregulated DA packaging, VMAT2 expression can be decreased through viral-mediated delivery of small-hairpin ribonucleic acids. This viral-mediated approach allows for specific neuroanatomical targeting, the manipulation of the extent of VMAT2 knock-down, and the dysregulation of DA packaging in an adult animal thereby circumventing the potential of compensatory changes that occur through development. Adult rats unilaterally injected with an AAV2 virus to knock-down VMAT2 within the nigrostrial pathway show decreased VMAT2 protein expression by 36.6% in transduced nigral neurons and by 44% in the striatal terminals (paired t-test, n=5, p<0.05). In the striatum, there was a corresponding loss of DA by 49.6%, increased DA turnover by 64.6%, and increased DA oxidation by 27.5% (paired t-test, n=5, p<0.05). The dysregulation of DA packaging resulted in a loss of TH+ neurons in the substantia nigra by 38.7% as determined by stereology (paired t-test, n=5, p<0.05). The administration of 5-HT agonists in animals demonstrated significant asymmetrical DA-mediated behavioral impairments through posturing and cylinder testing. These results demonstrate that viral-mediated targeting of VMAT2 can be used to dysregulate DA packaging, thereby generating a model by which cytosolic DA mediated toxicity can be studied.

2215 Activation of Lrrk2 and α-Synuclein in Specific Brain Regions after Chronic Exposure to Arsenite

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Increased life expectancy contributes to increased incidence of chronic neurodegenerative diseases such as Alzheimer’s and Parkinson’s diseases. The etiology of these disorders has not been elucidated; however, several genetic and environmental factors are known to contribute to the onset and/or development of these illnesses. Arsenic is a known neurotoxic metalloid adversely affecting neurodevelopment, cognitive and motor functions. As reported by the FRONTIER project, chronic exposure to low levels of arsenic correlates with a decline in cognitive functions and memory, reflecting the earliest manifestations of Alzheimer disease. Higher prevalence of Alzheimer disease was found in countries with higher levels of arsenic exposure. Epidemiological studies also suggest that the exposure to pesticides, including those containing arsenic, increase the risk of developing Parkinson’s disease. This progressive neurological disease is characterized by the loss of dopaminergic neurons in substantia nigra pars compacta leading to motor dysfunction. The hallmark of Parkinson disease is the presence of Lewy bodies in surviving neurons containing aggregates of the α-synuclein, which may result from LRRK2-mediated inhibition of chaperon-mediated autophagy (lysosomal) degradation pathway. Here, chronic exposure
of C57BL/6 male mice to arsenite negatively affected their grip strength. This may mimic neuropathy observed in population studies from arsineosis areas. Using organotypic brain slice cultures, short-term exposure to arsenite led to increased phosphorylation of Lrrk2 and α-synuclein in substantia nigra. Next, we evaluated the effect of arsenite on activation of Lrrk2 and α-synuclein after chronic exposure of C57BL/6 male mice to 50, 500 or 5,000 ppb of arsenite. Significant correlations between phosphorylated forms of Lrrk2 and a-synuclein (r2 = 0.85) were observed in substantia nigra and for Lrrk2 levels between substantia nigra and striatum (r2 = 0.9), which were completely disrupted by arsenite at either high or low exposure levels. These data suggest that arsenite interferes with process linked to α-synuclein pathology, which is associated with neurodegenerative diseases. This work was funded by CHHE grant P30ES025128.

2216 Expression of the Histone Methyltransferase Ezh2 Is Increased in Parkinson’s Disease Brain, and Ezh2 Inhibition Reduces the Astrocyte Inflammatory Response: Potential Role for Epigenetic Alterations in Neurodegeneration

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Neuroinflammation, mediated by microglia and astrocytes, is a hallmark for both Alzheimer’s (AD) and Parkinson’s (PD) diseases, and evidence indicates that it contributes to the progression of these diseases. Emerging evidence also indicates that reactive astrocytes play a prominent role in both AD and PD. Inflammatory cytokines can induce astrocytes to shift towards a reactive state, causing these cells to produce more inflammatory factors and increase neurotoxicity. Although a relatively new field, studies have found that epigenetic modifications, including DNA and histone methylation, contribute to the regulation of inflammation. Here, we used datamining techniques and found increased expression of the histone methyltransferase Ezh2 in the substantia nigra of PD patients with 5% more expression than age-matched controls. Treatment of mouse models with the dopaminergic neurotoxin MPTP increased Ezh2 gene expression by 70% in the striatum 2 days following treatment. In cultured mouse primary astrocytes and an immortalized mouse astrocyte line, treatment with a mix of pro-inflammatory cytokines (CM) caused a 9-fold increase of NO levels and significantly increased mRNA expression of cytokines (CM). This was followed by a 16-fold increase in Ezh2 gene expression. Next, we investigated the effects of GS126, a selective pharmacological inhibitor of Ezh2, on CM-treated cultured astrocytes. GS126 co-treatment significantly attenuated the levels for all of these inflammatory factors. Interestingly, our qRT-PCR, Western blot and ICC analyses also revealed that Ezh2 upregulated mRNA and protein levels of Rab27a, a key endosomal protein that mediates exosome release through fusion of MVBs with plasma membrane, suggesting Rab27a upregulation also contributes to Mn-induced exosomal release. Since aggregated αSyn can get degraded via autophagic/lysosomal degradation pathways, we examined if Mn impairs this pathway to promote exosomal αSyn release. Our Western blot analysis of M9N9D_αSyn cells shows that Mn upregulated expression of autophagosomal markers LC3-II and Beclin-1, but downregulated lysosomal marker LAMP2 suggesting impairment of autophagosome formation. Taken together, these novel findings suggest Mn compromises endosomal trafficking, leading to autophagic/lysosomal impairment, thereby promoting MB formation and the exosomal release of misfolded αSyn. NIH grants ES026892, NS088206 and Eugene & Linda Lloyd endowed Chair.

2217 Mechanism of Manganese Impairment Mitochondria Dynamics in Dopaminergic Neurons

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Excess accumulation of Manganese (Mn) in the brain results in a neurological syndrome with cognitive, psychiatric, and movement abnormalities called Parkinsonism. In addition, Mn accumulates in the basal ganglia triggering damage in dopaminergic neurons and gliosis. Thus, Mn neurotoxicity increases reactive oxygen species (ROS), damage in ATP synthase, disrupt Ca2+ homeostasis and apoptosis. However, few studies have evaluated the perturbations of Mn in mitochondrial dysfunction, the latter representing a site of predominant Mn accumulation. Thus, in this study, we have exposed in vitro rat embryonic primary cell culture derived from the midbrain for 6 and 24 h to Mn and the neurotoxicity was evaluated. The results showed that Mn exposure induced alternation in the dynamin-related protein 1 (DRP-1) as well as other proteins associated with susceptibility to Parkinson’s disease, such as PINK1 and Parkin. Moreover, we evaluated whether Mn exposure alters mitochondrial bioenergetics in cultured neurons. The results showed a variation in the basal mitochondrial oxygen consumption rate as well as the ATP-linked respiration. Finally, Mn produced a regulation in the expression of mitochondrial fission factor (Mff) as well as Park7. This study reports for the first time role of Mn in the abnormal imbalance in fusion/fission equilibrium in a neuronal cell culture. We expect that our results will contribute to therapeutic targets for Parkinsonism. This study was supported by NIHES R01ES01563 to MA and ABB.

2218 Manganese Exposure Enhances the Release of Misfolded α-Synuclein Via Exosomes by Impairing Endosomal Trafficking Machinery


Environmental exposure to excessive manganese (Mn) increases the risk for chronic neurological diseases including Parkinson’s disease (PD). Aggregated α-synuclein (αsyn) is a key pathophysiological feature of PD, and as summarized in our recent review in Toxicological Sciences; oligomeric proteins like αsyn can be released from neurons by exosomes, enabling misfolded proteins to propagate to neighboring cells, thereby inducing a neurotoxic response. We recently revealed that Mn increases the release of exosomes containing misfolded αsyn from dopaminergic neurons, but the underlying molecular signaling mechanisms are unclear. Herein, we examined how Mn modulates endosomal protein trafficking to promote exosomal αsyn release. MN9D dopaminergic neuronal cells stably expressing human wild-type (WT) αsyn (MN9D_αsyn) were exposed to Mn (300 μM) for 24 h. Mn significantly suppressed expression of key endosomal-recycling protein Rab11a, both at the protein and mRNA levels, suggesting Mn downregulates endosomal-recycling mechanisms, thus forcing large exocytic cargo to be internalized into multivesicular bodies (MVBs). Moreover, exocytic expression of WT Rab11a significantly attenuated exosomal release in both untreated and Mn-stimulated MN9D_αsyn cells, whereas ectopic expression of dominant negative mutant Rab11a (S25N) enhanced exosomal release. Interestingly, our qRT-PCR, Western blot and ICC analyses also revealed that Mn exposure upregulated mRNA and protein levels of Rab27a, a key endosomal protein that mediates exosome release through fusion of MVBs with plasma membrane, suggesting Rab27a upregulation also contributes to Mn induced exosomal release. Since aggregated αSyn can get degraded via autophagy/lysosomal degradation pathways, we examined if Mn impairs this pathway to promote exosomal αSyn release. Our Western blot analysis of M9N9D_αSyn cells shows that Mn upregulated expression of autophagosomal markers LC3-II and Beclin-1, but downregulated lysosomal marker LAMP2 suggesting impairment of autophagosome formation. Taken together, these novel findings suggest Mn compromises endosomal trafficking, leading to autophagic/lysosomal impairment, thereby promoting MBV formation and the exosomal release of misfolded αSyn. NIH grants ES026892, NS088206 and Eugene & Linda Lloyd endowed Chair.

2219 Mitochondria-Targeting Pesticides Modulate Elongator Protein 3 (ELP3) Function to Enhance Dopaminergic Neurotoxicity in Cell Culture and Animal Models of Parkinson’s Disease


Exposure to neurotoxic pesticides targeting mitochondria is linked to pathogenesis of Parkinson’s disease (PD), but the molecular mechanisms underlying the enhanced vulnerability of dopaminergic neurons to mitochondrial toxicants are not fully understood. In a serendipitous finding, we observed that mitochondria-targeting pesticides affect the novel lysing antigen ELP3 and map in the trafficking machinery of cell cultures and in rodent models of PD. Exposing N27 dopaminergic neuronal cells to tebufenpyrad (Tebu; 3 μM) increased ELP3 mRNA expression within 1 hr and then subsided over time. After further characterization of ELP3 expression in the mouse brain, we found high expression of full length ELP3 (62 KDa) in the cytosolic fraction from different brain regions, while the shorter form (~40 KDa) was detected in the mitochondrial fraction of the olfactory bulb (OB). Immunocytochemical analyses revealed that ELP3 is highly expressed in TH-positive neurons of the OB. Furthermore, OB slice cultures treated with Tebu showed reduced expression of short ELP3 in the mitochondrial fraction when treated for 6 hr. We also found that ELP3 expression was reduced significantly in the mitochondrial fraction of OB from A353 rat model as well as MitoPark.
While cell signaling mechanisms underlying neurotoxic injury have been actively studied in recent years, signaling molecules contributing to compensatory survival signaling are largely unknown. Recently, we reported that the secretory neuroepitope prokinetin-2 (PK2) is upregulated during early stages of neurotoxic stress and plays a major compensatory protective function in nigral dopaminergic neurons. In this study, we characterized the transcriptional regulatory mechanisms of MPP+-induced PK2 upregulation. In silico analysis of the PK2 promoter region detected binding sequences for the key oxidative stress-related transcription factor, hypoxia-inducible factor (HIF), and the early growth response (EGR) transcription factors associated with neuronal survival and differentiation. We validated the in silico findings by cloning the 5’-flanking region (1 kb) of the human PK2 gene into a luciferase reporter vector, which was then transfected into MIN9D dopaminergic neuronal cells to study the PK2 gene regulatory mechanism. Overexpression of EGR1 or HIF1α, and treatment with the HIF1α activator 3,4-dihydroxybenzoate (DHB), significantly upregulated PK2 promoter activity and upregulated PK2 expression in MIN9D dopaminergic neuronal cells, suggesting that EGR1 and HIF1α are potent transcriptional activators of PK2 expression. MPP+ treatment in N27 dopaminergic cells also induced PK2 upregulation, which correlated with HIF1α and EGR1 protein levels. Next, we generated stable EGR1 knockdown (KD) N27 cells using a CRISPR-Cas9-based method. Time-course studies with EGR1-KD cells revealed that MPP+-induced PK2 mRNA expression was not significantly reduced until the later stages of neurotoxicity (6-12 h). These results suggest that EGR1 is required to induce a sustained PK2 upregulation during neurotoxic stress. Taken together, these data suggest that HIF1α and EGR1 families of transcription factors play a key role in PK2 upregulation and its compensatory response during early stages of neurotoxic insults in dopaminergic neuronal cells. NIH grants NS0728247, ES027245, Eugene Linda Lloyd Chair and Salsbury Chair.

2220 Transcriptional Regulation of the Compensatory Signaling Neuropeptide Prokinetin-2 by HIF1α and EGR1 during Neurotoxic Stress in Dopaminergic Neuronal Cells

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2221 Pesticide Exposure Induces Mitochondrial Dysfunction and Inflammation in Enteric Glial Cells


Commonly used pesticides such as rotenone and tebufenpyrad are known to inhibit mitochondrial respiratory chain (complex I). Persistent pesticide-induced mitochondrial dysfunction and the resulting oxidative damage can promote chronic inflammation and have been linked to various disease conditions including Alzheimer’s disease (AD) and Parkinson’s disease (PD). In the present study, we examined the effects of rotenone and tebufenpyrad exposure on mitochondrial function and inflammation in enteric glial cells (EGCs). Despite evidence of gastrointestinal inflammation in PD, the mechanism of this inflammation in gastrointestinal abnormalities has not been studied in detail. Exposure to these two pesticides significantly reduced cellular metabolic activity with no effect on cell death as revealed by cell viability and cytotoxicity assays. Morphological analysis revealed that rotenone and tebufenpyrad significantly increased mitochondrial circularity, indicating augmentation of mitochondrial fission. Furthermore, pesticide exposure in EGCs led to a decrease in the mitochondrial fusion protein Mfn2, which according to colocalization studies, was shuttled out of mitochondria, indicating a loss of mitochondrial fusion. Furthermore, pesticide exposure further enhanced mitochondrial superoxide generation in EGC. The Seahorse mitochondrial bioenergetics analysis in pesticide-exposed EGCs showed impaired mitochondrial function as evident from reductions in ATP generation and basal respiratory rate. We recently demonstrated that mitochondrial dysfunction leads to inflammation in astrocytes and microglia. Here, we show that pesticide exposure increased pro-inflammatory factors in EGCs, and that this increase correlates with the loss in mitochondrial mass. ICC analysis revealed that rotenone and tebufenpyrad treatments led to formation of stress granules in EGCs, suggesting a possible role of stress-induced inflammation. Collectively, our studies demonstrate for the first time that neurotoxic pesticides impair mitochondrial bioenergetics, activate inflammatory pathways in EGCs, and induce stress granule formation, further augmenting proinflammatory events in the gut. Our findings have important implications in the pathogenesis and progression of environmentally-linked PD. NIH grants ES026892 and ES027245 and Eugene and Linda Lloyd Endowed Chair.

2222 Mechanisms of Harmane-Induced Selective Dopaminergic Neurotoxicity in Caenorhabditis elegans

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Dietary factors are of emerging interest in the etiology of Parkinson’s disease (PD). Heterocyclic amines (HCA) have attracted significant attention over the last few years due to structural resemblance to known PD-relevant toxicant and also because of prevalence in the diet. 1-methyl-9H-pyrido[3,4-b]indole (harmane) is a β-carboline (a HCA subclass). Harmane is a known tremorigenic agent which is found in cooked meat, roasted coffee beans, and tobacco. Given the structural similarity to other neurotoxicants and published clinical reports, we hypothesized that harmane would be selectively toxic to dopamine neurons. Here, we used C. elegans as a model system to allow determination of selectivity toxicity across numerous doses. Nematodes were exposed for 48 hours within dose range 100 to 500 μM. These doses were based on established neurotoxic nematode PD models and recommended guidelines for neurotoxicity testing in this species. Using numerous reporter strains, we found that dopaminergic neurons were selectively sensitive to harmane-induced neurodegeneration versus serotoninergic, GABAergic and cholinergic neurons. Further, we examined mechanisms of harmane-induced dopaminergic neurotoxicity. Harmane treatment caused decreased mitochondrial viability and increased reactive oxygen species. Inhibition of the dopamine transporter (DAT) was protective, suggesting that harmane does not enter dopamine neurons through DAT, as do many other dopaminergic neurotoxicants. In contrast, treatment with the mitochondrial complex I activator partially ameliorated neurodegeneration, reducing harmane-induced behaviors consistent with dopamine depletion (1-nanomol assay). Our studies suggest that in vivo harmane is selectively toxic to dopaminergic neurons. Further, mitochondria are a likely primary neurotoxic target. Our results suggest that additional mechanistic studies and neuropathology studies in higher order species be conducted to determine relevance in PD etiology.

2223 A Platform to Study the Effects of Complex Environmental Exposures on Aging and Neurodegeneration in Caenorhabditis elegans

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Neurodegeneration has a complex etiology, driven by genetic, environmental, and aging risk factors. Interactions between these factors remain unclear. We propose an experimental framework to assess the relationship between environmental exposure, dopamine homeostasis, and aging in C. elegans that uses a multi-faceted approach combining high-resolution metabolomics (HRM), exposomics, and high-content imaging through parallel electron microscopy over >350 environmental chemicals and their metabolites using gas-chromatography (GC) with high-resolution mass spectrometry and associated biological response by HRM, this framework provides a measure of exposure, internal dose, and biological response. As proof of principle, we measured effects of exposure in wild-type worms to a known neurotoxicant, manganese. Exposed worms showed depleted levels of glutathione, measured by HPLC. Using HRM, we were able to detect ∼3,000 features post-processing and blank subtraction. Using the Mummichog pathway analysis tool, pathways related to amino acids, glutathione and keratan sulfate metabolism were altered in exposed worms. These results support a role of HRM in detecting metabolic perturbations from environmental exposures in worms. Research from our lab and others has shown that disruption of vesicular monoamine transporter 2 (VMAT2; SLC18A2) confers vulnerability to dopamine toxicity in mice and Parkinson’s disease (PD) patients. Using automated lifespan analysis, wild-type worms had a median (SD) survival time of 11.4 (6.0) days, which increased to 13.5 (4.0) days in cat-1 (worm VMAT2 orthologue deletion) mutants. The short-lived
Parkinson’s disease (PD) is a devastating neurological disorder affecting over 1.5 million people in the United States. Over 85% of cases are idiopathic and exposures to certain environmental chemicals have been suggested to play a role in disease etiology. To this end, a screening of the published literature was developed using systematic review methods to identify studies reporting on neuropathological endpoints associated with PD and environmental exposures. A PubMed search identified 91,598 records potentially relevant to PD endpoints, which were further screened in a systematic manner. Evidence from in vivo and in vitro studies was also included. A total of 44,340 original research records reporting potential studies of PD and environmental chemicals was identified with most studies reporting well-established PD-related pharmaceutical and environmental chemicals including dopamine (DA), α-synuclein (α-syn) and environmental stress. Evidence from both PD patients and general populations was included. The results of this review are presented in a systematic evidence map that allows users to interactively visualize and query the dataset.

### 2222 Structure-Function Studies in Caenorhabditis elegans of the Organometallic Fungicide Manzate Indicate Mechanisms for Neurodegeneration

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Exposure to the commercial fungicide Manzate (cMZ) has been associated with the development of neurodegenerative diseases. The contribution of each component of this organometallic pesticide (manganese [Mn], zinc [Zn], and ethylene-bis-dithiocarbamate) to human toxicity remains unknown, although some epidemiology studies suggest Mn exposure may increase the risk of PD or parkinsonism. Since we previously observed neurodegeneration of dopaminergic and GABAergic neurons in Caenorhabditis elegans (C. elegans) treated with cMZ, we sought to investigate which chemical components of the active ingredient might promote this neurodegeneration. Therefore, we treated various strains of C. elegans with cMZ, the purified active ingredient, mancozeb (MZ), or two structural analogs, maneb (MB; Mn only, no Zn), or zineb (ZB; Zn only, no Mn). After a 24-h incubation with the respective compounds, general nervous system morphology was evaluated by assessing the pattern and intensity of green fluorescent protein tagged (GFP-labeled) neurons in transgenic worms. In these studies, treatment with MZ resulted in a statistically significant decrease in fluorescence compared to controls (**p < 0.01) in the nerve ring, which is predominantly dopaminergic neurons, compared to all other compounds. We then assayed the mitochodrial proton gradient using tetramethylrhodamine ethyl ester (TMRE). Although there were no statistically significant differences in overall fluorescence, we did observe a punctuated mitochondrial pattern in worm strains with PD-related genetic mutations. This pattern was qualitatively different from that of control worms, and is consistent with changes in mitophagy, mitochondrial fusion and fission, and general mitochondrial trafficking. Finally, we observed increases in oxidative stress, as assessed by increased fluorescence in worms treated with cMZ-labeled glutathione-S-transferase, when these worms were treated with cMZ. Data from all three studies are consistent with our previous work using the commercial formulation of this fungicide. Taken together, these data suggest that treatment with cMZ, as well as the purified MZ, may negatively affect the dopaminergic neurons in C. elegans, and may do so by altering mitochondrial trafficking and increasing oxidative stress.

### 2225 Parkinson’s Disease and Environmental Chemical Exposure: A Systematic Evidence Map

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Parkinson’s disease (PD) is a devastating neurological disorder affecting over 1.5 million people in the United States. Over 85% of cases are idiopathic and exposures to certain environmental chemicals have been suggested to play a role in disease etiology. To this end, a screening of the published literature was developed using systematic review methods to identify studies reporting on neuropathological endpoints associated with PD and environmental exposures. A PubMed search identified 91,598 records potentially relevant to PD endpoints, which were further screened in a systematic manner. Evidence from in vivo and in vitro studies was also included. A total of 44,340 original research records reporting potential studies of PD and environmental chemicals was identified with most studies reporting well-established PD-related pharmaceutical and environmental chemicals including dopamine (DA), α-synuclein (α-syn) and environmental stress. Evidence from both PD patients and general populations was included. The results of this review are presented in a systematic evidence map that allows users to interactively visualize and query the dataset.

### 2226 Autophagic Dysfunction in Brainstem Nuclei in a Preclinical Parkinson’s Disease Model


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A lesion to the nigrostriatal dopamine system is primarily responsible for cardinal motor features of PD. MRI studies of Parkinson’s disease (PD) patients have found abnormalities in many other brain regions are affected, where widespread α-synuclein pathology occurs. α-Synuclein is a pivotal protein in PD, as it is a major component of intracellular aggregates known as Lewy bodies. Interestingly, α-synuclein pathology occurs systemically and in other brain nuclei before the nigrostriatal dopamine system is affected, where it is postulated that pathology ascends from the lower brainstem, through the nigrostriatal pathway until it reaches the neocortex. Autophagy is one route of α-synuclein clearance and autophagy dysfunction occurs in PD prior to Lewy body formation. We hypothesized that a PD relevant dopaminergic neurotoxin would produce autophagic dysfunction in a pattern similar to that in PD. Thus, we expected brainstem nuclei to be affected prior to the substantia nigra. To test our hypothesis, we utilized a rat rotenone PD model, where rats were treated with 3.0 mg rotenone/kg per day for either 24 h, 5 d, or until death. The 24 h and 5 d rats were analyzed at PD phenotype developed. The 24 h and 5 d rats exhibited an abnormal brainstem morphology. We previously showed autophagic disruption and α-synuclein aggregation in this model is associated with co-localization of optineurin (OPTN), a known cargo adaptor in autophagy. OPTN has also been implicated as a genetic risk factor for PD. The present study focused brain regions identified in early-stage PD (DAVY, P97, RMG, LC, SNpc). Autophagic stress in these regions was assessed through LC3 staining. We detected OPTN expression in all tested regions, and OPTN was colocalized with LC3 or α-synuclein. Further, we found OPTN expression and average number of puncta were significantly increased in all stages and in multiple brain regions. Our results indicate these brain regions may be inherently distinct in their basal autophagic activity (e.g. the locus coeruleus had twice as many LC3 puncta than the nigra in control rats). Our data suggest that these regions likely vary in their autophagy response to rotenone, with the substantia nigra pars compacta being the most sensitive. These data suggest OPTN may have a role in PD. Further research is necessary to determine whether α-synuclein aggregates for autophagic degradation. Finally, these data implicate basal autophagic activity as a potential factor for neuronal vulnerability in PD pathogenesis.

### 2227 Levodopa and Dopamine Dynamics in Parkinson’s Disease Metabolomics


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Parkinson’s disease (PD) is a progressive neurological disorder caused by a combination of genetic and environmental factors. Metabolomics is a powerful tool that can help answer the question of how thousands of chemicals within a biological sample. Metabolomics is used to identify potential biomarkers, exogenous toxicants, and metabolic network changes that predict disease states. Here, we used liquid-chromatography-mass spectrometry-based high-resolution metabolomics to characterize and compare over 10,000 plasma metabolites from older adults with and without PD in an untreated way. We perform a network analysis that demonstrates that the presence of the PD drug levodopa influences variation observed between PD and control patients. We also perform metabolome wide t-tests, OPLS-DA, and PCA analyses. These analyses show a significant differentiation in the metabolomics profile of older adults with and without PD. Notably, 15 metabolites (ten of which we putatively identified) were significantly different between PD and control adults with p-values less than 0.05 and a corrected false discovery rate less than 0.2. Furthermore, 13 metabolic networks were identified to be functionally different between PD and disease patients. Lastly, dopaminergic toxic intermediates (DOPAL, m/z 153.0548) differed between patient populations, supporting the dopaminergic dysfunction in this model. This work suggests that levodopa may contribute to the metabolic perturbations observed in PD.
sequestration model of PD. These individual metabolites and metabolic networks have been implicated in past PD pathogenesis models, including the beta-carboline harmalol (m/z 223.0846) and the glycosphingolipid metabolism pathway (including the ganglioside GM2, m/z 1427.752). We recommend that future studies take into account the confounding effects of levodopa in metabolic analysis of disease versus control patients, and encourage validation of several promising metabolic markers of PD.

Epidemiological studies have given rise to possible etiologies of Parkinson’s disease (PD), especially farmers exposed to toxicants. The herbicide paraquat is similar in structure to the dopaminergic neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and redox cycling of MPTP to NADPH-oxidase to reactive oxygen species (ROS). Excessive ROS and microglial activation can damage neurons and is thought to contribute to the initiation and progression of neurodegenerative diseases, including PD. HV1 is a voltage-gated proton channel selectively found in microglia and other immune cells where it regulates NADPH-oxidase-dependent production of ROS. Deletion of HV1 in mice has been found to protect neurons from oxidative damage during ischemic stroke, but there have been no studies of HV1 in PD. The purpose of this study was to determine the role of HV1 in paraquat-induced neurodegeneration. In vitro studies with the BV2 mouse microglial cell line demonstrated that while paraquat does not directly activate microglia, it induces HV1 gene expression by 2-3-fold. To assess the effect of HV1 in the microglial response to paraquat-induced neuronal damage, we exposed primary microglial cells from C57BL/6J and HV1KO (HV1 knockout) mice to conditioned media from N27 dopaminergic neuronal cells treated with paraquat. The paraquat-treated N27 conditioned media induced HV1 and iNOS gene expression by 4- and 3-fold, respectively, in C57BL/6J microglia, which was significantly attenuated in HV1KO microglia. Similarly, conditioned media from paraquat-treated N27 cells increased ROS formation in C57BL/6J microglia, an effect that was completely abolished in HV1KO microglia. Preliminary data from in vivo studies with C57BL/6J and HV1KO mice exposed to repeated injections of paraquat showed that there was no significant dopamine neuron loss in HV1KO mice. These data demonstrate that paraquat regulates HV1 expression, HV1 is involved in the paraquat-induced production of microglial ROS, and genetic knockout of HV1 reduces this microglial inflammatory response. Thus, HV1 appears to be a key regulator of microglial activation and dopamine neuron vulnerability to paraquat. Supported by R01ES021800 and The Michael J. Fox Foundation.

Role of Reactive Metabolites of Dopamine in Pesticide Neurotoxicity

Pesticide exposure has been shown to be linked with Parkinson’s disease (PD) and other neurodegenerative disorders. One hypothesis is that pesticides lead to interuption in dopamine metabolism and trafficking, yielding a buildup of toxic intermediates such as aldehydes, which is termed the “catechol-aldehyde hypothesis.” Dopamine (DA) is metabolized to a toxic catechol-aldehyde - 3,4-dihydroxyphenylacetaldehyde (DOPAL) - by monoamine oxidase (MAO) and then detoxified by aldehyde dehydrogenase (ALDH). ALDH has been shown to be inhibited by lipid peroxidation products such as malondialdehyde and 4-hydroxynonenal which increase in the presence of pesticides such as dieldrin. Therefore, pesticide exposure can cause inhibition of ALDH leading to increased DOPAL concentrations. DOPAL can cause toxicity in several ways including modifying proteins or forming protein adducts, as well as oxidation to a reactive semiquinone radical which leads to additional toxicity. One protein of interest is the dopamine transporter (DAT). This work shows that treatment of dopaminergic N27 cells with DOPAL at non-toxic concentrations lead to decreased expression of DAT possibly through protein modification. DOPAL modified DAT could have decreased functionality leading to irregularities in dopamine (DA) cell trafficking. We have isolated proteins modified by DOPAL using an affinity resin. In addition, this study examined how DA metabolism is affected by organophosphate and pyrethroid pesticides, specifically chlorpyrifos and cypermethrin. These are both insecticides that are neurotoxic to insects as well as humans. Cypermethrin has been shown to cause nigrostriatal degeneration with long-term exposure and may be acting synergistically with chlorpyrifos. Using dopaminergic N27 cells, it was found that neither cypermethrin nor chlorpyrifos were toxic at low micromolar concentrations; however, a decrease in the level of DAT was observed following cypermethrin treatment. Understanding the mechanism of these insecticides and how they affect dopamine metabolism will further our understanding of PD and neurodegenerative diseases and be helpful in developing therapeutic strategies.

Toxicity, Recovery, and Resilience in a 3D Dopaminergic In Vitro Model Exposed to Rotenone

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The paradigm shift towards pathway-based toxicology involves moving away from animal tests into 3D in vitro models which could be more predictive for human toxicity. To date, most in vitro toxicity testing focuses on acute effects of compounds at high concentrations. This testing strategy does not reflect real-life exposures contributing to long-term disease outcome. In the area of Parkinson’s disease, we still do not understand the mechanisms behind gene-environment interactions which modulate risk. Studying recovery mechanisms can indicate which genes are important in repair after an environmental exposure. Using a well-characterized 3D-human, dopaminergic, in vitro model we investigated whether acutely-induced molecular effects are permanent or reversible. We analyzed the effects of rotenone (100 nM), a known Parkinson’s inducer, after acute exposure (24h) and 7 days after compound wash-out using cell viability, qPCR, cellular ATP, electrical activity, dopamine release and neurite outgrowth assays. We identified in vitro pathways (NOSA, OPTN and PKIN1) and reversible changes in gene expression (ATF4, ATP50 and KEAP1). Acute exposure to 100 nM rotenone (non cytotoxic) led to a decrease in neurite outgrowth (~50%) and cellular ATP (~25%), however, cells recovered after wash-out. To further study cellular neuroprotective mechanisms, cells were re-exposed after recovery. Pre-exposed cells showed higher metabolic activity (resilience, ~50% increase in resazurin reduction) than controls and changes in gene expression (VAMP1, PKIN1, PARK7) were observed. To compare cellular recovery to repeated-dose effects, we treated 3D Luhmes with 30 nM rotenone (a relevant in vivo concentration) every 48 h. This exposure is shown to induce Parkinson’s in animal models. Our results indicate that cells are able to recover from low-dose rotenone-induced decrease in ATP production and neurite outgrowth after wash-out, however, some changes to gene and protein expression are maintained after compound removal. Furthermore, pre-exposed cells may carry resilience to second exposures, shown by metabolic activity (resazurin reduction). This is the first study showing the complexity of recovery and delayed effects after compound removal and re-exposure in vitro.

The Effect of Paraquat on Cognitive Function and Emotional Reactivity in Mice Overexpressing Human a-Synuclein


Exposure to pesticides is an established risk factor for Parkinson’s disease (PD). PD is characterized by the degeneration of dopamine neurons in the substantia nigra and the development of alpha-synuclein (αsyn) positive Lewy bodies. Behaviorally, patients develop both motor and non-motor impairments. The non-motor impairments associated with PD can include cognitive and neuropsychiatric symptoms, and can be more debilitating to patients than the motor symptoms. Paraquat (PQ) is an herbicide strongly linked to PD, with systemic administration causing degeneration of nigrostriatal dopamine neurons and αsyn pathology in animals. However, the role of PQ in cognitive and neuropsychiatric function is unclear. In the present study, the effect of PQ on cognition and fear reactivity was tested in an established transgenic model of PD, αsyn overexpressing mice (Thy1-αsyn). Male and female Thy1-αsyn mice received injections of vehicle or PQ solution starting at 6-8 weeks of age (10 mg/kg b.w./day, 5x/week for 8 weeks, 5x/week for 8 weeks, and 5x/week for 8 weeks, 5x/week for 8 weeks, and 5x/week for 8 weeks, 5x/week for 8 weeks, and 5x/week for 8 weeks). One day following the third injection, mice were tested behaviorally for PQ alone or in combination with increased aSyn expression. In object recognition and elevated plus maze tests, PQ impaired sensorimotor function, object recognition, and emotional reactivity. PQ caused a significant decrease in spontaneous activity only in Thy1-αsyn mice. In object recognition and elevated plus maze tests, PQ impaired recognition and reduced reactivity in both WT and Thy1-αsyn mice. These data indicate that PQ alone or in combination with increased αsyn burden is associated with cognitive and neuropsychiatric alterations in vivo.
2233 Potential Protective Effect of Silybin against Central Nervous System Dysfunction


Silymarin is obtained from Silybum marianum (milk thistle), an edible plant with an excellent hepatoprotective action. The mayor component of the silymarin complex is silybin, which is the most active phytochemical. Silybin [(2R,3R)-3,5,7-trihydroxy-2-(2R,3R)-3,4-hydroxy-3-methoxyphenyl]-2-(hydroxy-methyl)-2,3-dihydro-benz[b][1,4] dioxin-6-yl]isochroman-4-one] is a flavonoid with anti-inflammatory and antioxidant activity of glutamatergic motoneurons. We will now use pharmacological and genetic probes to test this hypothesis. We suggest that interactions between aminergic and glutamatergic neurons we are investigating could be relevant to early events in the pathophysiology of PD.

2234 Determination of Pyrethroid Toxicodynamic Differences in Adult and Juvenile Rat Brain Tissue Microtransplanted into Xenopus Oocytes

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Microtransplantation of mammalian neurolemma is a tool to examine the endogenous structure and function of transmitter receptors and ion channels associated with the central nervous system. Microtransplanted neurolemma can originate from a variety of sources, posses ion channels and receptors in their native configuration, and are applicable to examine diseases associated with different channelpathies. In these experiments, we examined the age-related differences in VSSC expression and concentration-dependent responses to pyrethroids in juvenile and adult rat brain tissue microtransplanted in Xenopus oocytes. Automated western blotting results indicate that adult neurolemma exhibited 2.5-fold higher level of expression. These results correlated with juvenile neurolemma when normalized to the housekeeping protein β-tubulin. The predominate isoform expressed in both tissues was Na1.2 with both showing a significant difference from zero. Adult neurolemma, however, expressed 2.8-fold more Na1.2 than juvenile and also express Na1.6 at a higher level (2.2-fold). In addition, neurolemma tissue microtransplanted into Xenopus oocytes showed reconstituted native ion currents in the plasma membrane of oocytes that was sensitive to TTX and abolished by choline ion replacement, functionally demonstrating the presence of VSSC. Increasing concentrations of permethrin and deltamethrin exhibited concentration-dependent increases in TTX-sensitive current from both adult and juvenile tissues. Concentration-dependent response curves were analyzed using the equivalence test and the slopes of the curves were different. VSSCs associated with juvenile neurolemma was up to 2.5X more sensitive to pyrethroid than VSSCs in adult neurolemma. In contrast, VSSCs from juvenile neurolemma were less sensitive than adult VSSCs at lower concentrations (0.6-0.8X) and more sensitive at higher concentrations (up to 2.4X). However, because the expected brain concentrations in humans following realistic exposure levels are approximately 21- (deltamethrin) to 333- (permethrin) times below the threshold for response in rat neurolemma, age-related differences, if any, are not likely to be toxicologically relevant.

2235 Effects of Acute Exposure to Deltamethrin in the Nucleus Accumbens

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Deltamethrin (DM), a commonly used pyrethroid insecticide, exerts its effect on insects by delaying onset of inactivation in voltage gated sodium (Nav) channels fundamental for neuronal excitability. Due to species cross reactivity and the high evolutionary conservation of Nav channels, there is reason to study the effects of DM in humans. Epidemiological data showed a correlation between pyrethroid metabolites in urine and increased risk of ADHD diagnosis in children. In rats, exposure to DM results in behavioral phenotypes that mimic aspects of ADHD and are associated with the dopaminergic (DA) reward pathway in the nucleus accumbens (NAc). Disregulation of DA medium spiny neurons (MSNs) in the NAc is thought to play a critical role in neuropsychiatric disorders like ADHD, anxiety, and depression. The Nav 1.6 channel, critical in synaptic transmission, is abundant in the MSNs. All this evidence provided a strong scientific basis to investigate a possible causative link between exposure to DM and dysfunction of MSNs as the source of increased risks of ADHD. Previous electrophysiological studies in HEK 293 cells stably expressing human Nav 1.6 identified DM induced alterations in Nav channel function. After 1hr of 10 uM DM treatment, Nav 1.6-mediated persistent and tail currents were significantly potentiated. At ~20mV, Nav 1.6 encoded persistent current densities were increased from DMSO control (~0.4±0.6 pA/pF, n=6) with 10uM DM application (9.2±2.4 pA/pF, n=20, p<0.01). Tail currents were also significantly potentiated from 5.9±1.5 pA/pF (n=10) in DMSO control cells to 25.1±4.1 pA/pF (n=20, p<0.001) with 10μM DM. Use dependent activity was studied to determine how DM affects the fraction of Nav 1.6 channels at the open state. Use dependent protocol at 50mV for DM exposed cells, significantly increased amount of persistent (DMSO -3.5±1.8pA/pF, n=12, p<0.01). DM 11.8±2.5 pA/pF, n=19) and tail currents (DM 14.3±3.6 pA/pF, n=12, p<0.01. DM 45.5±5.7 pA/pF, n=20, p=0.01). Ongoing studies aim to investigate DM effects on MSN intrinsic excitability after acute exposure to brain slices. These studies will advance our knowledge of the toxic activity of DM in the brain and help assess risk exposure in the human population.
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Chronic exposure to organophosphates (OP) during deployment is considered a leading cause for Gulf War Illness (GWI), yet its pathobiology is not fully understood. Disruptions in neuronal calcium (\(Ca^{2+}\)) homeostasis are observed in many neurological disorders and in line with this, we recently observed chronic elevations in neuronal \(Ca^{2+}\) levels in an OP: diisopropyl fluorophosphate (DFP) based rat model of GWI. Sustained release of \(Ca^{2+}\) from the intracellular stores was found to be a major source for these \(Ca^{2+}\) elevations since in vitro treatment with levetiracetam, a mixed IP3/RyR antagonist, normalized \(Ca^{2+}\) levels in GWI neurons. In this study, we investigated whether treatment with levetiracetam could help improve neurological symptoms in GWI rats. Male Sprague-Dawley rats (9 wks) were exposed to DFP (0.5 mg/kg, s.c. 1x daily, 5 d). At 6 mos post-DFP exposure, rats were assessed using forced swim test (FST) for depression, elevated plus maze (EPM) for anxiety, and novel object recognition (NOR) for memory deficits. Levetiracetam (50 mg/kg, ip) was injected 30 min prior to behavioral assays in both GWI and age-matched control rats. Rats were tracked using Ethovision XT 10.2 and behavior was scored by two independent observers. DFP-treated rats exhibited significant deficits on FST, EPM, and NOR compared to control rats, indicating presence of chronic GWI neurological morbidities. GWI rats treated with levetiracetam exhibited significantly decreased immobility time on the FST (81 ± 7 vs 127 ± 12s respectively), exhibited increased exploration the open-arm of the EPM (12 ± 3 vs 29 ± 4% respectively), and spent more time with the novel object on the NOR test compared to non-treated GWI rats (69% vs 55% respectively, n= 6 rats). No such effects were observed in levetiracetam-treated age-matched control rats. Levetiracetam treatment in GWI rats significantly improved performance on FST, EPM, and NOR tests compared to non-levetiracetam-treated GWI rats. Interestingly, levetiracetam had no effect on the behavior of age-matched control rats. Our studies suggest that therapeutic targeting of intracellular \(Ca^{2+}\) stores with drugs like levetiracetam could be effective therapy for GWI neurological symptoms.

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About one-third of the Persian Gulf War veterans exhibit Gulf War Illness (GWI) symptoms, particularly depression and memory deficits. Chronic exposure to organophosphates (OP) is among multiple causes for GWI, yet its pathobiology remains ill understood. The role of calcium \(Ca^{2+}\) signaling in memory and mood is well established. In an OP:diisopropyl fluorophosphate (DFP)-based rat model of GWI, we observed disruptions in neuronal \(Ca^{2+}\) levels (\(Ca^{2+}\)i). This study is aimed at identifying mechanisms underlying elevated \(Ca^{2+}\) and investigating whether their therapeutic targeting could improve GWI neurological morbidities. Male Sprague-Dawley rats (9 wks) were exposed to DFP (0.5 mg/kg, s.c. 1x-daily for 5 d) and behavior was assessed at 3 mos post-DFP exposure. Ratiosmetric \(Ca^{2+}\) indicator Fura-2AM was used for \(Ca^{2+}\) estimation in acutely isolated hippocampal neurons. Pharmacological blockers for different routes of \(Ca^{2+}\) entry were used in vitro. Levetiracetam was used to target calcium stores in vivo and rats were assessed for behavioral symptoms. CA1 neurons from GWI rats manifested \(Ca^{2+}\)i of 399 ± 36 nM, that were significantly higher than \(Ca^{2+}\)i from age-matched control rats (208 ± 16 nM). Application of nifedipine (5 μM), DNQX (10 μM), or GdCl3 (100 μM) did not significantly affect \(Ca^{2+}\) in GWI neurons. In contrast, application of dantrolene (50 μM) or levetiracetam (100 μM) significantly lowered elevated \(Ca^{2+}\) in GWI neurons (240 ± 11 nM and 250 ± 19 nM, respectively, n= 5 animals, paired t-test). GWI rats treated with levetiracetam (50 mg/kg, ip) showed a significant reduction in immobility time on the forced swim test and improved performance on the open-arm of the elevated plus maze. Sustained \(Ca^{2+}\) elevations in GWI neurons had their origin in \(Ca^{2+}\) release from intracellular \(Ca^{2+}\) stores. The application of rydonanide (IP3 receptor antagonist) dantroline or levetiracetam produced greater than 50% reduction in their levels. Treatment with levetiracetam significantly improved symptoms of depression and anxiety in GWI rats. Since \(Ca^{2+}\) is a major second messenger molecule, such chronic increases in its levels could produce pathophysiological changes that express itself as GWI morbidities. Our studies show that treatment with drugs targeted at blocking intracellular \(Ca^{2+}\) release could be effective therapies for GWI.

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Chemical warfare nerve agents (CWNAs) such as sarin and VX continue to be a global threat. Animal models are critical for evaluating the efficacy of Cyna medical countermeasures, and a thorough characterization of available animal models is important for translating results to humans. Disruption of cholinergic function due to inhibition of acetylcholinesterase (AChE) is the primary mechanism of toxicity of CWNAs, and reactivation of inhibited AChE with pralidoxime (2-PAM) is one of the primary therapeutic strategies. CWNAs also inhibit butyrylcholinesterase (BChE) without any apparent toxic effects; rather, BChE acts as a bioscavenger that binds CWNAs and removes them from circulation. The degree of inhibition of AChE and BChE by CWNAs and selectivity of 2-PAM are known to vary between species. The objective of this study was to compare key properties of human AChE and BChE to the same enzymes derived from six commonly used large animal models. The enzyme activity and the characteristics of inhibition and reactivation of AChE and BChE were evaluated in isolated RBC membranes and plasma from the whole blood of human volunteers, as well as from different animal species. As these effects occur at concentrations below effect concentrations for AChE inhibition, the combined results indicate that other nerve agents may play a role in the neurotoxicity of OP insecticides. Funded by ZomMW (#114027/001) and the Faculty of Veterinary Medicine (Utrecht University, The Netherlands).
Temporal Variation in Rat Pulmonary Mechanics following Single Chlorpyrifos Exposure

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Occupational and environmental organophosphorus pesticides (OP) exposures are associated with increased incidence of asthma and other pulmonary diseases. While the canonical mechanism of OP neurotoxicity is inhibition of acetylcholinesterase (AChE), this inhibition is short-lived and likely not involved in persistent pulmonary dysfunction. It was previously demonstrated that the OP chlorpyrifos (CPF) causes airway hyperreactivity (AHR) in rats 24 hours after a single exposure to a dose that causes significant AChE inhibition in the lung, but not in the brain or blood. Preliminary data also suggested that neurogenic inflammation progresses after this exposure, as determined by an increased expression of the neuropeptides substance-P (SP) and calcitonin gene-related peptide (CGRP) in sensory neurons of the dorsal root ganglia (DRG) that innervate the lung. Based on these observations, it was hypothesized that AHR persists after 24 h, and that this persistence is driven by neurogenic inflammation. To test this hypothesis, eight-week old male Sprague Dawley rats were dosed with CPF (30 mg/kg s.c.) and their pulmonary mechanics in response to electrical stimulation of the vagus nerves were assessed at 1, 2, 3, or 7 days post exposure using a Flexivent mechanical ventilator. Following physiological assessment, DRG were collected, fixed, and immunostained for SP and CGRP. CPF significantly increased airway resistance response to electrical vagal stimulation at 24 hours post-exposure, but did not significantly increase this response at later time points. Unexpectedly, lung elastance response to electrical vagal stimulation was significantly increased at 7 d post-exposure. While changes in resistance correspond to constriction of the large conducting airways, increased elastance is consistent with greater constriction in the small distal airways. The latter coincides with an increase in SP and CGRP immunoreactivity in DRG. These data indicate that CPF-induced AHR may manifest in different lung compartments at different times post-exposure, and suggest that constriction of the distal airways at delayed times post-exposure is mediated by neurogenic inflammation. These findings have significant implications for assessing the risk posed by CPF, and potentially other OPs, to human health and safety. Work was supported by the NIH (grants 5RO1 ES017592 and T32 HL07013).

Copper Activation of Organophosphorus Compounds Detoxication by Chicken Serum Albumin

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O-hexyl O-2,5-dichlorophenyl phosphoramidate (HDCP) is a chiral analog of methamidophos insecticide that induced delayed neuropathy, the R(+-)HDCP inhibitor, and aging of the brain NTE. This enantiomer is not hydrolyzed by calcium-dependent phosphotriesterases in mammals tissues including the human serum paraoxonase-1 (PON1). Recently, our group has reported a relevant Cu2+-dependent hydrolysis of R(+-)HDCP in chicken serum using 20-250 µM copper in the ex vivo assays, calling it "antagonistic stereoselectivity". This study reported the identification of the responsible protein of "antagonistic stereoselectivity" in chicken serum using commercial animals metalloc-proteins with high affinity to Cu2+ and chiral chromatography method. Two hundred micrograms of human serum ceruloplasmin and horse kidney metallothionein did not show Cu2+-dependent hydrolysis of HDCP with low copper concentration (100 µM). Both proteins showed around 15% of hydrolysis of HDCP without stereoselectivity using 1 mM of copper. On the other hand, 10 µL of chicken serum or 216 µg of chicken serum albumin (CSA) (amount of albumin content in this serum volume) showed the same level (>30%) of Cu2+-dependent R(+-)HDCP hydrolysis at 60 and 120 minutes of incubation with 100 µM of copper. The incubation of CSA or human serum albumin (HSA) (216 µg) with 100 µM of different divalent cations (Cu2+, Zn2+, Fe2+, Ca2+, Mn2+ and Mg2+) showed that both proteins do not have this metal phosphotriesterase activity, except the copper in the CSA. These results confirm that the albumin is the protein responsible for "antagonistic stereoselectivity" in the chicken serum.

Increased Expression of Glial Cell-Derived Neurotrophic Factor (GDNF) and Transforming Growth Factor Beta 1 (TGF-β1) in the Hippocampus of Rats Exposed to Mancozeb Perinatally and during Adulthood

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Mancozeb is a widely utilized pesticide with millions of pounds applied annually in the United States to control plant diseases in the field and during storage. Mancozeb and its metabolite ethylendithionoureia are known for causing thyroid toxicity, leading to altered thyroid function. Thyroid hormones are essential for the development of the mammalian nervous system. Hypothyroidism may lead to the reduction of newly generated neurons and altered differentiation in immature neurons of the dentate gyrus of the hippocampus. Impaired neurodevelopment due to environmental toxins, in this case Mancozeb, may lead to increased sensitivity to neurodegenerative changes, especially upon further challenge to environmental stressors during adulthood. GDNF and TGF-β1, both neurotrophic factors, have potential neuroprotective and cell survival effects especially during an insult. This study investigated the effects of Mancozeb on the modulation of GDNF and TGF-β1 in the hippocampi of rats exposed perinatally followed by re-exposure during adulthood. Time-pregnant Long-Evans dams were exposed to 0, 50, or 100 mg/Kg body weight Mancozeb from gestational day 7 (GD-7) to postnatal day 21 (PND-21). Pups from each group were re-exposed with the same dose as dams from PND-60 to PND-90. At the end of exposure, total blood thyroid (T4) levels were measured. Hippocampi were isolated and stored for later analyses by either qPCR for gene expression profiling or immunoprobing. Whole brains were also preserved for histological evaluation. T4 levels in Mancozeb exposed dams and all re-exposed treatment groups were significantly decreased. Mancozeb exposure significantly up-regulated the gene expression of Gdnf and Tgfb1 in the hippocampus of 50, and 100 mg/Kg re-exposure groups and 100 mg/Kg groups with or without re-exposure, respectively. Immunoprobing revealed significantly elevated proteins levels of GDNF and TGF-β1, which correlated with the qPCR data. In addition, hematoxylin and eosin (H&E) stained dentate gyrus showed multiple foci of vacuolated distended neurons with increased eosinophilic Mancozeb treated animals. These findings indicate that Mancozeb injury in the hippocampus leads to increased levels of neuroprotective and cell survival factors such as GDNF and TGF-β1, which warrant further investigation to elucidate their exact roles.
2244 A Mouse Model for Human Poisoning by Tetramethylenedisulfotetramine (TMDT): Parameters Influencing Intoxication and Treatment

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TMDT is a seizure-producing neurotoxic rodenticide. Banned worldwide due to human toxicity and environmental persistence, it has been responsible for many individual and mass poisonings, often intentional. TMDT is easily synthesized, highly potent, water soluble, tasteless and odorless. Thus it is an ideal food or drink adulterant, raising concern regarding its utility for terrorist acts. No current standardized, effective treatment against TMDT intoxication exists. We sought to understand how exposure route and treatment time post-exposure alter intoxication and outcome. C57BL/6 adult male mice were exposed to TMDT via intraperitoneal (IP), subcutaneous (SC) and oral (PO) routes in a dose range of 0.1 to 0.7 mg/kg. Mice were observed for onset of twitches, clonic and tonic-clonic seizures, and survival 1 hr after exposure, and up to 24 hrs thereafter. Treatments were selected based upon performance in a Ca2+ flux assay using cerebral cortical cell cultures. TMDT produced a similar sequence of symptoms for different routes, however the doses at which the symptoms appeared varied. Seizure symptoms appeared at lower doses after IP as compared to SC exposure. Incidence of death at 1 hr peaked at 0.25 mg/kg for these routes. Two modes of PO administration demonstrated how rapidly and reliability of symptom appearance could vary by this route. While administration of selected treatments at the time of a tonic-clonic seizure was uniformly ineffective, treatment after the first or second clonic seizure yielded similar outcomes. To conclude, 1) exposure route, as well as mode of PO exposure, significantly influences key parameters of TMDT toxicity and 2) an effective treatment against TMDT intoxication exists. We sought to understand how exposure route and treatment time post-exposure alter intoxication and outcome.

2245 Amitraz Changes Monoaminergic Neurotransmitter Biosynthesis and Metabolism by E2 Content Alteration in Central Nervous System


Amitraz is a formamidine insecticide/acidaricide that alters different neurotransmitters levels, among other neurotoxic effects. Oral amitraz exposure (20, 50 and 80 mg/kg bw, 5 days) has been reported to increase serotonin (5-HT), norepinephrine (NE) and dopamine (DA) content and to decrease MAO and Metyoxytyramine (MTO) metabolites in the male rat brain, particularly in the striatum, prefrontal cortex, and hippocampus. However, the mechanisms by which these alterations are produced are not completely understood. One possibility is that amitraz monoamine oxidase (MAO) inhibition could mediate these effects. Alternatively, it alters serum concentrations of sex steroids that regulate the enzymes responsible for these neurotransmitters synthesis and metabolism. Thus, alterations in sex steroids in the brain could also mediate the observed effects. To test these hypothesis regarding possible mechanisms, we treated male rats with 20, 50 and 80 mg/kg bw for 5 days and then isolated tissue from striatum, prefrontal cortex, and hippocampus. We then measured tissue levels of expression and/or activity of MAO, catechoI-O-methyltransferase (COMT), dopamine-b-hydroxylase (DBH), tyrosine hydroxylase (TH) and tryptophan hydroxylase (TRH) as well as estradiol levels in these regions. Our results show that amitraz did not inhibit MAO activity at these doses, but altered MAO, COMT, DBH, TH and TRH gene expression, as well as TH and TRH activity and estradiol levels. The alteration of these enzymes was partially mediated by dysregulation of estradiol levels. Our present results provide new understanding of the mechanisms contributing to the harmful effects of amitraz.

2246 Effects of Low-Level Chlorpyrifos Exposure on Endocannabinoid Metabolism and Immune Function

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Prior research from our group demonstrated that neonatal rats treated with chlorpyrifos (CPF) exhibited decreased 2-arachidonylglycerol (2-AG) metabolism and increased levels of eCBs in brain at doses too low to inhibit acetylcholinesterase activity. Because eCBs are known to have immunomodulatory effects, we are investigating the link between the catabolism of these lipids and immunity in both adult and neonatal rodents. We hypothesized that CPF exposure would inactivate eCB metabolism leading to immunosuppressive effects in adult mice. In vitro and in vivo studies were conducted. Isolated naive splenocytes (SPLCs) from adult female C56BL/6 mice were pretreated with either vehicle (ethanol) or 1 μM chlorpyrifos oxon (CPO) for 30 min, followed by incubation in the presence or absence of lipopolysaccharide (LPS, 1 μg/ml, 3.5 h). Although LPS alone had no effect on 2-arachidonylglycerol (2-AG) hydrolysis activity in SPLCs, CPO treatment could significantly reduce this activity (>6-fold). CPO had no effect on LPS-induced IL-6 production. Next, adult female C56BL/6 mice (n=5 per group) were treated with vehicle or CPO (2.5 mg/kg, PO, 7 days); 4 hr after the final dose, tissues were harvested for analysis. Immunophenotyping of SPLCs by flow cytometry revealed that the proportion of CD8+ cells was significantly increased (1.3-fold) in CPF-treated mice compared to those of vehicle controls. CPF treatment did not affect 2-AG or anandamide hydrolysis activities in spleen and brain homogenates, while hepatic and lung carboxylesterase activity was inhibited in CPF-treated mice relative to vehicle controls. Resident peritoneal macrophages (RPMs) and SPLCs from vehicle- and CPF-treated mice were also stimulated ex vivo with LPS (100 ng/ml). No CPF-dependent differences were noted in IL-6 levels in the stimulated RPMs or SPLCs, although IL-6 levels were significantly lower in non-stimulated RPMs from CPF-treated mice compared to those from vehicle controls. 2-AG levels in non-stimulated RPMs from CPF-treated mice were significantly higher than those from vehicle controls. These data suggest that CPF can alter the immunophenotype of murine T-cell populations in the absence of effects on the splenic eCB hydroytic activity, while 2-AG levels were inversely associated with IL-6 levels in basal RPMs from CPF-treated mice. Further analyses are necessary to determine if CPF-mediated immunomodulatory effects are noted in other tissues. Studies funded by the MSU-CVM.

2247 Acetylcholinesterase Activity and Oxidative Stress Status of Subjects Occupationally Exposed to Organophosphate Pesticides in Nigeria

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Environmental contamination with pesticides is a problem of regional and worldwide significance. In Africa it has been estimated that 3.2% of workers suffer pesticide poisoning each year, with a possible further 0.8% suffering chronic effects of long-term exposure to pesticides. Organophosphate agents constitute about half of all pesticides used globally. Because of their actions, the public fear of risk of affected by occupational exposure to organophosphate pesticides. Fifty-two apparently healthy farmers occupationally exposed to organophosphate pesticides were compared with apparently healthy unexposed controls. Blood levels of acetylcholinesterase (AChE) activity were determined in peripheral erythrocytes using the spectrophotometric method described by Ellman. Total antioxidant status (TAS) by the method described by Koracevic et al., 2001, were assayed and Vitamins A, C, and E were assayed using HPLC, and total antioxidant production. Next, adult female C56BL/6 mice (n=5 per group) were treated with vehicle or CPO (2.5 mg/kg, PO, 7 days); 4 hr after the final dose, tissues were harvested for analysis. Immunophenotyping of SPLCs by flow cytometry revealed that the proportion of CD8+ cells was significantly increased (1.3-fold) in CPF-treated mice compared to those of vehicle controls. CPF treatment did not affect 2-AG or anandamide hydrolysis activities in spleen and brain homogenates, while hepatic and lung carboxylesterase activity was inhibited in CPF-treated mice relative to vehicle controls. Resident peritoneal macrophages (RPMs) and SPLCs from vehicle- and CPF-treated mice were also stimulated ex vivo with LPS (100 ng/ml). No CPF-dependent differences were noted in IL-6 levels in the stimulated RPMs or SPLCs, although IL-6 levels were significantly lower in non-stimulated RPMs from CPF-treated mice compared to those from vehicle controls. 2-AG levels in non-stimulated RPMs from CPF-treated mice were significantly higher than those from vehicle controls. These data suggest that CPF can alter the immunophenotype of murine T-cell populations in the absence of effects on the splenic eCB hydroytic activity, while 2-AG levels were inversely associated with IL-6 levels in basal RPMs from CPF-treated mice. Further analyses are necessary to determine if CPF-mediated immunomodulatory effects are noted in other tissues. Studies funded by the MSU-CVM.
2248 Corneal Effect in Animals Exposed to Inhibitor of 4-Hydroxyphenylpyruvate Dioxygenase: How Relevant for Human Risk Assessment?

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Isoxaloflote and several other herbicides inhibiting 4-Hydroxyphenylpyruvate Dioxygenase (4-HPPD) in plants have been designed in the last decades. These compounds also inhibit mammalian (and human) 4-HPPD, an enzyme involved in the tyrosine catabolism. Such inhibitors increase the free tyrosine in plasma which, in rats, is associated to the occurrence of corneal opacity. Interestingly, nitisinone (NTBC), a 4-HPPD inhibitor, has been used in human as drug for type 1 tyrosinemia; in those patients, no corneal lesions have been reported. The reason for the different sensitivity, has been hypothesized to derive from species differences in tyrosine metabolism. We carried out a further analysis of the tyrosine metabolism by evaluation of available toxicological studies considering the critical enzymes of tyrosine catabolism (TAT, 4-HPPD and MDHC) combined with in silico biochemistry approach, in order to better assess the relevance for humans of corneal lesions in animals. Comparative modelling showed a high sequence identity (> 87%) for the enzymes between rat, mouse and human; structures and binding sites are highly preserved too. Free energy values calculated by docking for enzymes binding to main physiological substrates (e.g. tyrosine and 4-Hydroxyphenylpyruvate for TAT) are comparable in the different species, as well as the calculated binding affinities. However, differences in the tyrosine aminotransferase (TAT) basal activity are evident among species (e.g. mouse is 3-5 times higher than in rats). Taking into consideration results from homology modelling and docking that showed no significant species differences, other factors should be investigated to explain different sensitivity of mammals in terms of accumulation of free tyrosine and corneal effects following 4-HPPD inhibition. A further step to better clarify one of the possible factor responsible of species differences could be an experimental study measuring TAT expression in different species using hepatocytes primary cultures.

2249 Comparative Toxicity of Ziram and Zineb on Rat Hippocampal Astrocytes

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Ziram and zineb are agricultural dithiocarbamate (DTC) fungicides used on a wide range of crops including nuts, fruits, and ornamentals. Although both compounds are complexed with zinc, they differ in their chemical background. Ziram is a zinc-(bis-dimethyldithiocarbamate) while zineb belong to the ethylene bisdithiocarbamate category of DTC pesticides. The widespread use of these fungicides are owed to their component metal moieties. Previous work has demonstrated that one mechanism of action of these compounds involves the induction of oxidative stress via Fenton-like reactions. In the current study, cell viability, metal accumulation, and oxidative stress were evaluated. Rat hippocampal astrocytes were chosen for this study because of the key role they play in normal brain physiology and their response to toxic injury. In this investigation, astrocytes were treated with concentrations of zineb (10-50 uM) and ziram (2-5 µM) for 24 hours. MIT and LDH analysis shows significant decreases in cell viability from treatment concentrations 30-50 µM of zineb and 4.5-5 µM of ziram. Phase contrast microscopy demonstrated morphological changes and decreased cell number consistent with cell viability data. Metal analysis was conducted using inductively coupled plasma optical emission spectrometry (ICP-OES) in order to determine the metal accumulation with each compound. Astrocytes treated with 20 and 30 µM of zineb showed significant increases in zinc levels while cells treated with 4 and 4.5 µM of ziram demonstrated significant increases only in the 4.5 µM treatment group. Previous work in our lab has demonstrated that DTC fungicides not only alter metal concentrations of the metal complexed with the compound, but also copper levels due to their ability to trans-chelate this metal. Cells treated with 4.5 µM of zineb and 20-30 µM of ziram showed significant increases in copper levels. Use of the total antioxidant capacity assay demonstrated significant increases in the antioxidant activity as compared to controls. These data lead us to conclude that exposure to these agents reduce cell viability and alter metal homeostasis. Induction of antioxidant proteins by Ziram indicates oxidative stress.

2250 Role of Chlorpyrifos in Generation of Oxidative Stress and Reduction of Cell Proliferation Capacity in Human Breast MCF-7 Cells

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Chlorpyrifos (CPF) is one of the most widely used organophosphate insecticides in the US. The primary target of CPF toxicity is the central and peripheral nervous system due to its ability to inhibit acetylcholinesterase activity. However, CPF is considered one of the endocrine-disrupting chemicals (ECDs) that alter the normal functioning of both wildlife and humans. EDCs can act at very low doses and affect the synthesis of natural hormones, their release and/or transport. Estrogen is mediated by the two receptors isomers: estrogen receptor alpha (ERα) and beta (ERβ). Both receptors are expressed in human tissues and have different action profiles. Estrogen receptor alpha (ERα) is the major regulator of breast cancer tumor behavior. In mammary gland, 17β-estradiol (E2) promotes cell proliferation in both normal and transformed epithelial cells by modifying the expression of hormone responsive genes involved in the cell cycle and/or programmed cell death. In addition, there are a number or reports that establish a link between estrogen-induced breast cancer and oxidative stress. Serum markers for oxidative damage have been shown to increase in women diagnosed with breast cancer. The aims of the present study were to examine the potential effects of CPF on estrogen-induced proliferation of human breast MCF-7 cells, and to test the effect of CPF on cellular production of oxidative stress (ROS). Also, we further examine whether the toxic effects would be mediated through ER. MCF-7 cells were treated for different exposure times and various concentrations of CPF in absence and presence of 17β-estradiol (E2). Cell proliferation, viability, ROS production and cell death were assessed. Exposure to CPF significantly decreased cell proliferation in a concentration-dependent manner in MCF-7 cells. The ERα antagonists, ICI 182,780, and ERβ antagonist, PHTPP, did not block these effects. By contrast, these antagonists inhibited E2-induced cell proliferation. Low concentrations (0.001µM to 10µM) of CPF significantly induced ROS production after exposure for one or three hours. This study demonstrated that exposure to CPF may cause toxic effects irrespective of ER receptor activation. It also suggests that CPF may act as an environmental risk factor for breast cancer. Supported by Title III.

2251 Maneb and Mancozeb Trigger Cellular Senescence via AKT and FOXO


Manganese ethylene-bis-dithiocarbamates, Maneb (MB) and mancozeb (MZ), are fungicides used to prevent blight and mildew on plants. Exposure to MB and MZ has been linked to the pathogenesis of neurodegeneration. However, the toxic mechanism of MB and MZ is not so clear. Preliminary studies from our laboratory demonstrated that exposure to MB and MZ in PC12 cells triggered cellular senescence and caused G1 phase cell cycle arrest. Studies have shown that down-regulation of a forkhead transcription factor, FOXO3a, will up-regulate p53 and p21 expressions to accelerate cellular senescence in human fibroblast cells. Our preliminary results also showed increases in p33 expression and p21 activation in response to MB and MZ in PC12 cells. In this study, we focused on unveiling the FOXO involvement in MB and MZ induced senescence in PC12 cells. Since phosphorylation of AKT can inactivate FOXOs resulting in their accumulation in the cytoplasm, western blot analysis was applied to evaluate the status of AKT. The FOXO reporter encoded with GFP from Qiagen was transfected into cells and used to determine the activity of FOXO. The results showed that phosphorylated AKT levels increased after MB and MZ treatment for 24 hours in a dose-dependent manner, and MZ has a stronger effect on AKT activation as compared to MB. FOXO reporter analysis showed that PC12 cells treated with 5-50 µM MB and MZ for 24 hours have lowered (at least 50% reduction) FOXO activity as compared to control cells. In summary, MB and MZ activated AKT by increasing the phosphorylated AKT level, and decreased the activity of downstream effector, FOXO. In the future, the relationship between AKT/FOXO and MB/MZ induced cellular senescence will be elucidated. This study will help to understand the role of environmental toxins in the pathogenesis of neurodegeneration.
2252 A Risk-Based Approach to an Accidental Exposure of Livestock to Chlorophacinone

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The mission US Environmental Protection Agency (EPA) is to protect the environment and human health. EPA is responsible for the safety of pesticides by implementation of several laws and regulations (Federal Insecticide, Fungicide, and Rodenticide Act, the Federal Environmental Pesticide Control Act, Food Quality Protection Act and the Pesticide Registration Improvement Act). We investigated a case of accidental exposure of a herd of 850 bison to the rodenticide chlorophacinone. Chlorophacinone, is an anticoagulant rodenticide. Its mode of action is through depressing hepatic synthesis of prothrombin and clotting factors VII, IX, and X. It causes direct damage to capillary permeability. Products that contain chlorophacinone are not registered for use near crops or where livestock may graze. A reference dose and final limit have not been established for chlorophacinone. In response to this, a reference dose of 0.005 µg/kg/day was calculated using standard uncertainty factors and risk-based screening level of 0.2 µg/kg was calculated using conservative exposure assumptions based upon reasonable maximum exposure for a susceptible population. The screening level is below analytical detection limits, residue levels in bison were modeled using worst case scenario exposure parameters. Based on the modeled residue levels and the half-life of diphacinone from a ruminant exposure study (Crowell et al. 2013), a hold time of 16 months for the potentially exposed bison was calculated to ensure that the risk-based screening levels were not exceeded.

2253 2,4-D (2,4-Dichlorophenoxyacetic Acid)-Altered Gut Microbial Pathways and Metabolic Profile Involved in Kidney Disease

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Gut microbiota critically contributes to various host metabolic functions, whereas environmental chemicals are able to affect its constitution and functionality thereby resulting in host metabolic disorders. In the present study, we aimed to evaluate the functional effects of a widely-used herbicide 2,4-Dichlorophenoxyacetic acid (2,4-D), on gut microbiome. A mouse model was used combined with metagenomic sequencing and metabolic profiling to investigate functional alterations in gut microbiome by 2,4-D treatment. The metagenomics result showed that 2,4-D significantly perturbed the gut microbial functions with pronounced changes in a series of bacterial pathways involved in urea cycle and amino acid metabolism. Moreover, the metabolomics result revealed distinct metabolic profiles in both fecal and serum samples. Additionally, these functional perturbations induced by 2,4-D were associated with the progression of kidney disease. These findings suggest that 2,4-D may lead to kidney disease by targeting the gut microbiota, indicating that the relationship between environmental contaminants and microbiota is largely underestimated for comprehensive consideration of the toxicity of environmental agents.

2254 Alpha-Cypermethrin (αCM) and Lambda-Cyhalothrin (λCH) Metabolism and Exposure Assessment in Egyptian Agriculture Workers

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Pyrethroids (PYRs), including αCH and λCH, are insecticides used in both agricultural and residential settings in Egypt and other parts of the world. PYR usage has increased during the past decade with the decline of organophosphate (OP) pesticide use, although they are often used in combination. αCH and λCH are neurotoxic, acting by prolonging the open time of voltage-gated sodium channels. They are metabolized by cytochrome P-450 (CYP) and carboxylesterase (CE), resulting in the formation of inactive metabolites that serve as biomarkers of exposure. Pooled human liver microsomes were used to assess kinetic parameters (Km and Vmax) for αCH and λCH metabolism, through the formation of non-specific metabolites, 3-phenoxbenzyl alcohol (PBO alcohol), 3-phenoxbenzonic acid (3-PBA), and the specific metabolites, cis-3,2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid (cis-DCCA) from αCM and lambda cyhalothric acid (LC acid) from λCH. The formation of 3-PBA and LC acid from αCH have Vmax values of 199 and 943 pmol/min/mg protein, and Km values of 14.1 and 36.3 μM, respectively. Similarly, the formation of 3-PBA and cis DCCA from αCH have Vmax values of 364 and 1476 pmol/min/mg protein, and Km values of 31.4 and 48.8 μM, respectively. Incubation in the absence of NADPH resulted in a marked reduction in the formation of 3-PBA and 3-PB alcohol, suggesting that these metabolites resulted from CYP-mediated metabolism, while CE mediated cis DCCA and LC acid formation. To assess the impact of co-exposure of αCH and λCH with an OP pesticide, incubations were conducted with CYP-oxon, the active metabolite of chlorpyrifos, resulting in an inhibition in the formation of all metabolites of αCM and λCH. These metabolites were also detected in urine samples of Egyptian adolescent agriculture workers that applied λCH and αCM to the cotton fields. Adolescent non-applicants primarily only had elevated levels of 3-PBA, suggesting environmental and domestic exposure to other PYRs. Together, in vivo exposure and in vitro metabolism can be used in risk assessment for occupational and residential exposures and to assist efforts to reduce exposures. NIEHS and the Fogarty Institute R21 ES017223 and R01 ES022163, Rohilman.

2255 Evaluation of Repellent Activities of Essential Oils Camphor, Norcamphor, and Thujone in Two Drosophila Species

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Essential oils derived from various plants have been shown to repel insects including flies and mosquitoes. Camphor and thujone are two essential oils found, respectively, in the wood of the camphor laurel, a large evergreen tree found in Asia, and in several plants, such as the arborvitae, cypress, and wormwood. Norcamphor is an analog of camphor, but without the three methyl groups. The objective of this study was to test the potential repellency of camphor, norcamphor and thujone against two species of the fruit fly D. melanogaster and Drosophila suzukii. D. melanogaster is a model insect and D. suzukii is an invasive pest known for its tendency to attack healthy, ripening fruit leading to crop loss and threatening fruit production. Electrophysiological recording experiments show that camphor, norcamphor and thujone activate specific olfactory neurons in both insect species. Preliminary T-maze behavioral assays showed that these compounds exhibit repellency against adult flies. Findings from this study suggest that camphor, norcamphor and thujone are potent repellents that may be further evaluated to be incorporated as a strategy for D. suzukii control.

2256 Effects of Dithiocarbamates, Mancozeb, and Disulfiram on Myocardial and Skeletal Muscle in Long Evans Rats

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Mancozeb (MZ) and Disulfiram (DS) are dithiocarbamates (DTC) used widely throughout agriculture, medicine, and industry. MZ is an ethylene bis-dithiocarbamate complexed with manganese and zinc and is used as a fungicide on fruits, vegetables, and seeds. DS, a diethylthiocarbamate, is used for alcohol aversion therapy under the brand name Antabuse®. Both of these compounds have been implicated in neurotoxicity however their effects on muscle have not been fully studied. The focus of this investigation was to determine the effects of MZ and DS on muscle degeneration in vivo. Adult, male Long Evans rats were treated with either 250mg/kg MZ or 750mg/kg DS via oral gavage in PEG 400 vehicle, three times a week for four weeks. Animals were euthanized four hours after final dose. Serum was separated from whole blood obtained via cardiac puncture for analysis of myoglobin (MYO). A significant increase in myoglobin (p < 0.05) was observed in DS treated animals [8.12 ± 1.27 ng MYO/mg Total Protein (TP)] when compared to control [4.31 ± 0.53 ng MYO/mg TP]. No difference was seen in MZ treated groups. Body weights of all animals were recorded before each treatment. As a result of DS treatment, a significant decrease in body weight gain was observed compared to control and MZ treated animals. Vastus medialis and right ventricular myocardium were isolated and fixed in 3.0% phosphate buffered glutaraldehyde followed by further processing for transmission electron microscopy (TEM). TEM micrographs of Vastus medialis from control rats showed typical skelatal muscle morphology of aligned sarcomeres with prominent, straight Vastus medialis micrographs of the ripening fruit leading to crop loss and threatening fruit production.
with defined sarcomeres and prominent intercalated discs. MZ-treated ventricular myocardium was seen to contain multi-vesicular bodies and extensive mitochondrial damage including destruction of the internal membrane. DS-treated ventricular myocardium showed myofibril splitting and disintegration and flocculent material within the mitochondria. This initial study demonstrates alterations to muscle architecture in both skeletal and cardiac musculature after treatment with MZ and DS however the myopathy is different for each toxicant.

2257 Toxicological Effects of Ethophrophos, Cadmium, and Their Mixture on Biochemical, Hematological, and Genotoxic Parameters in Albino Mice


Humans are usually exposed to a variety of chemical mixtures in the air he breathes, the water he drinks, and the food he eats. There are concerns regarding the presence of these toxicants individually and also interactive if co-exposure takes place due to their biochemical, mutagenic, and carcinogenic potential for humans and animals. The present study aims to evaluate the toxicity of ethophosphos and cadmium in male mice by determining the median lethal doses (LD50) and investigate the effects of sublethal doses (1/10 LD50) of ethophosphos, cadmium, or their combination either as a single dose or repeated doses for 7, 14, or 28 days on some biochemical, genotoxic, and reproductive toxicity parameters in Albino male mice. The results indicated that the oral LD50 values of ethophosphos and cadmium were found to be 8.12 and 31.50 mg/kg b.w, respectively. Sublethal doses of ethophosphos, cadmium, or their combination were found to alter the levels of WBCs, % of hematocrite, hemoglobin, and protein, as well as AST and AChE activities. Genotoxic studies revealed an increasing in total of chromosomal aberrations, mitotic index, and sperm abnormalities following single or repetitive treatment with either ethophosphos, cadmium, or their combination and the percentages of changes were dose-dependent. Moreover, the mixture had the most dramatic effects to produce the genotoxic effects. It can be concluded that the exposure to a pollutant alone or combined with others may produce a wide variety of toxic effects.

2258 Toxicokinetics (TK) of Permethrin as a Function of Isomer and Age of Rats

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Permethrin, a class I pyrethroid, is used extensively as an insecticide throughout the world. Nevertheless, there are limited TK data for development and validation of physiologically based pharmacokinetic (PBPK) models for subpopulations, notably children. The objective of this study was to assess the relative TK of cis- versus trans-permethrin (CIS vs. TRANS) as a function of stage of maturity. Groups of preweanlings (PND 15), weanlings (PND 21), and adult (PND 90) rats were given a series of doses of CIS or TRANS as a single oral bolus and euthanized at intervals for up to 24 hr for collection of plasma, brain, liver, muscle, and fat for isomer analysis by HPLC. Both CIS and TRANS were absorbed more rapidly and attained higher concentrations in plasma, brain, liver, and muscle of pups than in adults. The lower clearance and higher peak concentrations in pups resulted in significantly higher plasma and brain levels for both isomers. TRANS clearance and AUCs were inversely related to age in the PND 15, 21, and 90 animals. TRANS half-lives diminished from 5.2 to 3.6 to 0.9 h in the PND 15, 21, and 90 groups, respectively. CIS plasma Cmax and AUC levels were 3 to 4 times higher than TRANS levels, reflecting the more rapid metabolic clearance of TRANS. These data demonstrate that the TK of permethrin in rats is both isomer- and age-dependent. Supported by the Council for the Advancement of Pyrethroid Human Risk Assessment (CAPHRA).

2259 Redox Cycling Chemicals as Fungicides for the Prevention of Biodiesel Degradation

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Biodiesel is a renewable fuel composed of mono-alkyl esters of long chain fatty acids derived from vegetable oils or animal fats. It is also susceptible to biodegradation by contaminating microorganisms such as bacteria or fungi. Degraded fuel contains less overall energy and contains byproducts that can clog fuel filters and necessitate premature repairs. One approach to preventing fuel degradation is the addition of biocides that inhibit the growth of these fungi. Bactericides and fungicides may be added to biodiesel to prevent degradation, but these chemicals may come with potentially negative effects, such as being poisonous to humans or the environment, being non-degradable (such as metal-containing compounds), or by being detrimental to com-bustion of the fuel. One class of chemicals that may be of use are redox cycling chemicals which consist of carbon and oxygen and are readily combustible with no byproducts. Biodiesel is frequently contaminated with water, with the fungi primarily growing at the biodiesel/water interface. Selective choice of redox cycling chemicals that target the oil, water, or interface layer might therefore differentially inhibit fungal growth. We investigated several redox cycling chemicals with differing water/octanol coefficients to determine their efficacy in inhibiting the growth of two fungal strains isolates from contaminated biodiesel: Wickerhamomyces and Byssoschlamys. Wickerhamomyces was more susceptible to lipid soluble compounds including menadione, 1,2-naphthoquinone, and 2-methoxy-1,4-naphthoquinone, whereas Byssoschlamys was more broadly susceptible to toxicants and at lower concentrations. We next tested the effect of solvents on the toxicity of the menadione against both species. DMSO, ethyl acetate, and acetone all exhibited lower LD50 values, with ethanol, Tween 20, and biodiesel itself exhibiting higher LD50 values. These data show that solvents increase the toxicity of redox cycling fungicides and that the combination of a lipophilic toxicant with a solvent miscible in both oil and water exhibits the greatest toxicity to fungal contaminants. Taken together these data suggest that redox cycling chemicals may be useful in preventing the degradation of biodiesel by contaminating microorganisms.

2260 Evidence for Hepatic CYP4A1 Induction and Peroxisome Proliferation in Rats following Pyrethroid Insecticide Lambda-Cyhalothrin Exposure


Lambda-cyhalothrin, a synthetic pyrethroid insecticide, has been extensively used in the last two decades to control agricultural pests and insects of veterinary as well as human concern. Studies have reported links between insecticide exposure and adverse health effects including damage to the liver, endocrine disruption, fertility problems, neurological disorders, and cancer. Because there have been no data demonstrating whether lambda-cyhalothrin affects CYP induction and/or hepato cellular proliferation and because all peroxisome proliferators examined to date induce cytochrome CYP4A1 enzyme responsible for the omega-hydroxylation of fatty acids, this study examined lambda-cyhalothrin effects on CYP4A1 enzyme activity in liver microsomes and peroxisome proliferation determining hepatic peroxisomal enzyme activities (carnitine acetyltransferase and palmitoyl-CoA oxidation). Treatments of Wistar rats with lambda-cyhalothrin (1, 2, 4, and 8 mg/kg, i.p. over 2 days) gave rise to an increase in hepatic fatty acid hydroxylase activities reflecting CYP4A1/2 activity. Lambda-cyhalothrin produced, in a dose-dependent manner, a significant increase in the 12- and 11-hydroxylation of lauric acid (85% and 107%, respectively, P<0.001 for the dose 6 mg/kg bw). Lambda-cyhalothrin increased in rat liver the cyanide-insensitive β-oxidation of palmitoyl-CoA, enzyme marker for peroxisomes, and carnitine acetyltransferase activity (60% and 57%, P<0.001, respectively). The fact that lambda-cyhalothrin increased CYP4A1 activity and peroxisomal β-oxidation of lipids would support the classification of lambda-cyhalothrin as a potential peroxisome pro liferator with possible implications for oxidative stress. This subject is of considerable importance because many peroxisome proliferators are the prototype to exhibit epigenetic mechanism of hepatocarcinogenicity. Work supported by Projects (ALIBIRD-CM Program) Ref. S2013/ABI-2728 from Comunidad de Madrid, and Ref. RTA2015-00010-C03-03 from Ministerio de Economía, Industria y Competitividad, Spain.
Transferable Residues of Dinofuran, Pyriproxyfen, and Permethrin from Dogs Topically Treated with Vectra 3D to Humans

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The present investigation was undertaken with two specific objectives: one, to evaluate the safety of dinofuran, pyriproxyfen, and permethrin in dogs after topical application of Vectra 3D®, and two, to determine the transferable residue of these insecticides from dogs to humans. Six healthy, adult dogs with medium hair length received topical application of Vectra 3D® (3.6 mL; dinofuran, 4.95%; pyriproxyfen, 0.44%; and permethrin, 35.06%) on the back between the shoulder blades. At pre-determined intervals, dogs were given a full physical exam, and residues of these insecticides were determined in dog blood and cotton glove extracts using GC/MS. Peaks of dinofuran, pyriproxyfen and permethrin were identified and confirmed based on retention time and characteristic ions. At no time was dinofuran, pyriproxyfen or permethrin detected in blood samples. In glove extracts, residues of dinofuran, pyriproxyfen, and permethrin were detected 24 hr post-treatment (19.87±1.39; 25.79±3.65; and 399.6±100.45 ppm, respectively). At 48 hr post-treatment, the residues of dinofuran and pyriproxyfen were maximum (33.69±1.16 and 31.36±4.11 ppm, respectively), while the residue of permethrin was decreased (273.40±79.94 ppm). Permethrin residue persisted on canine coat in a significant amount until 3 weeks and in trace amount until 4 weeks post-treatment, while the residues of pyriproxyfen were undetectable after 1 week. At no time did dogs show any side effects. These findings suggest that Vectra 3D® is safe for dogs and transferable residues of dinofuran, pyriproxyfen, and permethrin pose no health concern to pet owners or veterinary personnel. Of course, veterinary personnel may require proper protection to avoid cumulative ecotoparasiticide exposure.

Exposure Levels of Organophosphorus Insecticides in 1.5- and 3-Year-Old Children in Japan

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Several epidemiological studies suggested that exposure to organophosphorus insecticides (OP) could elicit adverse health effects on children. It is thus important to clarify their exposure levels in each age group during infancy. In this study, we prospectively measured OP exposure level at 1.5 and 3 years old in a birth cohort in Japan. We recruited 283 children and 263 families participating in the Japan Environment and Children’s Study (JECS) at Aichi Regional Center of JECS when they became 1.5 and 3 years old between June 2015 and July 2017, and collected urine taken directly or extracted from disposable diapers by acetone. Six urinary dialkylphosphates (DAPs) and creatinine (C) were measured by ultraperformance liquid chromatography with tandem mass spectrometry. The absorption of OP into the diapers was corrected. The median concentrations (nmol/g Cr) of the urinary total dimethylalkylphosphates (DDMAP), total diethylalkylphosphates (DEAP), and total dialkylphosphates (DAP) were 165.3, 57.9, and 263.8 in 1.5-year-old children and 767.9, 66.1, and 257.8 in 3-year-old children, respectively. Reproducibility of exposure categories at both ages classified using quartile DAP concentrations was poor; the highest Cohen’s kappa coefficient was 0.052 for dimethylphosphate. The medians of DAP concentrations in each season (nmol/g Cr) at 1.5 and 3 years old were 215.2 and 214.3 for spring (Apr-Jun), 238.6 and 247.2 for summer (Jul-Sep), 345.8 and 476.9 for fall (Oct-Dec), and 293.2 and 260.9 for winter (Jan-Mar), respectively. The concentrations in fall at both ages were higher than those in other seasons (p<0.05 except for a difference between fall and winter at 1.5 years old). It was suggested that the difference in OP exposure levels between the ages was smaller than that between seasons.

Morphological Changes in Rat Colon after Mancozeb Exposure as Seen by Light and Scanning Electron Microscopy

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Mancozeb is a broad-spectrum fungicide whose polymeric structure consists of an ethylene bisdithiocarbamate backbone complexed to the metals manganese and zinc. The ratio of manganese to zinc in Mancozeb is 10:1. Oral exposure to Mancozeb can occur to manufacturers, applicators of the fungicide, and consumers of produce sprayed with this fungicide. Several human studies have shown immunomodulatory effects of Mancozeb in pesticide workers, and that uses of pesticide workers that handle Mancozeb have high concentration of manganese found in house dust, suggesting that families of Mancozeb applicators are also at risk for exposure. Oral exposure and the risk of reported potential toxicity of the agent warrants investigation of the effects of this compound on the gastrointestinal tract. Previous studies by our lab demonstrated the toxicity of Mancozeb on transformed human colon cells resulting in disrupted, rounded cells and showed increased caspase 3/7, 8, and 9, which indicated apoptosis. The purpose of the present pilot study was to conduct an in vivo histopathological examination of rat colon after treatment with Mancozeb. In this investigation, 8-week old Sprague-Dawley male rats were treated with either 100 mg/kg Mancozeb or vehicle control (50% v/v PEG 400) by oral gavage once a week. Colon sections were collected after four weeks of treatment and stained with hematoxylin and eosin. Scanning electron micrographs of H&E stained control tissues showed simple columnar epithelium with large nuclei and goblet cells. Mancozeb treated colon showed normal epithelium with the additional presence of cells containing pyknotic nuclei and increased eosinophilia. Microvilli on the surface of the colon were undamaged in the control group, while Mancozeb treatment caused a decrease in microvilli and increased eosinophilia on the surface of the colon. Damage to mucus pores was also observed. Toxicity observed in the current study is consistent with the gastrointestinal toxicity of Mancozeb as described in previous in vitro studies.
**Effect of Malathion on Bacterial Ingestion in Caenorhabditis elegans**

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Malathion is an insecticide that is widely used for agricultural and residential purposes. When malathion is introduced to the soil and water, it can be toxic to the organisms exposed to the compound. Malathion affects both the peripheral and central nervous system. We hypothesized that if *C. elegans* were exposed to malathion, then the amount of *E. coli* ingested by *C. elegans* will decrease. In order to test this hypothesis, WT *C. elegans* were exposed to four concentrations of malathion (0.2 mM, 0.5 mM, 1.5 mM, and 10 mM) in M-medium and LB broth with *E. coli* (OP50). The optical density (OD) of the bacteria was measured at 0.5 hours, 1 hour, and 24 hours after exposure. There was a trend of increased OD from the control across all timepoints (*p = 0.1*). There was also a significant increase in OD from the control at a 1 hour exposure time (1.5 mM, *p = 0.01*). There was also a significant increase in OD for the 1.5 mM malathion treatment from 0.5 hours to 1 hour (*p = 0.05*). The results suggest that high concentrations of malathion are toxic to organisms in the soil within 1 hour of exposure, in part by slowing feeding behavior, but that lower concentrations can be tolerated without significant adverse effects for at least 24 hours.

**Impact of Chronic Glyphosate Exposure on Hepatic and Ovarian Signaling in Female Mice**

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Representing approximately 50% of US herbicide usage per annum, glyphosate (GLY) usage is extensive. Previously, we discovered that chronic GLY (2 mg/kg; 20 weeks; 5 days per week) exposure decreased primordial follicle number and altered ovarian folliculogenesis and steroidogenesis associated proteins. This study investigated the hypothesis that shorter GLY exposure would also affect ovarian signaling pathways and deplete primordial follicles. Postnatal day 45 C57BL6 female mice were exposed to GLY (2 mg/kg) 5 days per week for 5 or 10 weeks. Mice were euthanized in the pro-oestrus phase of their estrous cycle. GLY exposure for 5 weeks did not impact (*P > 0.05*) body or any organ weight. After 10 weeks, there was no effect (*P > 0.05*) of GLY on body, kidney, heart, or uterus weight; however, ovarian and liver weights were reduced (*P < 0.05*). There was no impact (*P > 0.05*) of either 5 or 10 weeks of GLY exposure on healthy primordial, primary, secondary, or antral follicle numbers. The phosphatidylinositol-3 kinase (PI3K) pathway is critical for oocyte viability, primordial follicle activation, and steroidogenesis. GLY exposure did not impact (*P > 0.05*) abundance of ovarian mRNA encoding the PI3K members kit ligand (*Kit*), Kit proto-oncogene receptor tyrosine kinase (*c-Kit*), nor the insulin receptor (*Insr*), insulin receptor substrate 1 (*Irs1*) or *Irs2*. Neither ovarian thymoma viral proto-oncogene 1 (*AKT*) protein nor phosphorylated AKT protein were affected (*P > 0.05*) by GLY exposure. GLY did not alter (*P > 0.05*) ovarian mRNA or protein abundance of steroidogenic acute regulatory protein (*Star*), 3β-hydroxysteroid dehydrogenase (*Hsd3b*), cytochrome P450 (*Cyp11a1* or *Cyp19a*). Circulating 17β-estradiol and progesterone concentration were unaffected (*P < 0.05*) by GLY exposure. Hepatic protein analysis revealed that 42 proteins were increased and 26 decreased (*P < 0.05*) by GLY compared to control. KEGG pathway analysis revealed greatest alterations to proteins involved in metabolic pathways, carbon metabolism, and nonalcoholic fatty liver disease. These data support hepatic impacts of GLY exposure after 10 weeks, which could reflect metabolic changes in the organism. Unfortunately, we could not discover any effect on the ovarian endpoints examined by 5 or 10 weeks of chronic GLY exposure. Supported by R21ES026282 from the NIHES to AFK.

**Risk of Insecticides Usage to Control Grain-Stock Pests**

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Treatment of grain stocks with insecticides before storage is a common technology to control insect pests. Deltamethrin (pyrethroid), pyrimiphos-methyl, and chlorpyrifos-methyl (organophosphorus compounds) are used for disinfestation of grain stocks in Ukraine. The use of grain stocks with residues of insecticides can pose a significant risk to human health. Work purpose is risk assessment of insecticide residues for humans after the protection of grain stocks. To identify the hazard and assess the exposure, physicochemical characteristics, LD50 per os, application rates of insecticides active ingredients (AI), ratio of application rate and LD50 per os, results of residues measuring of AI in grain stocks (gas-liquid chromatography), period of insecticide retention in grain (DT50), ratio of possible daily intake (DI) of AI residues to acceptable daily intake (ADI), processing factors (PF), and daily consumption of grain processing products were analyzed. The integral exposure vector (combination of ADI, DI, DT50) was calculated and evaluated according to the proposed scale. Insecticides AI have sufficient selectivity of action: their effective application rates range from 180-640 times lower than LD50 per os. Low water solubility, hydrolytic stability, and high lipophilicity are promote resistance of AI, their concentration in brain and grain germ after processing of stocks. Due to the high persistence, the absolute residues amount in grain at the end of the study (up to 90 days) is affected by the initial application rate. The application rates and residues increase in the deltamethrin—chlorpyrifos-methyl—pyrimiphos-methyl series. With a conservative approach in 80-90 days after treatment, the theoretical DI of deltamethrin did not exceed ADI, while the pyrimiphos-methyl DI exceeded the ADI by 5 times, and chlorpyrifos-methyl by 11 times. When exposed on residual levels, insecticides with the highest values of the integral exposure vector— pyrimiphos-methyl and chlorpyrifos-methyl—are at greatest risk. With a more realistic approach, the largest exposure of pyrimiphos-methyl is associated with the consumption of bread from whole-meal flour (at the level of ADI), chlorpyri- fos-methyl—with consumption of bread from whole wheat and bran (2.4 and 2.3 times higher than ADI, respectively); the lowest level of exposure of pyrimiphos-methyl and chlorpyrifos-methyl is possible with the consumption of white flour and white bread.

**New Models to Assess Isoxaflutole Rodent Liver and Thyroid CAR/PXR Mode-of-Action**

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In rodent oncogenicity studies conducted with the herbicide isoxaflutole (IFT), increases in the incidences of hepato-cellular adenomas and carcinomas in rats and mice and of thyroid follicular cell adenomas in male rats were observed. Previously, traditional mode of action (MoA) studies (ex: mutagenicity, enzyme induction, BrdU proliferation, thyroid hormone kinetics) suggested constitutive androstane receptor (CAR) and/or pregnane X receptor (PXR) activation. Additional more novel studies were recently performed and found to support IFT as a novel thyroid and anti-thyroid MoA. The recent development of CAR/PXR double KO rats enabled the use of both mouse and rat to explore the essentiality of CAR/PXR causing the rodent-specific liver and thyroid proliferative effects of IFT. Wild type (WT) and KO rats and mice were administered IFT via the diet for 7 days at the highest doses from the oncogenicity studies (6,000 and 6,500 ppm, respectively). Results obtained in the liver with rats and mice confirmed that IFT acts through the CAR/PXR MoA. Induction of specific cytochrome P450 isoforms linked to the CAR/PXR receptors, increased liver weight, proliferation, and hepato-cellular hypertrophy were observed only in rats but not KO control rat models. The thyroid labeling index did not increase, increased relative thyroid weight, follicular hypertrophy, thyroid stimulating hormone (TSH), and induction of specific hepatic Phase II enzymes were observed in the male WT rats but not in the KO. While both triiodothyronine and thyroxine were decreased in the WT rat, high variability of TSH and unexpected thyroid hormone results in the KO rat require further investigation to assess the utility of this model for examining secondary thyroid effects. In vitro, replicative DNA synthesis indicating hepatocellular proliferation was only increased in rodent hepatocyte cultures, while specific P450 isoforms were increased in both rodent and human hepatocytes. These new models thus support that the hepatocellular and thyroid follicular cell tumors observed in IFT rodent oncogenicity studies are due to activation of the CAR/PXR MoA. As humans do not show the proliferative changes in the liver and are less susceptible to thyroid hormone perturbation secondary to liver induction, the tumors observed in rodent oncogenicity studies with IFT are not relevant for human risk assessment.

**Threshold Hormonal Disturbance Is the Cause of Leydig Cell Tumors in Rats after High Doses of Iprodione**

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Leydig cells tumors (LCT) were observed only after high doses (75 and 69 mg/kg) of iprodione, a fungicide active ingredient, in male rats in two 2-year studies. The primary objective of this study was to determine a NOAEL (threshold dose) based on changes of sensitive hormonal endpoints that regulate cell proliferation and functionality, and morphom-
etriy of Leydig cells in testis. Six groups of male SD rats aged at 13-14 weeks (30 rats/group) were administered 0 (control), 2, 6, 24, 140 or 250 mg/kg equivalent of iprodione via the diet for 90 consecutive days. Decreases in body weight (11% and 24%), body weight gains (29% and 77%), and epididymal sperm reserves (17% and 34%) were observed at the two high doses 140 and 250 mg/kg, respectively. Serum LH and FSH were significantly increased at both high doses (days 0, 21, 42, and 90) during the treatment. However, the contents of the beta subunits of LH and FSH were significantly increased only at the highest dose (250 mg/kg). Neither intra-testicular testosterone (T) concentrations nor the ability of isolated Leydig cells to synthesize T under simulated conditions was affected by any treatment. Furthermore, the mRNA or the protein concentrations for key steps in Leydig steroidogenesis was not affected even at the highest dose tested, at basal or stimulated conditions. Mitotic index of Leydig cells at the end of the 90 day treatment, as determined by EDU incorporation, revealed that none of the treatments resulted in a change in the low, basal rate of mitosis. The numbers of Leydig cells per testis and their volume density, as determined using morphometry, were not affected at doses up to 24 mg/kg/day; however, the number of Leydig cells per testis did increase to a similar extent (25%) with the two higher doses. The results clearly suggest a threshold hormonal disturbing mode of action for LCT formation in male rats, and therefore, a GI* based on LCT does not appear to be necessary; a reference dose approach should be protective of LCT.

**2270 Dissipation and Residues of Butralin in Tobacco under Field Conditions**


Butralin is applied as a plant growth regulator for the control of auxiliary bud growth on various types of tobacco in the maturation stage. The dissipation and residues of butralin in tobacco were investigated by modified QUECHERS method combined with gas chromatography tandem mass (GC-MS/MS). The average recoveries were in the range of 89-94% with relative standard deviations (RSD) less than 10%. The limit of detection (LOD) and limit of quantification (LOQ) of butralin was 3.3 and 11 μg/kg at the signal-to-noise ratio (S/N) of 3 and 10, respectively. Field experiments including residue dynamic experiment and final residue experiment were conducted in Enshi, Linyi, Qujing and Zunyi during the year of 2016. The dissipation of butralin appeared to follow the first-order kinetic reaction with half-lives of 2.78 - 3.38 days at four geographical experimental plots, which suggested that the dissipation of butralin in the field might be affected by some physical and chemical factors. The results clearly demonstrate that powdered butralin powder does not affect Leydig cell number or steroid production at doses up to 24 mg/kg. A NOAEL of 24 mg/kg determined from this study is consistent with observed LCT only at high doses but not at the mid doses (15/12 mg/kg) in two 2-year rat studies. These results strongly suggest a threshold hormonal disturbing mode of action for LCT formation in male rats, and therefore, a GI* based on LCT does not appear to be necessary; a reference dose approach should be protective of LCT.

**2271 A Species-Comparative Pharmacokinetic and Dynamic Investigation into the Inhibition of Glutamine Synthetase by the Herbicide Glufosinate**

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The mode of action for the herbicide glufosinate is through inhibition of glutamine synthetase (GS), which catalyzes the condensation of glutamate and ammonia to form glutamine. This reaction is involved in the glutamine synthase in processes as diverse as ammonia detoxification, energy metabolism, and neurotransmission. Despite conserved sequences and structures, dogs are very sensitive to intoxication by GS inhibitors when compared to rats and humans. The differences were hypothesized to be due to species-specific brain uptake and/or enzymatic processes. Our aim was to evaluate the pharmacokinetic and -dynamic factors involved in the inhibition of human, dog, and rat GS by glufosinate. PBPK analysis showed roughly 7:1 and 2:1 higher levels of glufosinate in dog and rat brain respectively compared to extrapolated human levels. Pharmacodynamic studies revealed the structure of human GS as a dimer of two concentric pentameric rings with 10 active sites; structurally conforming to earlier dog models. Glufosinate appears to be phosphorylated at the terminal phosphate upon entry to the active site, with the resulting pyrophosphate compound responsible for the inactivity of the GS. The human, dog, and rat enzymes also exhibit differential sensitivity to inhibition by glufosinate. Maximal inhibition occurs at inhibitor-to-active site ratios of 10:1, 1:1, 5:1 for the human, dog, and rat enzymes, respectively. Together, the pharmacokinetic and dynamic analysis shows the dog and rat are roughly 70 and 10 times more sensitive to GS inhibition compared to humans.

**2272 The Organophosphorus Pesticide Chlordrinfos Induces Neuron Differentiation of SH-SY5Y Neuroblastoma Cells**


The acute toxicity of the organophosphorus insecticide chloridrinfos (CPF) is primarily due to the irreversible inhibition of acetylcholinesterase (AChE). However, exposure of the developing brain to CPF levels that are insufficient to cause a substantial degree of AChE inhibition has been associated with neurological deficits in children and animal models (reviewed in Burke et al., J Neurochem 142,162, 2017). Yet mechanisms that contribute to the developmental neurotoxicity of CPF remain poorly understood. This study was designed to test the hypothesis that, acting via AChE-unrelated mechanisms, CPF disrupts neuronal differentiation, a critical process that shapes the nervous systems structurally and functionally from the earliest stages of embryogenesis. To address this hypothesis, SH-SY5Y cells (neuroblastoma cells used to model neuronal differentiation in vitro) were exposed for 7 days to CPF (0.1-30 μM) and processed for analysis of cellular differentiation, protein expression and phosphorylation, and histone modifications. In SH-SY5Y cell extracts, 0.1-3.0 μM CPF caused no significant inhibition of AChE, whereas 10-30 μM CPF caused a small, albeit significant degree of AChE inhibition. Following 7-day exposure of SH-SY5Y cells to 3 μM CPF, expression of nestin (a marker of progenitor cells) decreased while expression of NeuN (a marker of mature neurons) increased significantly. In addition, 3 μM CPF increased phosphorylation of a number of mitogen-activated protein kinases, including p38, and the p38 activity was required for CPF (3 μM)-induced increase in the levels of dimethylated histone 3 lysine 4. This is the first study to demonstrate that CPF, acting via AChE-independent mechanisms, may act at least in part as an oxidative stress activator and may involve p38 activation and histone modifications, induces neuronal differentiation. Supported by NIH ES019282 grant.

**2273 Effects of Glyphosate and Its Formulations on Markers of Oxidative Stress and Cell Viability in Heparg and Hacat Cell Lines**


Glyphosate (GLY) is the active ingredient found in herbicide formulations worldwide. GLY is toxic to plants by disrupting the shikimate amino acid synthesis pathway. The present day intensive use of GLY began with the introduction of GLY-resistant crops in the late 900s. A.C. Guthy GLY has a low toxicity profile for humans and mammals, conflicting reports exist as to whether it poses a cancer risk for humans. The US EPA and European regulatory agencies have described GLY as unlikely to pose a carcinogenic hazard to humans. However, the International Agency for Research on Cancer and the California EPA have classified GLY as “probably carcinogenic to humans” and “known to the State of California to cause cancer,” respectively. It has been proposed that oxidative stress may be a mechanism by which GLY could potentially cause cancer. To address this hypothesis, we are testing GLY in human cell lines using several assays that detect reactive oxygen species (ROS) or their effects. Studies were designed to compare the point of departure for the effects of GLY on cell viability (CellTiter-Glo assay) to the point of departure for effects in oxidative damage assays. We also directly compared the effects of GLY versus GLY salts, as well as GLY and adjunct active ingredients versus formulations. We used a high content, 384-well plate approach to generate extensive dose-response curves for multiple comparisons.
Assays (CellTiter-Glo, ROS-Glo, and JC-10) were performed after 1 or 24 h of exposure to test articles. GLY and GLY isopropylamine decreased cell viability and altered mitochondrial membrane potential (MMP) at ≥ 10 mM, but did not affect ROS production. The formulations were more efficacious and potent than GLY alone. Cell viability and MMP were significantly altered at 1 h by the formulations. Based on GLY concentrations, the mixture formulations were over 1000x more potent than GLY alone. In contrast to the robust induction of ROS by positive controls at both time points, formulations had no effect on ROS at 1 h and showed a marginal increase in ROS at 24 h. These data suggest that GLY does not induce oxidative stress. In addition, the formulations marginally increased oxidative stress only after significant loss of cell viability. The results were very similar for both HepaRG and HaCaT cell lines, suggesting that xenobiotic metabolism has little impact on toxicity.

2274 Histopathologic and Inflammatory Changes in the Respiratory Tract of Mice following Chlorine Gas Inhalation

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Chlorine (Cl2) gas is a cytotoxic pulmonary irritant that causes acute damage to the upper and lower respiratory tract. In the present studies we used a genetically modified model of chlorine poisoning to further investigate inflammatory mechanisms of lung injury. Spontaneously breathing male C57BL/6 mice were exposed to 325 ppm Cl2 for 15 min in whole-body chambers. Bronchoalveolar lavage fluid (BAL) and lung tissue were collected 24 h later. Exposure of mice to Cl2 resulted in peribronchial and perivascular edema, hemorrhage, and marked congestion in the pulmonary vasculature. Extensive loss of the epithelium was observed in the large bronchi resulting in denuded bronchial surfaces. This was correlated with increases in BAL cell and protein content, epithelial expression of phospho-histone H2AX, a marker of double-strand DNA breaks, and macrophage expression of heme oxygenase-1, indicating alveolar-epithelial barrier injury and oxidative stress. Cl2 exposure also resulted in increased numbers of lung macrophages and epithelial cells expressing cyclooxygenase, inducible nitric oxide synthase, and high-mobility group box 1 (HMGB1), proteins important in inflammatory lung injury; receptor for advanced glycation end products (AGE), the purine receptor for HMGB1, and tumor necrosis factor a were also increased in BAL. Whereas macrophage expression of mannose receptor was downregulated following Cl2 exposure, expression of Ym-1 was upregulated. A mechanistic understanding of Cl2-induced injury will be useful in the identification of efficacious countermeasures for mitigating morbidity and mortality following exposure to this highly toxic gas. Supported by NIH grants AR055073, ES004738, and ES005022.

2275 Inhibition of Chlorine-Induced Airway Fibrosis by Budesonide


Rationale: Chlorine gas is a commonly used, highly toxic compound that is also considered a chemical threat agent. Many victims of acute chlorine poisoning recover normal lung function, but a subset develops persistent adverse effects on respiratory health. We showed that chlorine-exposed mice developed fibrosis in large airways that was associated with inflammation and inefficient epithelial repair. In the current study, we investigated the effect of the corticosteroid budesonide on chlorine-induced airway fibrosis. Methods: FVB/NJ mice were exposed to 240 ppm-hr chlorine, and the effect of budesonide treatment was analyzed. Airway pathology was analyzed by H&E staining, and airway collagen content was quantified by image analysis of sections stained with picrosirius red. Analysis of pulmonary mechanics was performed by forced oscillation technique. Immunostaining was performed for markers for airway epithelial cells and alternatively activated macrophages. Results: Daily budesonide treatment starting 1 hour after the end of chlorine exposure prevented the development of airway fibrosis, inflammation, and abnormal lung function analyzed 7 days after exposure. In chlorine-exposed, budesonide-treated mice 7 days after exposure, large airways contained extensive denuded areas, indicating a poorly repaired epithelium. Examination at later times following 7-day budesonide treatment showed continued absence of fibrosis after cessation of treatment and regrowth of airway epithelium by 14 days after exposure. Staining for epithelial markers suggested a process of regrowth from isolated progenitor cells and revealed a poorly differentiated epithelium at 14 days after exposure. Delaying initiation of treatment until 1 or 2 days after chlorine exposure still resulted in significant inhibition of fibrosis. Conclusions: Damaged or poorly repaired epithelium has been considered a trigger for fibrogenesis, but the present results suggest that inflammation is the ultimate driver of fibrosis in this model. Budesonide represents a potential countermeasure for treating persistent effects of acute chlorine exposure.

2276 Heat Shock Protein 90 Is a Molecular Target for Sulfur Mustard and Nitrogen Mustard in Human Lung Epithelial Cells

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Heat shock protein 90 (Hsp90) is a molecular chaperone important in post-translational protein folding, stability and maturation. It is an essential mediator of proliferative and anti-apoptotic signaling, as well as cell cycle control. Mustard vesicants including nitrogen mustard mechlorothamine (bis[2-chloroethyl]methylyamine, HN2) and sulfur mustard (bis[2-chloroethyl] sulfide, SM) are bifunctional alkylating agents known to selectively bind to proteins on nucleophilic sites leading to the formation of mono-functional and cross-linked protein adducts, processes contributing to cytotoxicity and tissue injury. The present studies demonstrate that Hsp90 is a molecular target of mustard in human epithelial A549 and Calu-1 cells leading to inhibition of cell cycle progression and cytotoxicity. HN2 treatment (24 hr) was found to be cytotoxic for both lung cell types (LD50 = 22.3 ± 1.2 and 10.5 ± 0.7 μM for A549 cells and Calu-1 cells, respectively) and also induced a concentration-dependent arrest of the cells in the S and G2/M phases of the cell cycle. This was associated with decreases in expression of cell cycle regulatory proteins including p27Kip1, CDK2, cyclin D1, and Rb, as measured by Western blotting. HN2 and SM also induced the formation of high-molecular-weight Hsp90 cross-links in A549 cells, a process leading to protein dysfunction. This is supported by our findings that HN2 caused the depletion of Akt, an Hsp90 client protein, and induction of Hsp72. These data indicate that inhibition of Hsp90 by HN2 and SM is important in the mechanism of vesicant-induced cell cycle arrest and cytotoxicity. Understanding the mechanisms of action of mustards will be useful in the identification of efficacious countermeasures to mitigate tissue injury. Supported by NIH grants AR055073, NS079249, ES004738, and ES005022.

2277 Receptor for Advanced Glycation End Products (RAGE) Expression in Nitrogen Mustard-Induced Acute Lung Injury in Rats

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Nitrogen mustard (NM, mechlorothamine hydrochloride) is a toxic vesicant known to cause severe damage to the respiratory tract by alkylating DNA and proteins and generating oxidative stress. Exposure to NM results in rapid structural changes including perivascular edema and thickening of the alveolar wall followed by bronchiolization of the epithelium which progresses into lung fibrosis. Extensive loss of HMGB1, a critical mediator of proliferative and anti-apoptotic signaling, as well as high-mobility group box-1 protein (HMGB1) and promotes a pro-inflammatory response. In the current study, we determined if RAGE is involved in NM-induced lung injury. Male Wistar rats were exposed intratracheally to 0.125 mg/kg NM or PBS control. Bronchoalveolar lavage fluid (BAL) and lung tissue were collected 3 d later and analyzed for markers of injury and inflammation. Exposure of rats to NM caused an increase in BAL protein and cell content indicating alveolar epithelium injury and inflammation. Significant increases in soluble RAGE and HMGB1 were detected in BAL from NM-exposed rats when compared to PBS treated controls. This was associated with increased expression of RAGE in alveolar epithelial cells and HMGB1 in alveolar macrophages. These findings suggest that RAGE and HMGB1 play a role in regulating mustard induced lung inflammation and injury. Understanding the mechanisms of action is essential to develop novel strategies to counter the pneumotoxic effects of mustard exposure. Supported by NIH grants ES020721, US4AR055073, R01ES004738, and P30ES005022.
Inhalation of sulfur mustard (SM, 2-bis-chloroethyl) sulfide), a bifunctional alkylating agent, is known to cause severe debilitating lung injury in humans. Previously, we have demonstrated that TNFα, a pro-inflammatory cytokine, plays a key role in the pathogenic response to mustard injury. In these studies, we analyzed the ability of anti-TNFα antibody to mitigate SM-induced lung injury. Spontaneously breathing male Wistar rats were anesthetized, intratracheally intubated, and exposed to 0.4 mg/kg SM by vapor inhalation. Animals were sacrificed 3 days post-exposure and bronchoalveolar lavage fluid (BAL) and lung tissue collected. SM inhalation resulted in increases in TNFα in BAL; increases in soluble receptor for advanced glycation end products (sRAGE), a pattern recognition receptor that binds to High Mobility Group-1 protein (HMGB1) to promote pro-inflammatory vascular responses, were also observed. This was associated with increased levels of BAL cell and protein content and tissue expression of HMGB1, indicating damage to the alveolar-epithelial barrier, and inflammation. SM exposure also resulted in increased numbers of lung macrophages expressing heme oxygenase (HO)-1, consistent with oxidative stress. Treatment of rats with anti-TNFα antibody (15 mg/kg, i.v., 1x) 15 min after SM inhalation reduced lung injury and inflammation, as measured by BAL cells and protein content and SM-induced expression of sRAGE and TNFα. SM-induced levels of HO-1 and HMGB1 proteins were also suppressed by anti-TNFα antibody treatment. These data demonstrate that that blocking TNFα represents an important approach to mitigating acute lung injury induced by vesicants. Supported by NIH Grants U54AR050573, 8R01ES004738, and P30ES005022.

Nitrogen Mustard Modulates Cell Cycle Progression via the DNA Damage Response in Human Lung Epithelial A549 Cells

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The nitrogen mustard mechlorethamine (bis[2-chloroethyl][methyl] amine; HN2) and sulfur mustard are bifunctional alkylating agents that target cellular macromolecules leading to cytotoxicity and tissue injury. HN2 is known to damage DNA and result in induction of DNA damage signaling pathways including ataxia telangiectasia mutated (ATM), ataxia telangiectasia, and Rad3-related (ATR), as well as DNA-dependent protein kinase (DNA-PK). In the present studies, the crosstalk between the HN2-induced DNA damage response and cell cycle progression were investigated in human lung epithelial A549 cells. HN2 treatment (1-20 µM; 24 hr) caused a concentration-dependent arrest of cells in the S and G2/M phases of the cell. This was associated with an inhibition of DNA synthesis as measured by incorporation of 5-ethynyl-2’-deoxyuridine (EdU) into cells in S phase. HN2-mediated cell cycle arrest was correlated with the activation of DNA damage and cell cycle checkpoint signaling. Thus, immunoblotting revealed that HN2 treatment led to time- and concentration-dependent increases of the expression of DNA damage response and checkpoint proteins including phosphorylation on ATM (S1981), H2AX (Ser139), and p53 (ser15). The activation of DNA damage signaling was most pronounced in S-phase cells followed by G2/M-phase cells. The induction of cell cycle arrest was partially suppressed by pretreatment with the ATM and DNA-PK inhibitors, KU55933 and NU7441. These data indicate that activation of ATM and DNA-PK signaling may be effective countermeasures for vesicant-induced tissue injury. Supported by NIH grants AR055073, NS079249, ES004738, and ES005022.
that genetic knockout of reganase-1 and roquin by their siRNA significantly enhances arsenical-induced production of inflammatory mediators IL6, IL18, and COX2. The 5Gs disassemble with concomitant degradation of roquin and reganase-1 provides a novel molecular mechanism by which warfare arsenicals cause skin inflammation and tissue damage. oxidative stress. Taken together, these data indicate that NM-induced DNA damage is associated with epidermal oxidative stress. Antioxidants may be an important strategy for the development of countermeasures to mitigate mustard-induced epidermal injury. Supported by NIH/NIEHS grants AR055073, ES050022, and T23ES007148.

2282 Ebselen Oxide Reduces the Dermatotoxicity of Mechloethamine In Vivo: A Comparison with Ebselen

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Mechloethamine (HN2) is an alkylating agent and sulfur mustard mimetic which is also used in antitumor therapy. Dermal exposure of HN2 leads to epidermal necrotic tissue blistering. Previous work in our lab has shown that ebselen (EB-1) possesses anti-vesicant, anti-inflammatory, anti-bacterial, anti-fungal and cytoprotective properties, both in vivo and in vitro. We recently reported that ebselen oxide (EB-2), an analog of ebselen with a tetravalent selenium atom, also possesses anti-inflammatory and anti-fungal activity and confers cytoprotection against HN2 in vitro. The purpose of the present study was to determine the vesicle countermeasure potential of EB-2 in comparison to EB-1. To this end, the mouse ear vesicle model (MEVM) was used, with male Swiss Webster mice serving as the test strain. Compared to control ears, mouse ears exposed to a single dose of HN2 (0.500 µmol/ear) showed an increase in wet weights, ear thickness, edema, hyperplasia, vesication and inflammatory cell infiltration after 24 h. Fluorescence microscopy of TUNEL stained sections showed that the occurrence of apoptosis extended from the epidermis of the HN2 treated side all the way to the contralateral epidermis. In contrast, HN2 exposed mouse ears treated topically with EB-2 at a test dose of 0.250 mg/ear showed a significant decrease in wet weight (12% less compared to HN2 alone), morphometric thickness (13% less than HN2 alone) and vesication. Moreover, TUNEL staining revealed that HN2 ears treated with EB-2 (0.250 mg/ear) showed a decrease in apoptosis and vesication as compared to the HN2 group. In comparison to EB-2, topical treatment with EB-1 decreased HN2-induced changes in ear wet weights and morphometric thickness by 10% and 13%, respectively. Taken together, our study demonstrates that EB-2 is an equally efficacious test countermeasure to EB-1. Additional studies will be required to determine whether or not EB-2 exhibits more favorable pharmacokinetic parameters than EB-1 (e.g., bioavailability, duration of action, etc.).

2283 Oxidative Stress and DNA Damage in Mouse Epidermis following Exposure to Nitrogen Mustard

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Nitrogen mustard (SM, bis[2-chloroethyl] sulfide) is a potent vesicant that has been used in chemical warfare. It is a bifunctional alkylating agent known to target many tissues, particularly the skin. SM can cause inflammation, edema, necrosis, and blistering, depending on dose and time following exposure. Epidermal damage is associated with delayed wound healing and persistent dermatitis. The precise mechanism leading to skin damage induced by SM is not known. In the present studies, a modified semi-occlusive patch test model was used to analyze alterations of oxidative stress and DNA damage markers in mouse ear skin following exposure to the SM surrogate, nitrogen mustard (NM, bis[2-chloroethyl]methylene). NM (40 micromoles dissolved in 80% acetone/20%water) or vehicle control was applied onto 24 mm glass microfiber filters affixed to the shaved dorsal skin of CD-1 mice for 6 min. Epidermis was removed and analyzed 1-24 hr post-treatment by western blotting and immunohistochemistry. Within 1 hr of NM exposure, Akt, a serine/threonine-specific protein kinase known to play a role in apoptosis, was downregulated. This was associated with a 2.5-5-fold increase in expression of the double-strand DNA break marker, phosphorylated H2AX (pH2AX). And the DNA chain break-activated poly (ADP) ribose polymerase (PARP). Heme oxygenase-1, a marker of oxidative stress, also increased (1.5-4.5-fold) 1-12 hr post-NM exposure, along with proteins modified by 4-hydroxynonenal (2-3.6-fold over 1-24 hr), a lipid peroxidation end product. This was correlated with increased epidermal expression of 8-oxodeoxyguanosine (8-oxo-dG), an oxidized derivative of deoxyguanosine and a product of DNA oxidation, as well as expression of cytochrome oxidase-2 and inducible nitric oxide synthase. Infiltration of macrophages and neutrophils into the skin as well as mast cell degranulation suggested that cytokines and chemokines, as well as lipid mediators released in the tissue post-NM exposure contributed to

2284 Induction of DNA Damage and Stress Responses by the Sulfur Mustard Analog Mechloethamine in Human HaCaT Keratinocytes

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Mustard compounds, including sulfur mustard and nitrogen mustards, are vesicants known to cause inflammation and blistering in the skin. These agents have been used in chemical warfare and continue to be chemical threats. Nitrogen mustard (mechloethamine, bis[chloroethyl][methyl]amine; HN2) and several derivatives including chlorambucil and melphalan, are also used in cancer chemotherapy. In the present studies, the effects of HN2 on DNA damage/stress responses were assessed in HaCaT human keratinocytes with the goal of elucidating mechanisms of cytotoxicity. We found that HN2 caused a time- and concentration-dependent inhibition of cell proliferation as well as a 2-50µtka of S/G2/M phases of the cell cycle. Western blotting revealed that HN2 treatment caused time-dependent changes in cell cycle regulatory proteins including increased expression of the cyclin-dependent kinase inhibitor, p27 Kip1, and decreased expression of cyclin D1 and the Ser/Thr cyclin-dependent kinase, CDK2. In addition, HN2 treatment increased expression of DNA damage/stress response proteins including auto-phosphorylation on H2AX (Ser139), p53 (ser15), HSP27 (Ser82), and protein acetylation on histone H4 (Lys16). HN2 also caused an upregulation of heme oxygenase-1 (HO-1). HN2 induced differential protein expression of DNA damage markers, but not stress-response markers, within cell cycle phases. The induction of H2AX (Ser139) and p53 was most pronounced in S-phase cells followed by G2/M-phase cells. Taken together, our studies demonstrate that HN2 modulates cell cycle checkpoints and activates DNA damage responses. We hypothesize that these signaling pathways are critical to the mechanism of vesicant-induced cell cycle arrest and cytotoxicity. Supported by NIH grants ES020721, AR055073, NS079249, ES047438, and ES050022.

2285 Role of Chemokine Signaling in Delayed Skin Inflammation following Exposure to Nitrogen Mustard

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It is well established that dermal wounding is delayed following exposure to the vesicants nitrogen mustard (NM) and sulfur mustard. The molecular mechanisms underlying this effect remain poorly defined. Hallmarks of early responses to chemical burns are noted for infiltration of immune cells, which clear the tissue of dead cells and debris, these cells also release mediators that promote wound healing. Compelling evidence supports the thesis that malfunction of cyto-kine/chemokine signaling results in impaired neutrophil recruitment to sites of injury and contributes to skin destruction. We hypothesize that these signaling pathways are critical to the mechanism of vesicant-induced cell cycle arrest and cytotoxicity. Supported by NIH grants ES020721, AR055073, NS079249, ES047438, and ES050022.
Sulfur mustard (SM; bis (2-chloroethyl) sulﬁde) is a bifunctional alkylation agent used in chemical warfare. Depending on the dose and duration of exposure, SM can induce ocular irritation, tearing, photosensitivity, and short-term blindness. In the present studies, we examined the effects of ocular SM exposure on meibomian glands and goblet cells in the conjunctival eyelid borders of New Zealand white male rabbits. Twentyeight days post-SM exposure (0.4 microliters of neat SM applied directly to the central cornea), rabbits were sacriﬁced, eyelids removed, trimmed, and ﬁxed in paraformaldehyde/sucrose, embedded in parafﬁn, sectioned, and stained with hematoxylin and eosin (H&E) and Gomori’s trichrome (for analysis of collagen III). H&E staining revealed erosions in the epithelial surface of the eyelid borders along with an inﬂammatory cell inﬁltrate in the palpebral conjunctiva. The grape cluster-like appearance of the meibomian glands was still evident although the acini had lost their rounded structure and the specialized sebocytes were not as prominent as in control. These structural changes correlated with altered expression of fatty acid synthase (FAS) within the meibomian gland sebocytes of the lower eyelids of the SM exposed rabbits. Trichrome staining of the upper and lower eyelids revealed SM induced compaction of the dermis surrounding the meibomian glands. Interestingly, there were no differences in the size or total number of goblet cells in the conjunctiva of the upper and lower inner eyelid borders post-SM exposure when compared to the control. Periodic acid-Schiff (PAS, a differential mucin stain) staining revealed a 32% and 40% increase in neutral mucin containing goblet cells in the lower and upper eyelids of the SM exposed rabbits when compared to the control, respectively. Taken together these data suggest that SM injures the palpebral conjunctiva may in part modify goblet cell and meibomian gland function such that ocular barrier integrity may be compromised. Supported by NIH/NIEHS grants AR055073, ES05022, and T32ESE007148 (GMC).

2289 Warfare Agent and Pesticide Chloropicrin-Induced Toxic Reponses in Ex Vivo Rabbit Cornea and Primary Human Corneal Epithelial Cells

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Lack of research efforts to evaluate the mechanism of corneal toxicity in relevant injury models following chloropicrin (Trichloronitromethane; PS, CP), exposure has hampered the development of effective treatments against ocular injury from this ubiquitous agricultural pesticide which was employed during World War I as a chemical warfare agent. In addition to its accidental exposure, toxic effects of CP together with easy availability and lack of antidotes make it a potential agent for warfare and terrorism. Here, we analyzed the effects of CP exposure in ex vivo rabbit corneas and primary human corneal epithelial cells (HCE) to understand the mechanisms involved in CP-induced corneal injury. In ex vivo rabbit corneas, CP exposure induced signiﬁcant epithelial degradation and cell death. In HCE cells, CP exposure caused a dose-dependent decrease in viability and apoptotic cell death. These toxic effects could be related to DNA damage (p53 and H2AX phosphorylation) observed in HCE cells. In addition, CP exposure also induced caspase-3 and poly (ADP-ribose) polymerase (PARP) cleavage, suggesting their involvement in CP-related HCE cell death. CP exposure also led to increased cyclooxygenase-2 (COX-2) levels in both ex vivo rabbit cornea and HCE cells, indicating the role of cyclooxygenase and related pathways in CP-induced epithelial degradation. CP exposure in HCE cells also instigated an increase in phosphorylation of mitogen activated protein kinase-JNK as well as an increase in hemeoxygenase-1 (HO-1) expression and lipid peroxidation, further suggesting the role of oxidative stress in the activation of signaling pathways. In conclusion, CP caused an increase in protein carbonylation in ex vivo rabbit cornea and HCE cells. Proteomic analysis revealed an increase in the carbonylation of 179 proteins following CP exposure and further bioinformatics analysis found enrichment of the Pathways and cell processes affected by CP exposure in rabbit corneas and HCE cells.

2288 Corneal Injury following Ocular Exposure to Vesicating Agent Nitrogen Mustard

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Sulfur mustard (2,2’-dichloroethyl sulﬁde; SM) causes severe ocular injury, especially to the outermost corneal layer. Ocular exposure results in devastating acute and delayed injuries including inﬂammation, corneal edema, ulceration, opacity, neovascularization, and impaired vision. Effective therapies for SM-induced ocular injuries, mainly to the most affected corneal tissue, are not available. Hence, for screening therapeutic agents, we have established primary human corneal epithelial cells and rabbit corneal organ culture models with the SM analog nitrogen mustard (NM; Bis (2-chloroethyl) methyamine, HN2), which have helped to test the efficacy of potential therapeutic agents. To further test the therapeutic agents in vivo, establishment of suitable animal model and detailed understanding of the acute and chronic pathophysiology are essential. In the present study, using cornea from relevant ocular (in New Zealand white rabbits) and established clinical, histopathological and molecular markers. Right eye of each anesthetized rabbit was exposed to 100µL NM, and left eye was exposed to saline served as control. Clinicopathological observations and lesion quantiﬁcation showed that NM exposure induced corneal opacity (peak 1 day post-exposure), corneal ulceration (peak at day 14 and 21 post-exposure), eyelid, and conjunctival swelling (peak at day 1 post-exposure). NM induced an increase in corneal thickness, epithelial degradation, epithelial-stromal separation, and a surge in the number of inﬂammatory cells and blood vessels from day 3 to day 28 post-exposure. NM exposure also caused a signiﬁcant keratocyte cell death at 3 to 28 days post-exposure. NM also induced the expression of proteolytic and angiogenic mediators Metalloproteinase-9 and vascular endothelial growth factor, and elevated the levels of inﬂammatory cytokine IL-8, with maximal response at day 14 post-exposure. The ongoing studies in this ongoing effort will help in understanding the pathophysiology involved in NM-induced corneal injury, and in the evaluation of effective therapies.
proteasome pathway, catabolic process, intracellular protein transport, and tRNA aminoclylation for protein translation. Major pathways and processes identified in the molecular and proteomic analyses following CP exposure could be lead-hit targets for further biochemical characterization and therapeutic intervention.

2290 Development of a Novel Yorkshire Swine Model of Potassium Cyanide Poisoning with Improved Relevance toward Mass Casualty Care

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Exposure to cyanide (CN) poses a threat to both civilian and military populations, either through unintentional distribution involving plastic-containing fires or through intentional distribution as a weapon by terrorists. Current CN countermeasures are administered through intravenous (IV) infusion, ultimately complicating rapid and safe treatment in a mass casualty environment. The difficulties involved in the medical treatment of CN exposed patients are important to model when developing animal platforms for CN countermeasure testing. To address this, the study objective was to establish a well characterized large animal model that provided a more accurate representation of care during a mass casualty event to properly evaluate medical countermeasures for potassium cyanide (KCN) exposure. To properly represent this scenario, criteria for the animal model included: delayed onset of care, delayed lethality (>20 mins), and sufficient lethality to occur in >75% of untreated animals to allow for countermeasure efficacy comparisons in treated animals. Secondary endpoints evaluated arterial lactate, methemoglobin, pH, blood gases, glucose, blood pressure, heart rate, STsegment depression, body weight, and effectiveness of F2A (dimethyl trisulfide) during challenge. Dose rates of KCN ranged from 0.17 to 0.32 mg/kg/min. Models were fitted to the data to identify any significant effects among the dose administered, isoflurane concentration, SpO2, fraction of inspired oxygen (FiO2), and body weight. Control of these parameters prior to KCN challenge provided less variability for a sound animal model. The final model challenged anesthetized Yorkshire swine IV at a target rate of 0.185 mg/mL/min with an approximate 4.0 mg/mL KCN solution via a central line catheter. Upon injection, DMTS serves both as a sulfur donor forCN and as a metal chelator. Cyanide Antidote Dimethyl Trisulfide (DMTS) is a potent next-generation antidote against CN poisoning. Upon injection, DMTS serves both as a sulfur donor for CN and as a metal chelator.

2291 Developing and Testing a New Intramuscular Formulation for the Cyanide Antidote Dimethyl Trisulfide


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Cyanide (CN) is an extremely toxic inorganic molecule that can readily cross biological membranes. It has been shown that dimethyl trisulfide (DMTS) is a potent next-generation antidote against CN poisoning. Upon injection, DMTS serves both as a sulfur donor for CN and as a metal chelator. Cyanide Antidote Dimethyl Trisulfide (DMTS) is a potent next-generation antidote against CN poisoning. Upon injection, DMTS serves both as a sulfur donor for CN and as a metal chelator.

2292 Transcriptomic Profile Analysis of Brain Inferior Colliculus following Acute Hydrogen Sulfide Exposure

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Hydrogen sulfide (H2S) is a gaseous molecule produced endogenously and in the environment. Endogenously, it plays important roles in many physiological functions. Nonetheless, acute exposure to high concentration of H2S causes severe brain damage and induces long-term neurological sequelae. The cellular and molecular mechanisms underlying the H2S-induced dysfunction of central nervous system have not been clearly elucidated. To better understand the mechanisms of H2S-induced neurodegeneration, we performed RNA sequencing analysis to identify differentially expressed genes (DEG) and pathways that contribute to H2S-induced neurotoxicity. CS7BL/6j black mice were exposed by whole body inhalation to 765 ppm of H2S for 1, 2 or 4 days for 40 min on the first day and 10 min on subsequent days. The H2S-treated groups showed behavioral motor deficits and developed lesions in inferior colliculus (IC), among other regions. The IC was dissected at 2 hr post H2S exposure for each group and used for the transcriptomic analysis. RNA-Seq libraries were prepared using poly (A) enrichment and miRNAs were sequenced at 20 million reads per sample on an Illumina Hiseq2000 system. Acute exposure to H2S induced 283, 193 and 296 DEG (q-value < 0.05, fold-change > 1.5) for day 1, 2 and 4, respectively. Dysregulated biological pathways were further analyzed using Ingenuity Pathway Analysis. Transcriptomic analysis results and key molecular mediators deregulated biological pathways were validated using quantitative RT-PCR and Western blot assay. Acute exposure to high concentration of H2S was shown to activate Akt, Fas, c-Fos, ATF2, and TNFα in the IC. These results indicate that exposure to H2S induces dysregulation of multiple pathways leading to activation of the inflammation response. The obtained transcriptomic data may hold a role in elucidating the mechanisms of H2S-induced neurotoxicity, and neurodegeneration following acute H2S exposure.

2293 ButyrylcholinesteraseG117H as a Catalytic Bioscavenger: Structure-Dynamics-Activity Relationships of Surface Peptide Network Variants

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The use of human butyrylcholinesterase (BChE) as a bioscavenger for organophosphorus (OP) anticholinesterases is limited by its stoichiometric reaction with OP toxicants. After over 2 decades of searching for catalytic variants, the most promising mutant (BChE G117H) has a turnover rate that is too slow to be toxicologically relevant. There is a growing body of evidence that links protein dynamics to enzyme catalysis. Surface peptide networks exhibit dynamic fluctuations in concert with the catalytic reaction and may be hypothesized to play a role in energy transfer from the solvent to the active site. Molecular simulations with paraoxon as the substrate identified a catalytically active surface peptide network at residues 276-286 of human BChE G117H. We hypothesized that increasing the number of peptides in this network with long side-chain hydrophobic peptides could lead to higher transfer to catalytic residues and increased catalytic activity. We constructed six His4-tagged mutants with three peptide insertions into this region with an identity of ENX, where “X” is one of 6 different amino acids (A, G, I, P, R, and T). These mutants were produced by transfecting Spodoptera frugiperda Sf9 cells with a bacmid DNA-CellFECTIN mixture, and purified using a Ni2+-NTA agarose column. Substrate kinetics with butyrylthiocholine iodide were determined using a modified Ellman assay and fit to the Michaelian model. Resistance to inhibition was determined as rate of butyrylthiocholine hydrolysis in the presence of concentrations of paraoxon, ecothiophate and disopropylfluorophosphate (DFP) that elicited complete inhibition of wild-type BChE. Substrate kinetics indicated a reduction in kcat in all six surface network mutants compared to BChE G117H. In contrast, some BChE G117H mutants were relatively more resistant to inhibition by echaithiophate (three mutants; 12-18%) and DFP (five mutants; 15-19%). BChE G117H was more resistant to inhibition by paraoxon than all surface network mutants. These results provide further evidence that this network of surface peptides may influence OP catalysis. Further computational and biochemical studies could aid in optimizing catalytic bioscavenger development.
Seizure Behavior and Neurodegeneration Are Not Predicted by Acetylcholinesterase Inhibition in a Rat Model of Acute Disopropylfluorophosphate (DFP) Intoxication

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Several organophosphates (OP) can trigger seizures that rapidly progress to status epilepticus (SE) in humans and preclinical models. It is thought that inhibition of acetylcholinesterase (AChE), the enzyme responsible for terminating cholinergic neurotransmission in the central and peripheral systems, mediates OP-induced seizures. However, in a rat model of acute DFP intoxication, 10-15% of animals exhibit minimal to no seizure behavior upon injection with a dose of DFP that typically induces robust seizure behavior in rats of the same strain, sex, and age. Therefore, this study examined whether varying levels of AChE inhibition explained the differences in seizure response between low and high responders to DFP. Adult male Sprague-Dawley rats were acutely intoxicated with DFP (4 mg/kg, s.c.) followed by a combined injection of atropine sulfate (2mg/kg, i.m.) and 2-PAM (25 mg/kg, i.m.) and monitored for seizure activity for 4 h post-exposure. AChE activity was measured in the cortex, hippocampus, cerebellum, and amygdala using the Ellman assay at 1 and 4 d post-exposure. Neuronal damage was measured using Fluoro-Jade C staining at 1, 2, 4, and 6 d post-exposure. No significant differences in AChE activity between high-responding and low-responding animals were detected in any brain region at any time point, with the exception of the cerebellum at 4 d. At 1 d post-exposure, high responders showed significant neuronal damage in the somatosensory cortex, piriform cortex, and hippocampus, whereas low responders did not. However, delayed neuronal damage was observed in the piriform cortex of low responders at 2 and 4 d post-exposure. At 60 d, degenerating neurons remained present in the cortex of high responders but were no longer detected in low responders. These findings suggest that AChE activity is not predictive of seizure behavior or neurodegeneration in animals intoxicated with DFP, and suggests that there are SE-independent mechanisms contributing to the delayed neurodegeneration caused by acute OP intoxication. Supported by the NIH CounterACT program (NS079202); predoctoral fellowship to EAG (GM5676520, Initiative for Maximizing Student Development); predoctoral fellowship to MAG (NHMG099608, David and Dana Looury Foundation).

Novel Brain-Penetrating Oxime Acetylcholinesterase Reactivators Attenuate Organophosphate-Induced Neuropathology in the Rat Hippocampus

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The nerve agent sarin and the active metabolite of the insecticide parathion, paraoxon (PXN), inhibit acetylcholinesterase, causing excess synaptic acetylcholine and seizures which, if not stopped, result in neuronal damage in the rodent brain. Our lab invented substituted phenoxyalkyl pyridinium oxime acetylcholinesterase reactivators (US Patent 9,227,937) that penetrate the rat blood-brain barrier in vivo tests with a sarin surrogate (nitrophenyl isopropyl methylphosphonate, NIMP) or PXN, and reduce the time to cessation of seizure-like behaviors compared to 2-PAM. To test the ability of our novel Oxime 20 to prevent neuropathology, changes in the levels of Nissl, NeuN, and Fluoro-Jade B histological staining were used as indicators of neuronal damage in the CA1 hippocampal region after either 0.6 mg/kg NIMP, 0.8 mg/kg PXN, or vehicle (MultiSol) SC and 1 hour later 146 µmol/kg of a novel oxime or 2-PAM in vehicle (MultiSol) IM. Animals were euthanized 4 days after challenge and assigned a numeric code to blind participants to their positive charge and the highly restrictive blood-brain barrier (BBB), thus allowing the potential for brain damage to occur. A box containing the BBB is to deliver the oximes via this route can provide direct nose-to-brain drug transport along the olfactory and trigeminal nerves while bypassing the BBB, potentially allowing for rapid therapeutic action and protection against CNS damage. Our laboratories have synthesized a series of novel phe-noxyalkyl pyridinium oximes (US patent 9,227,937) that have shown the ability to reactivate AChE, with novel Oxime 20 showing promise as an effective reactivator. This study explored the intranasal delivery potential of Oxime 20 and 2-PAM, the current oxime for the US, to decrease brain AChE inhibition after exposure to a sublethal challenge (65-75% brain AChE inhibition) of a sarin surrogate (nitrophenyl isopropyl methylphosphonate, NIMP). Sprague-Dawley rats received 1.46µmoles in 10µl multisol vehicle of either Oxime 20 or 2-PAM bilaterally 1 hr after OP administration and were euthanized 5 min after oxime delivery. Ofactory bulbs were harvested, and brains were dissected into three regions: midbrain, midbrian, and hindbrain. Acetylcholinesterase (AChE) reactivation in the olfactory bulbs, and 20% reactivation in each of the forebrain, midbrain, and hindbrain. These data suggest rapid delivery of the oximes into all regions of the brain and a fast therapeutic onset. Use of this alternative delivery could provide a novel way in protecting against OP neurotoxicity and address the current deficiencies in OP therapy. Supported by the Defense Threat Reduction Agency HDTA 1-15-0-0046.

Assessment of Nerve Agent Reactivators Using the Mouse Phrenic Nerve Hemidiaphragm Preparation


Nerve agents are highly potent acetylcholinesterase (AChE) inhibitors, which lead to an inability of the AChE enzyme to hydrolyze the neurotransmitter acetylcholine (ACh). The resulting build-up of ACh leads to overstimulation of neuromuscular junctions, glands, and central nervous system, which results in salivation, bronchoconstriction, tremors, seizures, and central respiratory failure. Treatment with atropine, a reactivator and an anticonvulsant, can provide a fast recovery. We have employed an ex vivo mouse phrenic nerve hemidiaphragm preparation to evaluate a variety of reactivators for their ability to restore physiologic function following inhibition by the nerve agent sarin (GB). Male C57BL/6 mice 8-10 weeks old were used for all studies. Hemidiaphragm/phrenic nerve bundles/ribs were dissected and maintained in tissue baths with oxygenated (95%O2/5%CO2) Tyrode's buffer solution at 37°C. Tetanic stimulation parameters are 100 Hz, 2 sec train, 5 volts, resting tension ~3.5 g every 10 min. The experimental protocol consisted of five phases: Acclimation (buffer only), 1 hr; Baseline (buffer only), 30 min; GB exposure, 30 min; Reanimation, 1 hr; and Recovery (buffer only), 30 min. The GB bath concentration was used 5 x 10-6 M, and bath concentrations for each reactivator were 200, 100, or 50 µM. The reactivators evaluated, in relative order of efficacy at the 200 µM dose, were Hlo-7, MMB-4, H-6, SWI-80A(+), SWI-80A(-), SWR-80A, and 2-PAM. All reactivators significantly improved peak area, action potential amplitude, and twitch tension during the Reanimation phase in the absence of treatment. The oxime reactivators currently fielded by the US Army is 2-PAM. Several of the reactivators tested provided an improvement over 2-PAM and have the benefit of also being centrally active, which could better protect the CNS in an in vivo model. Disclaimers: The views expressed in this abstract are those of
the authors and do not reflect the official policy of the Army, DoD, or the US Government. The experimental protocol was approved by the Animal Care and Use Committee at the US AMRICD and all procedures were conducted in accordance with the principles stated in the most current Guide for the Care and Use of Laboratory Animals; Animal Welfare Act and implementing Animal Welfare Regulations, as amended; and Public Health Service Policy on Humane Care and Use of Laboratory Animals.

2298 Delayed Treatment with Midazolam, Allopregnanolone, and Perampanel Terminates Benzodiazepine-Refractory Seizure Activity following Acute Diisopropylfluorophosphate (DFP) Exposure

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Organophosphorus (OP) nerve agents are potent neurotoxins. OPs can trigger seizures that rapidly progress to status epilepticus (SE). If not terminated within minutes, OP-induced SE leads to extensive neuropathology and long-term behavioral deficits. Current standard-of-care includes atropine, a cholinesterase reactivator (e.g., 2-PAM), and a benzodiazepine (e.g., midazolam). In a rat model of acute intoxication with DFP, delayed administration of midazolam (40 min after seizures begin) reduces EEG amplitude but does not terminate electrographic seizures. To identify a better treatment for benzodiazepine-refractory OP-induced seizures, we are investigating adjunct therapies to standard-of-care. Adult male Sprague-Dawley rats were permanently implanted with cortical EEG leads prior to administration of DFP (4 mg/kg, s.c.). Immediately following DFP, animals were treated with atropine (2 mg/kg, i.p.), diazepam (2-PAM; 25 mg/kg, i.p.), or perampanel (2 mg/kg, i.p.), singly or in combination. Combining midazolam with allopregnanolone, a neurosteroid that acts as a positive allosteric modulator of the GABAA receptor, modestly attenuates electrographic seizure activity compared to midazolam alone. Combined treatment with midazolam, allopregnanolone, and a low dose of perampanel, a selective non-competitive antagonist of AMPA receptors, completely eliminates electrographic seizures. Neuropathology was examined by magnetic resonance imaging (MRI) and histology in a separate cohort of animals who were not surgically implanted for EEG. The combination treatment stopped behavioral seizures in 100% of DFP-treated animals within a few minutes of administration. MRI and histology data suggest that combined therapy is more efficacious than midazolam alone in mitigating the neuropathological effects associated with DFP-induced SE. In summary, post-intoxication treatment with a combination of midazolam, allopregnanolone, and perampanel holds significant promise as a more effective medical countermeasure for acute OP intoxication relative to current standard-of-care. Supported by NIH CounterACT Program (NS079202).

2299 Acute Diisopropylfluorophosphate (DFP) Intoxication Promotes Degeneration and Senescence in Neurons in the Rat Hippocampus and Thalamus

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Organophosphates (OPs) can cause acute neurotoxicity by irreversibly inhibiting acetylcholinesterase (AChE), the enzyme responsible for terminating cholinergic neurotransmission by hydrolyzing acetylcholine (ACh). Acute intoxication with the OP, DFP, leads to excessive buildup of ACh at synaptic clefts resulting in cholinergic crisis, which in preclinical models includes seizures that can rapidly develop into status epilepticus. It is postulated that traumatic brain injury increases the risk for late-onset neurodegenerative disease, but whether acute OP-induced status epilepticus promotes neuropathological phenotypes consistent with late-onset neurodegeneration is not known. To address this question, this study investigated whether acute DFP intoxication promotes cellular senescence in rat brain. Adult male Sprague-Dawley rats were randomly divided into 2 groups: (1) animals that received a single dose of DFP (4 mg/kg) and (2) animals that received an equal volume (300 microliter) of vehicle (saline). Both groups were administered atropine sulfate and 2-PAM one minute later. Brain tissues were evaluated at 3 months post-DFP intoxication. Neurodegeneration was assessed using Fluoro-Jade C staining; senescence, by immunohistochemistry for the senescence marker p16. Senescent cell type was determined by co-localization of p16 immunoreactivity with biomarkers for endothelial cells (CD31/PECAM-1), astrocytes (S100beta), and neurons (NeuN). The data indicate that a single acutely toxic dose of DFP induces senescence in neurons but not endothelial cells or astrocytes. Moreover, neuronal expression of p16 was especially upregulated to the hippocampus, particularly the hilus region of dentate gyrus and the CA3 region, and thalamus. Significant neurodegeneration was also detected in these same brain regions as determined by Fluoro-Jade C staining. These data suggest that acute DFP intoxication may increase risk of neurodegenerative disease. Supported by NIH CounterACT grant # NS079202.

2300 In Vitro Reactivation of Organophosphate-Inhibited Acetylcholinesterase by Novel Pyridinium Oximes in Brain Tissue from Juvenile and Adult Rats

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Organophosphorus (OP) compounds are best known for their acute neurotoxicological effects as acetylcholinesterase (AChE) inhibitors. Treatment includes an oxime, such as pralidoxime (2-PAM), which is utilized to reactivate AChE phosphorylated by an OP compound. Novel OPPs (OPPAs) and pyridinium pyrophosphonates (US Patent 9,203,905) have been developed for penetration of the blood-brain barrier to prevent or attenuate seizures and central nervous system damage. To further enhance our understanding of OP intoxication with the goal of developing more effective antidotes, information regarding the effective- ness of the novel oximes in brain tissue from both sexes and age differences is needed. Previous studies in rats have indicated no differences in the sensitivity of brain AChE to oxon inhibition at different ages. Paraoxon (the active metabolite of the insecticide parathion) and a nerve agent (sarin surrogate, 4-nitrophenyl isopropyl methylphosphonate (NIMP), were incubated with pooled rat brain homogenate from five animals in adult males, females, and 12-day-old male rats. At 40 min post-exposure, the animals were pretreated with pyridostigmine (0.1 mg/kg, im) prior to DFP (4 mg/kg, s.c.) and at 40 min post-exposure, diazepam (DZP, 5 mg/kg, ip), midazolam (MDZ, 0.7 mg/kg, im) or vehicle. Acute DFP intoxication caused calcification in the thalamus and hippocampus as early as 14 days post-exposure, as detected using micro-CT and confirmed by Alizarin staining. The area of calcium deposition was positively correlated with increased time post-DFP, but not with behavioral seizure severity. Calcium deposition paralleled the appearance and amount of immunoreactivity for osteopontin and B-amyloid, both markers of neurodegeneration. Post-DFP treatment with DZP or MDZ did not prevent calcium deposition or other neuropathological abnormalities at 90 and 180 days. Neuroinflammation (microglial activation and astrogliaosis as detected by IBA-1/CD68 and GFAP/S100B immunoreactivity, respectively) and neurodegeneration (assayed by Fluoro-Jade C labeling) were significantly increased in the thalamus, hippocampus, periform cortex, and amygdala at 90 and 180 days. Neither DZP nor MDZ effectively pro-
Novel phenoxyalkyl pyridinium oximes (US Patent 9,227,937) designed to increase their ability to cross the blood-brain barrier (BBB) have previously shown the ability to reactivate inhibited brain cholinesterase (ChE) in vitro and in vivo.

In vivo, the novel oximes at the onset of seizure-like behavior (25 min) scored for signs of seizure-like behavior was scored on a scale of 1-5. Twenty-four-hour survival was moderate for the novel oximes. Five of the novel oximes alone yielded 30-65%, and 30-85% survival and a novel oxime in combination with 2-PAM (33-50%) but due to its inability to cross the BBB, seizure-like behavior persisted over the 8 hours.

Exposure of guinea pig brain ChE reactivation was generally lower than in human equivalent dosages of atropine (0.65 mg/kg) and the currently approved oxime, PAM-146 (µmoles/kg), or novel oximes at the onset of seizure-like behavior (25 min).

The HPLC and GC-MS-SPME analysis revealed good retention of DMTS formulations in glass ampules. The DMTS stability of a DMTS formulation (IM-DMTS) was stored at 4, 25 and 37 °C temperatures in hermetically sealed glass ampules. The DMTS concentrations of these samples were measured by HPLC-UV at periodic time intervals. Each sample was analyzed by breaking open an individual ampule. Over the nine month period of the study, no significant DMTS loss was observed in the samples stored at 4 and 25 °C.

It has been previously reported that GABA<sub>A</sub> receptor (GABA<sub>A</sub>R) antagonists, including pentylenetetrazole (PTZ), tetramethylenedisulfotetramine (TETS), and picrotoxin (PTX), which are potent convulsants in mammalian models, trigger seizure-like behavior in zebrafish (Danio rerio) larvae. Extracellular field potential recordings obtained from the optic tectum of 5 days post-fertilization (dpf) zebrafish confirmed that acute exposures to all three GABA<sub>A</sub>R antagonists elicited extra-cellular spiking patterns consistent with seizure activity. However, the pattern of electrical activity varied between the individual GABA<sub>A</sub>R antagonists, which is consistent with data from primary mammalian neuronal cell cultures and rodent models of TETs and PTX-induced seizures. Collectively, these data suggest the possibility of differential GABA<sub>A</sub>R subunit profile binding for each convulsant. To address this question, genetic knockdown using morpholinos (MO) targeting GABA<sub>A</sub>R subunit-specific mRNA was used to delineate the full receptor subunit profile critical for TETs- and PTX-induced seizures. At 3 dpf, tropical wildtype zebrafish injected with MO were acutely exposed to seizure-causing concentrations of TETs or PTX added to fish water. Behavior was recorded for 20 min post-exposure using the Noldus automated tracking system to determine whether MO knockdown prevented chemical-induced seizure behavior. Based on these experiments, a differential GABA<sub>A</sub>R subunit binding profile was identified for TETs vs. PTX. The subunits a2, b2, and extrasynaptic δ are essential to the seizure-inducing activity of both TETs and PTX, whereas α1 and β3 were important for TETs but not PTX-induced seizures, while γ2 was uniquely important for PTX-induced seizures. Phylogenetic analyses demonstrate homologous subunits between zebrafish and mammalian GABA<sub>A</sub>R.
The aryl hydrocarbon receptor (AhR) mediates the toxic effects of dioxin-like compounds, such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Sequence and structural features of AhR can underlie species- and population-specific differences in its affinity for TCDD and other agonists. While most vertebrates possess at least one AhR that binds TCDD tightly, all characterized amphibian AhRs bind TCDD with very low affinity. Our previous analyses of AhRs from Xenopus laevis (a frog; order Anura) and Ambystoma mexicanum (a salamander; order Urodela) identified three key amino acid residues in the ligand-binding domain (LBD) that underlie low-affinity binding. In X. laevis AhR1β, these are A354, A370, and N325. In studies of the AhR of the amphibian Gymnopus multiplicata, we sought to determine if AhRs from all three amphibian orders share the low-affinity phenotype. *G. multiplicata* represents Order Apoda (caecilians), a clade of legless amphibians that diverged early from the class’s common lineage, prior to the split between frogs and salamanders. cDNA was isolated by RT-PCR from tissue provided by the Museum of Vertebrate Zoology at the University of California, Berkeley. The encoded protein (92.7 kDa) is monophyletic with vertebrate AhR1s, sharing 59% identity with *X. laevis* AhR1β and 63% identity with *A. mexicanum* AhR. An LBD homology model suggests that the basic architecture of the caecilian AhR closely resembles that of other species, and the LBD sequences include the three signature residues of low TCDD affinity. We measured the TCDD responsiveness with transactivation assays employing pGudLuc6.1, a luciferase reporter construct driven by the mouse *CYP1A1* enhancer region. For *G. multiplicata* AhR, the EC50 for reporter gene induction by TCDD was 28.46 nM, similar to *X. laevis* AhR1β (26.8 nM) and dramatically less than a chimeric frog AhR containing the mouse LBD (0.2 nM). Taken together, low TCDD responsiveness and sequence conservation with the frog and salamander AhRs suggest that this caecilian AhR also binds TCDD with low affinity. We predict *G. multiplicata* is similarly resistant to the toxic effects of TCDD and other xenobiotic AhR agonists.

### 2307 Metatranscriptomic and Metabolomic Investigation of Dietary 2,3,7,8-Tetrachlorodibenzo-p-dioxin Exposure in Mice

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Understanding the interaction between the gut microbiome and the host following toxicant exposure is an important, emerging area of investigation and one that will hopefully improve the understanding of toxicological mechanisms. 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is a persistent organic pollutant that has been reported to cause compositional changes within the gut microbiome while also promoting liver damage and intestinal inflammation. In the current study, we conducted metatranscriptomic, 16S rRNA amplicon sequencing, and metabolomic analysis to further understand how TCDD modulates the host-gut metabolic axis. Following a newly optimized bacterial ribosomal RNA depletion method to remove 16S and 23S RNA and RNA sequencing of the isolated mRNA on the Illumina HiSeq, several metatranscriptomics analysis pipelines were used including MetaTrans, SAMSA (Simple Annotation by Metatranscriptomes by Sequence Analysis) and COMAN (Comprehensive Metatranscriptomics Analysis). Due to the depth of sequencing, specific mRNA were matched to the bacterial species that created them, further increasing analytic potential. Within the same isolated cecal contents we completed long read 16S RNA sequencing to better facilitate species level identification of the gut microbiome community. Additionally, 1H NMR, gas chromatography-coupled mass spectrometry and liquid chromatography-coupled mass spectrometry were used to measure host and microbiome metabolic changes after TCDD exposure. Through an integrated network analytics approach, the metabolomics, 16S rRNA amplicon sequencing, and metatranscriptomic data were integrated to uncover microbiome and metabolite interactions that help provide a more detailed view of the toxicological mechanisms associated with TCDD exposure in mice.

### 2308 Inducible Loss of Hepatocyte Aryl Hydrocarbon Receptor Increases Epididymal White Fat Thermogenesis through the Activation of Fibroblast Growth Factor 21

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The aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor with numerous physiological roles. Fibroblast growth factor 21 (FGF21) is an important metabolic hormone that has recently been shown to be under the regulation of AhR. FGF21 is secreted from the liver in response to prolonged cold exposure and promotes thermogenesis in white fat deposits. Exogenous FGF21 administration results in weight loss, reduced fat mass, and improved insulin sensitivity in various models of obesity. Consistent with recent data showing that a hepatocyte-targeted loss of the AhR results in elevated hepatic FGF21 mRNA and protein, we utilize a novel tamoxifen-inducible AhR knockout mouse model (AhR<sup>fl/fl</sup>Alb<sup>CreERT2</sup>) to demonstrate that a loss of hepatocyte AhR results in significant 2-fold induction of hepatic FGF21 transcription and significant 3-fold increase of serum FGF21 protein concentrations relative to AhR<sup>fl/fl</sup> control mice. Epididymal white adipose tissue (eWAT) isolated from AhR<sup>fl/fl</sup>Alb<sup>CreERT2</sup> mice further reveals that increased hepatic FGF21 is associated with the promotion of thermogenesis, as evidenced through greater immunohistochemical staining for uncoupling protein 1 (UCP1) in AhR<sup>fl/fl</sup>Alb<sup>CreERT2</sup>- and a significant 2.1-fold increase in the rate of mitochondrial respiration relative to AhR<sup>fl/fl</sup>. To demonstrate that FGF21 is necessary for the thermogenic phenotype observed in eWAT, we generated a doubleconditional, tamoxifen-inducible AhR-FGF21 knockout mouse model (AhR<sup>fl/fl</sup>FGF21<sup>-/-</sup>Alb<sup>CreERT2</sup>). Compared to AhR<sup>fl/fl</sup>FGF21<sup>-/-</sup> control mice, AhR<sup>fl/fl</sup>FGF21<sup>-/-</sup>Alb<sup>CreERT2</sup> mice do not exhibit any evidence of increased thermogenesis within eWAT. The data therefore suggest that the AhR can influence white adipose physiology directly through the modulation of hepatic FGF21. Given the ability of increased FGF21 to counteract the obese phenotype, our laboratory is currently investigating the potential use of AhR antagonists and/or selective aryl hydrocarbon receptor modulators as a therapeutic means of increasing de novo FGF21 production. This work is supported by grants R01ES026874 and T32ES007254.

### 2309 Agonist-Specific Aryl Hydrocarbon Receptor-Mediated Differential Gene Expression

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The aryl hydrocarbon Receptor (AhR) is a ligand-activated transcription factor known to regulate adaptive and toxic responses to a variety of chemicals, pollutants including the polycyclic aromatic hydrocarbons, most notably 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Upon activation by TCDD, the AhR translocates into the nucleus and binds to DNA at the Xenobiotic Response Element (XRE) in partnership with the Aryl hydrocarbon Receptor Nuclear Translocator (ARNT) to drive target gene expression, most notably cyp1a1. Our recent studies identified stanniocalcin 2 (st2c) as a novel AhR target gene responsive to the endogenous AhR agonist cinnamonic acid (CA). CA-dependent AhR-XRE-mediated st2c upregulation is responsible for cytoprotection against endoplasmic reticulum/oxidative stress-induced apoptosis both in vitro and in vivo. Significantly, CA but not TCDD induces expression of st2c in hepatocytes. In contrast to TCDD, CA is unable to induce the cyp1a1 gene, thus revealing an agonist-specific mutually exclusive transcriptional response. Studies reported here provide a mechanistic explanation for this dichotomous response by identifying an interaction between the AhR and the metastasis-associated protein 2 (MTA2). Immunoaffinity purification using an anti-AhR antibody followed by LC-MS/MS identified MTA2 as a novel - agonist specific - cofactor recruited by the AhR. Moreover, co-IP and ChIP studies confirmed the AhR-MTA2 interaction reported here provide a mechanistic explanation for this dichotomous response by identifying an interaction between the AhR and the metastasis-associated protein 2 (MTA2). Immunoaffinity purification using an anti-AhR antibody followed by LC-MS/MS identified MTA2 as a novel - agonist specific - cofactor recruited by the AhR. Moreover, co-IP and ChIP studies confirmed the AhR-MTA2 interaction...
gene-editing strategy was employed to assess role of chromatin architecture in agonist-specific stc2 expression. Our data suggest that the stc2 XRE cassette can transfer CA responsiveness to the cypl1a1 gene in context of chromatin packaging but not in vitro. In summary, this study describes a novel physiological role for AhR and highlights the complexities and potential biological ramifications resulting from agonist-specific AhR activation.

2310 Synergistic Interactions of Ah Receptor Ligands and Short Chain Fatty Acids in Colon-Derived Cells

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Short chain fatty acids (SCFAs) including acetate, propionate, and butyrate are gut microbiota metabolites that are highly produced in animals on fiber-rich diets and SFCA production is associated with health-protective responses in the intestine. Microbiota-derived tryptophan metabolites that act through the aryl hydrocarbon receptor (AhR) also induce similar protective effects in the intestine and this study investigates interaction of SCFAs and AhR ligand on expression of Ah-responsive genes such as Cyp1a1/CYP1A1 in YAMC mouse colonocyte and Caco2 human colon cancer cell lines. Butyrate slightly induced CYP1A1 but synergistically enhanced AhR ligand-induced Cyp1a1/CYP1A1 in these cells with comparable enhancement being observed for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and also microbiota-derived AhR ligands tryptamine, indole, and 1,4-dihydroxy-2-naphthoic acid (DHNA). The effects of butyrate on enhancing induction of Cyp1b1/CYP1B1, AhR repressor (Ahhr/Ahrr) and TCDD-inducible poly(ADP-Ribose) polymerase (Tiparp/TIPARP) by AhR ligands were gene- and cell-context-dependent with Caco-2 cells being the most responsive cell line. Like butyrate and propionate, the prototypic hydroxyacids acid-derived histone deacetylase (HDAC) inhibitors Panobinostat and Vorinostat also enhanced AhR ligand-mediated induction and this was accompanied by enhanced histone acetylation. Acetate also enhanced basal AhR ligand-inducible Ah responsiveness and histone acetylation demonstrating for the first time that acetate is an HDAC inhibitor. These results demonstrate SCFA-AhR ligand interactions in YAMC and Caco-2 cells where SCFAS synergistically enhance basal and ligand-induced expression of AhR-responsive genes.

2311 Urolithin A Is a Natural Human AhR Antagonist That Exhibits Anti-Inflammatory Activity

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Aryl hydrocarbon receptor (AhR) is characterized as a xenobiotic receptor. However, recent studies have pointed to a role of AhR in myriad of different cellular mechanisms, including cell proliferation, tumor invasiveness, and cytokine signaling. We have previously demonstrated that AhR antagonism attenuates aggressive phenotype in head and neck tumor cell lines. Discovery of its numerous physiological functions raised the hypothesis of the existence of endogenous ligands for AhR. Over the last decade, a number of endogenous and natural AhR ligands has been identified, including the agonists indole, kynurenic acid, indirubin, and natural antagonists flavonoids that are abundant in plants. Here, we investigated whether urolithins, gut microbiota-derived AhR ligands tryptamine, indole, and 1,4-dihydroxy-2-naphthoic acid (DHNA). The effects of butyrate on enhancing induction of Cyp1b1/CYP1B1, AhR repressor (Ahhr/Ahrr) and TCDD-inducible poly(ADP-Ribose) polymerase (Tiparp/TIPARP) by AhR ligands were gene- and cell-context-dependent with Caco-2 cells being the most responsive cell line. Like butyrate and propionate, the prototypic hydroxyacids acid-derived histone deacetylase (HDAC) inhibitors Panobinostat and Vorinostat also enhanced AhR ligand-mediated induction and this was accompanied by enhanced histone acetylation. Acetate also enhanced basal AhR ligand-inducible Ah responsiveness and histone acetylation demonstrating for the first time that acetate is an HDAC inhibitor. These results demonstrate SCFA-AhR ligand interactions in YAMC and Caco-2 cells where SCFAS synergistically enhance basal and ligand-induced expression of AhR-responsive genes.

2312 Agonist-Activated Aryl Hydrocarbon Receptor Suppresses Fibroblast Growth Factor 21 Gene Expression Through Binding to a Novel Upstream Putative Xenobiotic Response Element within the Promoter Region

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The aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor that plays a critical role in many physiological processes. Recent data implicate the AhR in transcriptional modulation of the metabolic hormone fibroblast growth factor 21 (FGF21). FGF21 is secreted from the liver in response to various stressors, promotes a brown adipocyte-like phenotype in white fat deposits, and improves glucose metabolism when administered to various models of obesity. The precise mechanism by which the AhR regulates Fgf21 transcription remains unclear, given previous observations that show AhR agonists can both induce and suppress Fgf21 transcription. The data from these studies nevertheless consistently demonstrate that the AhR binds to a xenobiotic response element (XRE) located 72 bp upstream of the Fgf21 transcriptional start site. Here, we demonstrate that the AhR can also bind to two additional upstream putative XRE sequences located at -862 bp and -1744 bp. We hypothesize that these sites may be critical for directing the AhR agonist-mediated induction or suppression of Fgf21 transcription. Through chromatin immunoprecipitation studies and quantitative PCR in mouse hepatic tissue, we observe that maximal, agonist-driven Fgf21 transcription is associated with decreased AhR binding to the distal putative XRE from constitutive levels detected in vehicle-treated animals. Additional data reveal that agonist-dependent Fgf21 transcription is transient, reaching a minimum at 24 h. Ligand-induced AhR binding to the proximal XRE is increased relative to vehicle, whereas AhR binding to the mediol XRE is constitutive and not influenced by agonist. Introduction of a point mutation targeting the distal putative XRE in a luciferase reporter construct that abolishes AhR binding showed that loss of constitutive binding increased promoter activity. We therefore conclude that AhR binding to the distal putative XRE may be required for agonist-mediated suppression of Fgf21 gene expression. We are currently working to further elucidate this novel mechanism of agonist-mediated repression and XRE selectivity, as well as its potential therapeutic applications within the context of hepatic FGF21 expression and obesity. This work is supported by grants RO1ES266874 and T32ES007254.
The aryl hydrocarbon receptor (AHR) is a basic helix-loop-helix, PER-ARNT-SIM (bHLH-PAS) cytosolic transcription factor that is responsible for mediating the toxic effects of halogenated hydrocarbons (such as 2,3,7,8-tetrachloro-dibenzo-p-dioxin, TCDD) and polycyclic aromatic hydrocarbons (such as benzo[a]pyrene, BaP). Xenobiot-bound AHR dimers interact with aryl hydrocarbon receptor nuclear translocator (ARNT) to activate the transcription of targets that include cytochrome P450 genes. In this way AHR is the principle factor responsible for the biological toxicity, carcinogenicity, and mutagenic effects of a large number of environmental pollutants. A complete picture of the genetic pathways that regulate AHR expression and activity has yet to be determined. Using a BaP sensitivity assay based on (ARH-induced) CYP1A1 metabolism of BaP in mouse Hepa1 cells, previous work in our laboratory identified AHR signaling mutants that fell into four complementation groups. Complementation groups A, C and D were found to correspond to mutations in CYP1A1, ARNT and AHR, respectively. The gene responsible for BaP resistance in complementation group B mutants remained unidentified. Here we report the use of whole-exome sequencing (WES) to identify four candidate genes that have undergone loss of heterozygosity from parental BaP-sensitive Hepa1 cells in each of three independently derived B mutant lines. We are now using CRISPR-Cas9 reverse-genetics approaches to target these genes with multiple synthetic guide RNAs (sgRNAs) to determine which, if any, of these genes correspond to complementation group B. We further report that we have simultaneously performed multiple genome-wide screens of Hepa1 mouse and HepG2 human hepatoma cells using CRISPR-Cas9 and sgRNA libraries targeting all the known protein coding genes and microRNAs to identify the totality of possible gene disruption mutations, other than (and including) the complementation group B mutations, that affect AHR signaling and expression, in an effort to identify all possible genes that influence AHR signaling in response to xenobiotic binding. Supported by NIHES grant 1R21ES026392, and a postdoctoral fellowship to GDS from NIEHS training grant T32ES051547.

The aryl hydrocarbon receptor (AHR) is a physiologic sensor of both chemical environmental pollutants and ligands of natural origin. Quinones, which may be of xenobiotic or endogenous origin, are a less well-defined chemical class of AHR activators. Among the large diversity of quinone chemical structures, it is unclear whether all activators occur through reversible, bimolecular interactions or through interactions involving AHR alkylation. In this study, differences were observed in the toxicity and AHR activation profile of fully substituted and unsubstituted para-quinones. For example, we found that alpha-tocopherylquinone (TQ), a complex, fully substituted para-quinone showed low toxicity. Gene expression analyses showed a clustering of AHR responsive genes, of CYP1A1 by TQ, while wild-type MEF cells responded to TQ treatment established where knockout MEF cells showed little change in the expression of CYP1A1 by TQ, while wild-type MEF cells responded to TQ treatment with increased CYP1A1 expression. Though TQ showed a low toxicity profile, simple unsubstituted para-quinones, such as tert-butyl quinone (tBQ), were found to be more toxic. We and others found tBQ to be a moderately potent activator of the AHR. Initial studies support a mechanism in which simple unsubstituted para-quinones, such as tBQ, may alkylate the AHR through electrophilic sites present in these structures. As fully substituted quinones, such as TQ, do not possess electrophilic carbons, they cannot alkylate proteins. Thus, substituted quinones that activate the AHR may do so through reversible, bimolecular interactions similar to known ligands of the AHR. These results support that simple, unsubstituted and complex, fully substituted quinones may activate the AHR through distinct mechanisms.

Vitamin D (1α, 25-dihydroxyvitamin D3) is a steroid hormone traditionally associated with mineral ion homeostasis; however, accumulating evidence suggests a wider biological role for VD and its importance in immune function, xenobiotic metabolism, cell differentiation, and neurodevelopment. Like other members of steroid hormones, the biological effects of VD are mediated through the binding of 1α, 25-dihydroxyvitamin D3 ligand to its hormone receptor, VDR. In this study, we examined the Tox21 qHTS data set generated against VDR and have identified 21 potential VDR agonists and 19 VDR antagonists with putative VDR activity. Select orthogonal assays including transient transfections, mammalian two hybrid and molecular modeling were conducted to assess VDR ligand interactions. In vivo studies were additionally conducted to demonstrate proof of principle for a role of VD/VDR signaling axis in obesity. Three dietary cohorts of zebrafish were established and placed on engineered diets: a standard lab diet (1.4 iu/g) as a control, a VD null diet (0 iu), and a VD enriched diet (400,000 iu/g). When zebrafish switch over from a standard lab diet to a VD null diet at 2 months of age, they develop obesity within 3-4 months of age. Histopathological examinations consistently show that this phenotype is attributed to significantly elevated levels of visceral and subcutaneous fat compared to controls. Concordantly, preliminary gene expression data shows an overall upregulation of adipogenic and lipogenic markers presence of VD deficient fish at 4 months of age. The gene expression data shows a significant differential regulatory of genes specific to lipid metabolism. Hepatic triglycerides are also significantly elevated in the VD deficient fish at 6 months of age. RNA-Seq data is currently being utilized to identify key obeseogenic genes being regulated by VD and to understand the molecular mechanisms associated with both VD deficiency and its effects in progression of obesity and associated diseases such as diabetes.
2319 Coordinated Regulation of Molecular Chaperones and the Ubiquitin-Proteasome System Determines the Activation and Translocation of Human Constitutive Androstane Receptor

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2320 Nuclear Co-Regulator Interactions with the Constitutive Androstane Receptor

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2321 Monepantel is a Non-Competitive Antagonist of Nicotinic Acetylcholine Receptors from Ascaris suum and Oesophagostomum dentatum

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2322 Characterization of Aryl Hydrocarbon Receptor Knockout HepG2 Cell Lines Using CRISPR-Cas9 Genome Editing Technology


Each compound. Further characterization used 100 µM and 10 µM concentrations. Results: Several compounds were shown to have significantly greater inhibition than the previously studied monoterpenoid, Carvacrol (maximal current 80.6% of acetylcholine control), including geraniol (maximal current 63.0% of acetylcholine control). No monoterpenoids showed agonist activity on the ACR-16 receptor. Concentration response curves showed that the compounds, across the array, had a significant effect on the EC50 values of the acetylcholine response. Several monoterpenoids gave significantly lower maximal currents than the control. Conclusions: This research confirms previous findings that monoterpenoids as a class of compounds have varied but noticeable neurotoxic effects at a nicotinic acetylcholine receptor. We plan to characterize those compounds most active at our chosen target site.

The constitutive androstane receptor (CAR; NR1I3) is a member of the nuclear receptor family members (e.g., AHR, PXR). Previous studies demonstrated that CAR’s interaction profile with CoRegs likely differs between rodents and humans, among the human CAR splice variants, and even between hepatocytes across the liver lobule. To begin to test this hypothesis, we used a MARCoNi platform (micro array assay for real-time CoReg-NR interaction). CAR interactions across a solid phase matrix of 154 CoReg peptides were simultaneously quantified. Since wild type CAR is constitutively active, the 5 amino acid insertion present in the ligand-activated human CAR3 splice variant was used to assess CAR interactions, in the presence and absence of both direct and indirect CAR activators. Similarly, the 4 amino acid-substituted human CAR2 splice variant was also tested. A protocol was developed enabling ε-coli expression of His-tagged ligand binding domains of various CARs from human and mouse as a non-competitive antagonist on the expressed receptors. ACR-16 competitively and non-competitively inhibited hCAR transactivation in a dose-dependent manner. Although several CAR-CoReg interactions have been identified, over 300 proteins to modulate transcriptional activity across CAR target genes. Although several CAR-CoReg interactions have been identified, over 300 proteins to modulate transcriptional activity across CAR target genes. Although several CAR-CoReg interactions have been identified, over 300 proteins to modulate transcriptional activity across CAR target genes. Although several CAR-CoReg interactions have been identified, over 300 proteins to modulate transcriptional activity across CAR target genes. Although several CAR-CoReg interactions have been identified, over 300 proteins to modulate transcriptional activity across CAR target genes. Although several CAR-CoReg interactions have been identified, over 300 proteins to modulate transcriptional activity across CAR target genes.

As a pyrantolet/tribendimidine preferring heteromeric subtype comprising UNC-29, UNC-38 and UNC-63 subunits; and a levamisole preferring subtype comprising UNC-29, UNC-38, UNC-63 and ACR-8 subunits. For each subtype tested, monepantel applied in isolation produced no measurable currents. When monepantel was continuously applied, it reduced the amplitude of acetylcholine induced currents in a concentration-dependent manner. In all three subtypes, monepantel acted as a non-competitive antagonist on the expressed receptors. ACR-16 from A. suum was particularly sensitive to monepantel inhibition (IC50 values: 1.6 ± 3.1 nM and 0.2 ± 2.3 µM). We also investigated the effects of monepantel on muscle flaps isolated from adult A. suum. The drug did not significantly increase baseline tension when applied on its own. As with acetylcholine induced currents in the heterologously expressed receptors, contractions induced by acetylcholine were antagonized by monepantel. Further investigation revealed that the inhibition was a mixture of competitive and non-competitive antagonism. Our findings suggest that the mode of action of monepantel is more complex than previously described.

Characterization of Aryl Hydrocarbon Receptor Knockout HepG2 Cell Lines Using CRISPR-Cas9 Genome Editing Technology


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Knock It Down: Improved Methods for Functional Genomics in the Parasitic Nematode, *Brugia malayi*, Reveal Diverse nAChRs That Can Be Exploited for Selective Toxicity

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Parasitic nematodes are a scourge on animals and humans and for control require treatment with selective chemicals (anthelmintics) that kill the parasite but not their host. To identify suitable drug targets, many techniques for studying functional genomics of target sites of anthelmintics have been restricted to *Caenorhabditis elegans* because they have failed when used on animal or human parasites. To overcome these limitations, we have focused our research on the human nematode parasite, *Brugia malayi*, which causes elephantiasis. We have combined single-cell PCR, whole-muscle cell patch-clamp, motility phenotyping (Worminator), and RTNAI for functional genomic studies that reveal *in vivo*, four different muscle nAChRs (*M*-, *L*- and *N*-). Selective cholinergic nematode anthelmintics have different selectivities for these receptors. We find that motility and patch-clamp responses to the compounds levamisole and pyrantel, but not morantel or nicotine, require unc-38 and/or unc-29 genes. Derquantel behaves as a competitive antagonist (*M*- and *L*-nAChRs) activated by morantel (Kᵩ 13.9 nM), *P*-nAChRs activated by pyrantel (Kᵩ 126 nM) and *L*-nAChRs activated by levamisole (Kᵩ 0.96 µM) and bephenium. Derquantel is a non-competitive antagonist of nicotine, and exposes *N*-type nAChRs. The presence of four distinct nAChRs in the muscle is surprising and not predicted by the *C. elegans* model. The diverse nAChRs represent distinguishable drug targets with different functions: knock down of *unc-38-unc-29-16-acer-26* (M- and N-receptors) inhibited motility but knock down of *ac-16-acer-26* (M- and N-receptors) did not. These studies, describing improved methods for the field, are relevant to toxicologists interested in the developing selective nematocides and anthelmintics. Supported by NIH ROI AI047194-15 to RJM and the E. A. Benbrook Foundation for Pathology and Parasitology.

Knockdown of Diacylglycerol Kinase Zeta in Human T Cells by Short Hairpin Ribonuclease Technology

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When T cells are activated through the T cell receptor (TCR), phosphorylase C (PLC) is phosphorylated and activated. Activated PLC produces the second messengers diacylglycerol kinase or DAG. DAG assists with T cell development and function. In T cells, DAG activity is regulated by diacylglycerol kinases (DGKs). DGK catalyzes the conversion of DAG into phosphatidic acid (PA). In CD8 cells, reduced DGK activity is associated with enhancing primary T cell responses against viruses and tumors. We hypothesized that knock down of DGK, expression in human T cells, will increase their effector function. The goal of this study was to test lentivirus encoding four different short hairpin RNA (short hairpin RNA) complementary to human DGK, for the ability to reduce expression of DGK in Jurkat (immortalized human) T cells. The lentiviral vector also encodes green fluorescent protein (GFP) (used to identify transduced cells using flow cytometry), and a puromycin resistance gene. The methodologies included the transfection of Stable 3 E coli with the shRNA lentiviral plasmids, followed by a plasmid preparation to purify the plasmid DNA. The plasmids were then transfected into 293FT cells (immortalized human embryonic kidney cells) to produce the virus. Jurkat cells were transduced with each of the four viruses produced. Transduced Jurkat cells were selected in the antibiotic, puromycin, and the percentage of transduced cells was assessed by GFP expression using flow cytometry. The transfected 293FT virus producing cells were green which indicated the expression of GFP. The initial transductions of the Jurkat cells were suboptimal as only a small percentage of GFP expressing cells (0.9% to 3.3%) survived selection with puromycin. The transduction efficiency was improved by concentrating the virus using centrifugal devices. Using this technique, transduction efficiency increased GFP expression to 98%. Transduced cells are being selected to be used for analysis of DGK expression by western blot. We produced virus using shRNA constructs and successfully transduced Jurkat cells. Future short-term goal is to analyze the effector functions of human T cells with reduced DGK expression, and long-term goal is to use these cells for adoptive cell therapy for patients with leukemia.

Bystander Effects of Antibody Drug Conjugates on the Hematopoietic Compartment

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ADCs represent a relatively new class of cancer therapeutics. Their design involves a tumor-specific antibody, a linker and a potent payload. In addition to the targeted killing of antigen-positive tumor cells, some ADCs appear to have significant off-target effects, which necessitated some clinical trials to be terminated early. However, the success of some ADCs at treating diseases has stimulated the continued research of ADCs as well as increases in the complexity of ADC development. Publications by Zhao et al. (2017) suggested that extracellular cleavage of the linker was responsible for cytotoxicity to differentiating neutrophils, whereas internalization of the ADCs by macrophagocytes induced thrombocytopenia. We developed a platform to assess ADCs and address the specific bystander effects of the payload. Both Trastuzumab and Trastuzumab-vc-MMAE (Creative Biolabs), neither of which target hematopoietic cells, were incubated in medium containing bone marrow nucleated cells derived from normal human donors (NorCal Biologics) and NHP (non-human primates from SNBL). The compounds were added at concentrations ranging from 30 to 0.1 µg/mL for 72 hours and then the treated cells were transferred to methylcellulose-based or collagen-based media for 14 days to support the growth of erythroid, myeloid and megakaryocyte progenitors. Trastuzumab did not cause inhibition of colony growth in human or NHP cultures over the concentration range tested but Trastuzumab-vc-MMAE significantly impacted all three lineages with IC₅₀ values ranging from 0.4 µg/mL (human megakaryocyte progenitor) to 3.8 µg/mL (NHP megakaryocyte progenitor) to 8 µg/mL (NHP megakaryocyte progenitor) to 11 µg/mL (NHP megakaryocyte progenitor). This specialized primary cell based platform can evaluate differences between antibodies with and without payloads, can rank lead compounds in terms of progenitor toxicity and can identify differentiating features of molecules in human and NHP systems. These assays may be of use pre-clinically for screening antibodies to identify potential off-target effects.

The Effect and Mechanism of Hypoxia-Inducible Factor-1a in Benzene-Induced Hematopoietic Toxicity

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Benzene exposure can cause bone marrow toxicity and hematopoietic disorders by oxidative stress. In this study, the effect and mechanism of Hif-1α on hematopoietic toxicity by benzene exposure were explored. Male C57BL/6 mice were exposed to 0 and 150 mg/kg/d benzene by subcutaneous injection for 13 days. Blood parameters, ROS level and Hif-1α expression were then detected. ChIP-Seq analysis in bone marrow cells was performed to identify Hif-1α downstream target genes and pathways associated with benzene hematotoxicity. The K562-NC cell and K562-HIF-1α+ cell, which express high HIF-1α, were treated with 1,4-BQ for 24h. The cell proliferation, apoptosis, and cell cycle were detected. The results revealed that mice in 150 mg/kg/d benzene group displayed decreased red and white blood cell counts, reduced platelet count, diminished hemoglobin content, and lower number of hematopoietic stem cells in bone marrow (BM). In BM there was a significant increase in ROS levels at 150 mg/kg benzene exposure. Genes associated with Hif-1α-binding sites showed a highly significant enrichment for certain gene ontology classifications, including cellular process, phosphorus metabolic process, organelle organization, cellular response to stress. ChIP-Seq results showed Hif-1α peaks were significantly associated with 245 downregulated genes after benzene exposure. Of the 40 genes contain Hif-1α specific binding site HRE, including 24 annotated genes and 4 micRNA. Hif-1α targets genes were significantly enriched for KEGG pathways specific for Jak-STAT signaling, natural killer cell-mediated cytotoxicity, Fc epsilon RI signaling, pyrimidine metabolism, and T cell receptor signaling. It revealed the relative growth rate was significantly increased in K562-HIF-1α+ cell than in the controls group, when used along with wild-type cells and may eventually serve as an tool for the field, are relevant to toxicologists interested in the developing selective nematocides and anthelmintics. Supported by NIH ROI AI047194-15 to RJM and the E. A. Benbrook Foundation for Pathology and Parasitology.
Cadmium Exposure Inhibits Branching Morphogenesis and Causes Alterations Consistent with HIF-1α Inhibition in Human Primary Breast Organoids

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Breast cancer is the most common cancer in women, yet the environmental risk factors for it are still not well understood. The toxic heavy metal cadmium is a naturally occurring known human carcinogen, but its role in breast cancer is controversial. Developmental cadmium exposure in vivo disrupts mammary gland differentiation, while exposure of breast cell lines to cadmium causes invasion consistent with the epithelial-mesenchymal transition (EMT). The effects of cadmium on normal human breast stem cell development have not been measured. Here, we sought to quantify the effects of cadmium exposure on normal breast stem cell proliferation and differentiation. Using the mammosphere assay and organoid formation in 3D hydrogels, we tested the effects of two commercially available (iCell® Hepatocytes 2.0) 250nM and 2.5μM on mammosphere and organoid formation by 68%. RNA-seq showed cadmium treatment downregulated HIF-1α target genes and genes associated with extracellular matrix formation by 83%, respectively. Despite no changes in mammosphere formation, 250nM cadmium inhibited branching organoid formation in hydrogels by 68%. RNA-seq showed cadmium treatment downregulates HIF-1α and HIF-1α target genes and genes associated with extracellular matrix formation and EMT. To validate these findings, we used a HIF-1α activity assay and target genes and genes associated with extracellular matrix formation. Cadmium treatment significantly inhibited HIF-1α activity in a luciferase assay, and the HIF-1α inhibitor acriflavine ablated mammosphere and organoid formation while producing a similar phenotype as cadmium treatment. These findings show that cadmium, at doses that do not affect cell health, inhibits HIF-1α in mammospheres and organoid formation assays. Cadmium treatment significantly inhibited HIF-1α activity in a luciferase assay, and the HIF-1α inhibitor acriflavine ablated mammosphere and organoid formation while producing a similar phenotype as cadmium treatment. These findings show that cadmium, at doses that do not affect cell health, inhibits HIF-1α in mammospheres and organoid formation. Our flow cytometry results accordingly suggest that support cadmium being a breast cancer initiator via induction of stem cell proliferation, but do implicate cadmium as an inhibitor of mammary gland morphogenesis.

Comparison of Different Sources of Stem Cell-Derived Hepatocyte-Like Cells with Respect to Hepatocyte Functionality

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Human induced pluripotent stem cell-derived hepatocytes (hiPSC-HLCs) are suggested as in vitro model for drug research and development. However, compared to freshly isolated human primary hepatocytes, currently available hiPSC-HLCs exhibit a fetal phenotype, as revealed by e.g. expression profiling (1) and CYP activity. Yet since basal and inducible CYP activity is a prerequisite of a functional hepatocyte model, we tested the effects of two commercially available (iCell® Hepatocytes 2.0 (CDI-HLCs)) and in-house derived HLCs for CYP activity as well as other hepatocyte characteristics. Our in-house differentiation efforts are based on a published protocol (2) with modifications shown to enhance definitive endoderm (DE) differentiation (3), a key prerequisite in HLC differentiation. Our flow cytometry results accordingly suggest that inclusion of several pathway inhibitors appears beneficial for DE differentiation as reflected by increased CXCRI4/SOX17 expression on day 3 and 7. qPCR analysis confirmed DE differentiation by increasing levels of SOX17/CXCRI4/GATA4 expression at day 7 and decreasing levels of these genes at later stages. Further differentiation up to the hepatocyte stage was indicated by increasing albumin, α-fetoprotein and CYP expression levels. Concerning CYP3A1 inducibility we were able to observe up to 10-fold induction with the reference inducer Rifampicin at the mRNA level. Yet when measuring effective enzyme activity, hardly any increase was detected in a 3D spheroid model of CDI-HLCs. Therefore, our current research will be focused on enhancing the maturation of HLCs, especially CYP activity/inducibility, from both commercial sources and in-house activities. This includes culture of HLCs in 3D configuration and further molecular analyses of critical hepatic characteristics to potentially identify inadvertently activated, or inhibited but required pathways, which might be targetable. (1) T. Waltermann et al. (2017) Poster no. 117, NRW Stem Cell Network Congress (2) Hannan et al. (2013). Nature Protocols 8, pp430 (3) Loh et al. (2014) Cell Stem Cell, 14, pp237.

Transcriptome Profiling Reveals That the Aryl Hydrocarbon Receptor Influences Distinct Gene Regulatory Networks in Long-Term Hematopoietic Stem Cells and Multipotent Progenitors

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The continuous production of blood cells is orchestrated by tightly controlled cellular quiescence, proliferation, and differentiation. Mounting evidence indicates that the aryl hydrocarbon receptor (AHR) plays a key role in hematopoiesis and hematopoietic stem cells (HSC) function. However, the molecular mechanisms and signaling pathways regulated by AHR remain poorly defined. Long-term HSC (LT-HSC) can self-renew and differentiate. Multipotent progenitors (MPP) arise from LT-HSC, are highly proliferative, and differentiate to all lineage cell. To better understand the role of AHR in LT-HSC and MPP function, we used transcriptome profiling by RNA-Seq to identify differentially expressed genes (DEG) in cells from global AHR knock-out (AHR-KO) and wild type (WT) mice. There were 103 DEG in LT-HSC of AHR-KO mice, compared to WT; yet there were only 8 DEG in MPP. In addition to a markedly different number of DEG, Ingenuity Pathways Analysis (IPA) revealed distinct pathways among the DEG from LT-HSC versus MPP. In LT-HSC, DEG represented pathways of oxidative stress, notch signaling, hematopoiesis, cell cycle, and DNA repair. However, DEG in MPP were primarily involved in hematopoiesis and hematopoietic regulation. As LT-HSC transition from quiescence and differentiate to MPP, many signaling pathways are involved. To better understand how AHR regulates differentiation from LT-HSC to MPP, we compared the gene expression changes between LT-HSC and MPP from AHR-KO and WT, with gene changes between LT-HSC and MPP from WT. This comparison revealed 1643 DEG in WT and 2474 in AHR-KO, with 1138 DEG common between genotypes. AHR gene expression was down regulated in WT MPP, in comparison to LT-HSC, which indicates that AHR is more highly expressed in quiescent LT-HSC cells than in highly proliferative MPP. These analyses indicate that AHR regulates signaling pathways that are essential in HSC function, but it may play a less central role in MPP. Understanding cell stage specific pathways in LT-HSC and MPP provides critical insight regarding how AHR signaling regulates hematopoiesis and influences hematopoietic diseases.

The Use of Phytochemicals to Combat the Detrimental Consequences of Cigarette Smoke on Osteogenic Differentiation


Cigarette smoking has been shown to inhibit bone healing and increase the risk of pseudarthrosis after spinal fusion. We previously reported that activation of the aryl hydrocarbon receptor (AhR) by dioxin inhibits bone regeneration and spine fusion in rat model and in vitro. Since dozens of dioxin-like AhR ligands are present in cigarette smoke, here we investigated the downstream mechanisms of cigarette smoke action on osteogenic differentiation to identify potential therapeutic options to mitigate the effects of cigarette smoke on bone. Particulate phase extract (PPE) was prepared. Primary rat bone marrow stromal cells (BMSC) were cultured under basal standard or osteogenic media. BMSC were subsequently exposed to either the vehicle control (DMSO) or dioxin or PPE. Some cells received co-treatment with AhR antagonists, including α-naphthoflavone (ANF), resveratrol (Res), and 3,3-diindolylmethane (DIM). PPE-treated cells showed increased activity of CYP1A1 subfamily proteins, demonstrating AhR activation. PPE treatment inhibited ALP activity and part of the co-treatment with each of the AhR antagonists. A similar response was seen in and mineralization and cell proliferation assays. RNA expression studies showed that PPE downregulated numerous pro-osteogenic genes, such as ALP, Runx2, OCN, and Phex, whereas co-treatment with AhR antagonists prevented PPE-mediated inhibition of those RNA. Interestingly, we observed a trend in the ratio of active/inactive β-Catenin, an effect which was recapitulated by treatment with dioxin (TCDD). Our results suggest that AhR hyper-activation may play an important role in the adverse effects of cigarette smoke on bone healing—potentially by perturbation of canonical Wnt pathways.
signaling—and that Ahr antagonists may be protective in combating cigarette smoke-mediated inhibition of bone regeneration and healing. This study identified naturally available phytochemicals that antagonize the AHR receptor to provide a protective effect against the harmful consequences of compounds found in cigarette smoke on bone healing.

### 2331 Impact of the Aryl Hydrocarbon Receptor on Mitochondria in Hematopoietic Stem Cells

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We published that Aryl hydrocarbon receptor (AHR) activation by the environmental contaminant 2,3,7,8 Tetrachlorodibenzo-p-dioxin (TCDD) during fetal development leads to a loss of long-term self-renewal potential of hematopoietic stem cells. This loss was due in part to elevated reactive oxygen species (ROS) in these cells. These studies combined with the work of others are consistent with the hypothesis that the AHR regulates hematopoiesis by acting as a negative regulator of oxidative phosphorylation-dependent ROS. To test this hypothesis we conducted a series of studies on mitochondria function in murine hematopoietic stem cells that lacked the AHR. Specifically, we analyzed mitochondria superoxide production, number, and performed a mitochondria stress test to measure spare respiratory capacity in hematopoietic stem cells from WT, and AHR-/- mice. We found that in fetal hematopoietic stem cells that lack the AHR, there is a significant increase in mitochondria-produced superoxides. This particular assay is impacted by both superoxide level and number of mitochondria and so we next used a quantitative PCR-based assay to enumerate mitochondria DNA copy number relative to genomic DNA as a proxy for mitochondria number. We found that the number of mitochondria was elevated in AHR deficient fetal hematopoietic stem cells for six times fewer mitochondria than WT cells using this assay. These data are consistent with the conclusion that superoxide production is not an artifact of elevated mitochondria number and suggests elevated superoxides are coming from fewer mitochondria per cell. Finally, we performed a mitochondria stress test on AHR-deficient cells to measure spare respiratory capacity. Using a Seahorse extracellular flux analyzer for these assays we found a significant reduction in the spare respiratory capacity of hematopoietic stem cells from both fetal AHR-/- and AHR DNA binding domain mutant (DBD-/-) mice. It is critical to note that the reduced superoxide production is not suggestive of the mitochondria DNA copy number. Superoxide production was normalized by itself explain this data. This is because the spare respiratory capacity is based on the basal rate of each cell population and thus normalizes any potential differences in mitochondria content. The potential implications of this data are that the AHR acts as a negative regulator of oxidative phosphorylation in hematopoietic stem cells. This conclusion is made in part from the stress test failing to raise oxygen consumption above basal levels indicating that the AHR-/- cells are already at maximal electron transport capacity.

### 2332 Inter-Individual Differences in Electrophysiology, Pharmacology, and Drug Responses in a Cohort of hiPSC-Derived Cardiomyocytes—Endorsing Clinical-Trials-in-a-Dish

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The concept of “clinical trials in a dish” or in vitro clinical trials has received significant attention lately because of emerging technologies that allow testing novel compounds on patient cells before they move into actual clinical trials. Moreover, recent studies have shown that individual serious adverse events (SAEs) susceptibility in a population of volunteers with unknown genetic background can be recapitulated in human-inducible pluripotent stem cell cardiomyocytes (hiPSC-CMs) derived from the same individuals, providing a proof of concept for in vitro preclinical trials. Hence, leveraging genetic diversity in preclinical testing may hold the key to reducing clinical attrition, based on an increased ability to predict SAEs in a population. In this study, we examined the electrophysiological and pharmacological properties of hiPSC-CMs derived from 12 healthy neonatal donors. We first established the full transcriptome profiling of all of our cell lines, and found significant inter-line differences in the expression levels of important cardiac markers, and key ion channels. We then defined individual electrophysiological and pharmacological fingerprints of each line by measuring a number of parameters extracted from recording spontaneous beating activity (beat rate and amplitude, pulse width, pulse kinetics), and extracellular field potential (EFP) signals. All studies were performed on at least five technical replicates. Our results showed consistency for beating activity and EFP across technical replicates, but significant differences in these endpoints when compared across lines. Furthermore, we studied six different CiPA drugs (from all three risk categories for arrhythmia liability), for cardiac effects on our cohort of hiPSC-CMs. Our results showed similarities in the magnitude of responses for beating activity and EFP across technical replicates, but significant dispersion when studied across different lines. Our data demonstrate that hiPSC-CMs derived from healthy donors exhibit electrophysiological and pharmacological profiles that are unique to each donor, resulting in a clear distribution of drug effects and potencies. Our study supports the validation and implementation of testing for safety and toxicity of drugs across a cohort of donors, rather than a single cell line, endorsing the concept of clinical trials in a dish.

### 2333 CXCR7 Agonist TC14012 Improves Angiogenic Function of Endothelial Progenitor Cells in Diabetic Limb Ischemia

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Stromal cell-derived factor 1 (SDF-1)- chemokine receptors CXCR4/7 axis plays a vital role in diabetic limb ischemia. Our previous studies demonstrated that elevated CXCR7 improves therapeutic efficacy of EPCs in diabetic limb ischemia. The effects of a specific CXCR7 agonist TC14012 on diabetic hind limb ischemia remains untested. We hypothesize that CXCR7 expression is reduced in diabetic EPCs and study the angiogenic function of EPCs in diabetic limb ischemia. Early WT-EPCs were transfected with siRNA against CXCR7, and early db/db-EPCs were infected with CXCR7 lentivirus. Tube formation was assessed by capillary density of ischemic gastrocnemius muscle (GS) and soleus muscle (SS) at day 28 post-ischemic surgery. Total and (C-X-C motif) chemokine receptor type 4 (CXCR4) expression of CXCR7 were significantly decreased in EPCs from db/db mice compared with those from WT mice, whereas no significant differences of CXCR4 expression were observed. Tube formation by EPCs from db/db mice was also impaired compared with WT-EPCs. siRNA knockdown of CXCR7 was accompanied by impaired tube formation and increased CXCR7 levels in diabetic EPCs completely reversed impaired tube formation function. High glucose (HG) dose-dependently decreased the expression of CXCR7 in vitro but not that of CXCR4. HG also impaired tube formation of EPC from WT mice. Tube formation function of HUVEC was impaired after treated with HG (25 mM) for 24 hours, which could be prevented by TC14012. Most importantly, TC14012 significantly improved blood flow recovery in diabetic hind limb starting from 2 weeks after ligation and promoted angiogenesis of ischemic GS and SS. Diabetes attenuates CXCR7 expression and impairs angiogenesis of EPCs. TC14012 improves blood reperfusion of diabetic hind limb ischemia.

### 2334 Toward a More Mature hiPSC Model for Physiologically- Relevant In Vitro Cardiotoxicity Testing

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Human induced pluripotent stem cell (hiPSC)-derived cardiomyocytes are an attractive model for in vitro cardiotoxicity testing; however, cell immaturity and limitations surrounding the panel of assays available hamper a disease phenotype characterization that translates in vivo. While fatty acid oxidation (FAO) can be responsible for up to 90% of ATP in vivo, cardiomyocytes in vitro are typically maintained in high glucose. This negatively impacts physiological relevance, leading to a functional insensitivity to mitochondrial insult; a significant gap considering the implication of mitochondrial dysfunction in the etiology of drug-induced cardiotoxicity. To address these issues, we have developed and characterised an FAO-conditioned hiPSC-derived cardiomyocyte model (ConHiCM), and, using a multiparametric assay suite, have evaluated their utility in assessing the impact of drug treatment on cardiomyocyte function and metabolism. This is achieved by integrating impedance measurements (CardioExyte) with a fluorescence-based bioenergetics assessment, measuring O2 consumption (MitoXpress®-Intra), cellular oxygenation (MitoXpress®-Intra). O2 consumption is used as an interrogation parameter, with sensitivity to the CPT-1 inhibitor etomoxir used to identify the portion of respiration linked to long chain FAO. While current cell models show minimal etomoxir sensitivity, this increases on FAO conditioning, with additional
sensitivity observed when substrate demand is increased (FCCP treatment). FAO-conditioned cells also exhibit reduced reserve capacity, suggesting increased dependence on oxidative metabolism. Treatment with a panel of classical mitochondrial modulators (antimycin, rotenone, FCCP), reveals that, while glucose-grown cells are functionally insen-
sitive to perturbation, FAO-conditioned cells show impaired contrac-
tility due to a reduced capacity to maintain mitochondrial repair.
FAO-conditioning also reduces cell oxygenation (<15% O2) which
decreases further at increased beat rate (<5% O2). Data is also presented
demonstrating that beat rate modulation (Isoproterenol, Amiodarone,
Nifedipine) impacts metabolic flux, with increasing beat rate increasing
FAO. This was demonstrated through physiologic relevant in vitro
model for the identification of drug-induced cardiotoxicity.

2335 Leveraging Genetic Diversity In Vitro: A New Approach in Cardiac Safety Assessment
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Although models for detecting preclinical toxicity and safety have
improved in recent years, predicting serious adverse events (SAEs) in
preclinical studies remains a challenge because these events often occur
in a small subset of patients. In addition, cellular or animal models cur-
rently being used are not suited for the interrogation of inter-individual
differences in drug susceptibility. Over the past few years, cardiomyo-
cytes derived from human induced pluripotent stem cells (hiPSC-CMs)
have shown promise for cardiac safety screening. Moreover, recent
studies have shown that individual SAEs susceptibility in a population
of volunteers with known genetic background can be recapitulated in
hiPSC-CMs derived from the same individuals, providing a proof of
concept for in vitro preclinical trials. Hence, leveraging genetic diversity
in preclinical testing may hold the key to reducing clinical attrition by
predicting SAEs in that population, potentially resulting in reducing clin-
ical attrition of drugs.

2336 Assessment of Gut Organoid Models from Several Species Ability to Predict Intestinal Toxicity of Drug Development Candidates
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*An in vitro* organoid model was developed from intestinal epithelial
cells in order to predict predictivity of *in vivo* gastrointestinal
toxicity. Historically, *in vitro* models of the GI used tumor derived, trans-
formed intestinal cell lines (e.g., HT-29, Caco2, and HCT-8), which can be
limited in the cell morphology, cell type distribution, and ultimately,
suboptimal system for studying drug induced toxicity. Small intestinal
organoids (enteroids) were generated from commercially available
small intestinal epithelial cells (C57BL/6 mouse, rat, canine, cynomolgus
monkey, and human) using established culture reagents for organoids
(Ibiza E et al. 2016). The morphology of the absorptive (enterocytes)
and secretory (goblet, Paneth, enteroendocrine, and tuft) cells, assessed
with hematoxylin and eosin, PAS, and Alcan blue staining, confirmed
that enteroids have similar cell types and morphologic attributes to
*in vivo* intestinal epithelium. The toxicity of various known GI toxicants
was assessed using two independent cell death assays, CellTox Green
and CellTiter-Glo. Interestingly and correlated with *in vivo* findings, the
toxicity (IC50 values) was species dependent, with dogs being the GI
most adversely amongst the five-tested species. In particular, a, develop-
ment test compound QC8222, which was toxic proximal to dog GI
epithelial barrier in *in vivo* was also toxic in vitro. The most susceptible
cell type to toxicity was examined using a cell death (anti-caspase 3) and
a proliferation (anti-Ki67) markers combined with cell-specific immuno-
taining (mucin 2 for Goblet cells, lysozymes for Paneth cells, chromog-
arin A for enteroendocrine cells, and succrose isomaltase for absorptive
enterocytes). This study showed that QC8222 generally prevented the
expression of stem cells from stem cells and induced caspase 3 medi-
ated cell death, which prevents the protective mechanism of secreting
anti-microbial peptides, and may be related to its epigenetic mecha-
nism of action. Elevated cell death marker gene expression in the Paneth
cells was confirmed by RNAseq. In conclusion, this study reveals that
enteroids models from multiple species can be used to infer potential
effects in whole organisms but additional validation is needed.

2337 3D Organotypic Co-Culture System: A Simple High-Throughput Mini-Liver Tissue for Toxicology Study
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The liver is the primary site for drug metabolism, and cultured hepatocytes
are indispensable for drug development and toxicity testing. Traditionally,
liver slices, microsomes, and primary hepatocytes are used for such purposes,
but a large gap exists between demand and availability of primary human hepatocytes. This problem of availability is further complicated by differdetermination of primary cells in vitro. The advent of hepatocytes derived from induced pluripotent stem (iPS) cells and embryonic stem cells may help close that gap, but they function more like fetal hepatocytes than mature cells and do not display many metabolic behaviors. In this study, we studied the use of hepatocytes in co-culture for cell-cell interaction on human iPS-derived hepatocytes maturation in a 3D organotypic culture system. The all the cell types originate from human origin. To our knowledge this is the first 3D in vitro model that co-cultures iPS derived hepatocytes with non-parenchymal cells all from within one species. Historically, hepatic stellate cells and biliary epithelial cells (cholangiocytes) on human iPS-derived hepatocytes. We tested the hepatocyte matu-

2338 Multiparametric Assessment of Networked Electrical Activity Using Induced Pluripotent Stem Cell-Derived Glutamatergic Neurons
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The lack of a predictable preclinical test system to identify CNS adverse
effects greatly hinders the drug development process and contributes to
the drug attrition rate. Three-dimensional *in vitro* test system of human neuronal cultures with synchronized network electrical (bursting) for assessment of compound effects on neuronal function and network communication including seizurogenic liability. Human induced pluripotent stem cell (iPSC) derived neurons, mixture of 75% glutamatergic and 25% GABAergic were cultured for 3 days on multi-electrode array (MEA), alone or in combination with human iPSC-derived astrocytes, and assessed for compound effects. Known excitatory compounds were measured for concentration-dependent effects, at clinically relevant concentrations, including bicuculline, picro-
toxin, glutamate, penetrenetrazol, 4-aminopyridine, and chloroproma-
mine. Activity parameters displaying concentration-dependent changes
with pharmacology include: mean firing rate, ‘single-channel’ burst rate,
together with pharmacology include: intensity duration, ‘network-level’ burst rate, intensity and duration, and synchrony measures. Furthermore, while both cultures of gluta-
marergic and GABAergic co-cultures with neurons and astrocytes
exhibited the expected pharmacology, the bursting behavior of neu-
ronal and astrocyte co-culture was more clearly defined. The presented
data demonstrate how iPSC technology coupled with MEA technology
create a noninvasive human neuronal test system, previously limited to
rodent models, and provide an unprecedented investigatory space for

Drug development. Furthermore, overall activity can be modulated by titrating different levels of neuronal subtypes and support cells (astrocytes). Together, this methodology allows quantification of neuronal networked electrical activity in a human model which should be valuable to identify CNS liability and support preclinical toxicity programs.

### 2339 Induction of Epileptiform Activities in Cultured Human iPSC-Derived Neuronal Networks

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The functional network of human induced pluripotent stem cell (hiPSC)-derived neurons is a potentially powerful in vitro model for evaluating drug toxicity. Epileptiform activity is one of phenomena in neuronal toxicology. To evaluate the dynamics of epileptiform activities and the effect of anti-convulsant drug in cultured hiPSC-derived neurons, we used the high-throughput multielectrode array (MEA) system, where we simultaneously record extracellular potentials for 16 channels per well across 24-well plates. We examined chemically evoked epileptiform activity. Epileptiform activities were induced by 4-Aminopyridine (4-AP), pilocarpine, chlorpromazine, and pentylenetetrazole (PTZ). The number of synchronized burst firings were increased in a concentration dependent manner at 4-AP, Pilocarpine, and Chlorpromazine administration. On the other hand, the duration and spikes in a synchronized burst were increased at PTZ administration. Phenytoin used in anti-convulsant drug suppressed electrophysiological activities. From these results, we suggest that the electrophysiological assay in cultured human iPSC-derived neuron using MEA system has the potential to investigate the neuronal toxicity in drug discovery.

### 2340 Electrophysiological Pain Responses in Cultured Human iPSC-Derived Sensory Neurons Using High-Throughput Multi-Electrode Array System


Dorsal root ganglion (DRG) sensory neurons are pain-related neurons and have a variety of sensory receptors that are activated by chemical, thermal, and mechanical stimuli. Establishment of a pharmacological assay in pain research and drug screening is an important issue. In addition, human-induced pluripotent stem cell (hiPSC)-derived sensory neurons may be effectively used for drug discovery and toxicity testing. The purpose of this study was to evaluate the physiological responses against typical pain-related molecules, temperature change, and anticancer drugs in cultured sensory neurons using the high-throughput multi-electrode array (MEA) system. Firstly, we confirmed human iPSC-derived sensory neurons expressed typical sensory neural markers Nav1.7, TRPV1, and TRPA1 using immunostaining. Next, we detected the physiological responses to temperature change, capsaicin, menthol, and wasabi by change of spike rate using MEA system. Phenytoin administration. We examined chemically evoked epileptiform activity. Epileptiform activities were induced by 4-Aminopyridine (4-AP), pilocarpine, chlorpromazine, and pentylenetetrazole (PTZ). The number of synchronized burst firings were increased in a concentration dependent manner at 4-AP, Pilocarpine, and Chlorpromazine administration. On the other hand, the duration and spikes in a synchronized burst were increased at PTZ administration. Phenytoin used in anti-convulsant drug suppressed electrophysiological activities. From these results, we suggest that the electrophysiological assay in cultured human iPSC-derived neuron using MEA system has the potential to investigate the neuronal toxicity in drug discovery.

### 2341 Simultaneous Measurement of Contractility, Electrophysiology, and Biomarker Secretion to Determine Cardiotoxicity in hiPSC-Derived Cardiomyocytes


Drug-induced cardiotoxicity has been the major cause of drug withdrawal from the market. For example, many novel oncology therapeutic agents are associated with cardiodepressive effects, including in vitro overexpressing human cell lines or use in vivo animal models. These models often lack the complexity of human cardiomyocytes, whereas animal models may lack predictivity due to inherent species differences. Therefore, there is a need for the development of more predictive and specific assays that allow for multi-parametric assessment of potential cardiotoxic side effects of new drugs in humans. Using proprietary hiPSC-derived ventricular cardiomyocytes (Pluricyte® Cardiomyocytes) that recapitulate a human myocyte’s contractile and electrophysiological profile, as well as mature sarcomere organization, we developed a multiparametric assay to measure potential cardiotoxic effects in vitro. Here, both acute and long-term drug effects on contraction, electrophysiology, and cardiac troponin I release are determined simultaneously from each well of a 48-well plate. To assess the effects of anticancer drugs on the physiology of Pluricyte® Cardiomyocytes, the cells were incubated with concentration ranges of nilotinib, lapatinib, doxorubicin, and ponatinib for up to 64 hours, and analyzed simultaneously using microelectrode array (MEA), impedance, and cardiac troponin I (cTnI) release assays. This resulted in defined cardiotoxicity profiles for each compound. Whereas treatment with nilotinib and lapatinib caused a transient, short-term (functional) contractile/electrophysiological deficit, doxorubicin exhibited a continuous long-term toxic effect in both MEA and impedance measurements. While lapatinib and nilotinib did not cause structural toxicity as measured by cTnI release, ponatinib induced a cTnI dependent increase in cTnI release. The dose-dependent increases in cTnI release correlated with reduced cell index values obtained in the impedance assay. These data suggest that a multiplexed analysis is crucial to investigate short- and long-term cardiac liabilities as it provides a more comprehensive readout that generates mechanism-specific cardiotoxicity profiles, leading to better prediction of drug-induced cardiotoxicity.

### 2342 Optimization of Optogenetic Transduction of Stem Cell-Derived Cardiomyocytes with Adeno-Associated Virus for Optically Paced Cardiac Electrophysiology Assays


Commercially available stem cell derived cardiomyocytes beat spontaneously, offering a simple assay solution for evaluation of cardiac risk in vitro. However, the spontaneously beat rate may differ across wells, particularly after pharmacological manipulation, necessitating the reliance on correlation formulae for determining the relationship between repolarization timing and beat frequency. Optogenetics, a technique whereby light sensitive ion channels allow depolarization of electro-active cells upon incident light, allows pacing of cardiomyocytes to control beat frequency. Here, we use a multi-well microelectrode array (MEA) assay with integrated multi-well light delivery to optimize the viral transduction of commercially available stem cell derived cardiomyocytes. Increasing amounts of virus were added to wells across the 48-well plate in half-log increments, with six replicates per multiplicity of infection (MOI). At high MOI’s, the spontaneous beat frequency decreased, and the depolarization amplitude decreased, suggesting an effect of cHR2 on the cardiomyocyte electrophysiology. Meanwhile, low MOI’s produced no change from control for beat frequency or amplitude. A pacing light stimulus was delivered simultaneously to each well on the plate, with increasing light intensity, such that the number of wells paced at each light intensity could be quantified. As expected, the wells with highest MOI responded to optical pacing at the lowest intensity and the wells with the lowest MOI did not respond differently than control wells. Using the optimal MOI, the cardiomyocytes were paced at increasing rates to quantify the relationship between repolarization timing and the paced beat frequency.

### 2343 Human iPSC-Derived Hepatocytes with Advanced Functionality and Longevity: An Innovative In Vitro Model for Studying Metabolic Diseases

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Generating accurate liver disease models requires a reliable and reproducible source of well-characterized, functionally mature, and long-lasting human hepatocytes. However, existing primary cell and tumor-derived model systems lack proper functionality, do not last long in culture, have limited batch sizes, or have high batch-to-batch variation. To pave the way for better liver disease models, we have created human iPSC-derived hepatocytes (hiPSC-HEP) that are more mature and show improved functionality, and consistent performance between batches and over extended time in culture. These cells secrete albumin and urea and overexpress human cell lines and are used in vivo animal models. These models often lack the complexity of human cardiomyocytes, whereas animal models may lack predictivity due to inherent species differences. Therefore, there is a need for the development of more predictive and specific assays that allow for multi-parametric assessment of potential cardiotoxic side effects of new drugs in humans. Using proprietary hiPSC-derived ventricular cardiomyocytes (Pluricyte® Cardiomyocytes) that recapitulate a human myocyte’s contractile and electrophysiological profile, as well as mature sarcomere organization, we developed a multiparametric assay to measure potential cardiotoxic effects in vitro. Here, both acute and long-term drug effects on contraction, electrophysiology, and cardiac troponin I release are determined simultaneously from each well of a 48-well plate. To assess the effects of anticancer drugs on the physiology of Pluricyte® Cardiomyocytes, the cells were incubated with concentration ranges of nilotinib, lapatinib, doxorubicin, and ponatinib for up to 64 hours, and analyzed simultaneously using microelectrode array (MEA), impedance, and cardiac troponin I (cTnI) release assays. This resulted in defined cardiotoxicity profiles for each compound. Whereas treatment with nilotinib and lapatinib caused a transient, short-term (functional) contractile/electrophysiological deficit, doxorubicin exhibited a continuous long-term toxic effect in both MEA and impedance measurements. While lapatinib and nilotinib did not cause structural toxicity as measured by cTnI release, ponatinib induced a cTnI dependent increase in cTnI release. The dose-dependent increases in cTnI release correlated with reduced cell index values obtained in the impedance assay. These data suggest that a multiplexed analysis is crucial to investigate short- and long-term cardiac liabilities as it provides a more comprehensive readout that generates mechanism-specific cardiotoxicity profiles, leading to better prediction of drug-induced cardiotoxicity.
over a two-week assay window and show mRNA expression levels of albumin and urea cycle enzymes that are comparable to those of human primary hepatocytes (hphep). Moreover, activity of cytochrome P450 enzymes and expression of genes essential for the drug metabolizing machinery are stably detected for up to 19 days in hiPS-HEP compared to hphep after one day in culture. Finally, we demonstrate that our iPSC-derived hepatocytes exhibit critical metabolic features. First, genes involved in both glucose and lipid metabolism are expressed at similar levels to hphep. In addition, the cells respond to insulin by phosphorylation of AKT and show capacity to take up low-density lipoproteins and become steatotic if incubated with fatty acids. The availability of robust and consistent human pluripotent cell-derived hepatocytes (hphep) with mature hepatic functions represents an important step toward advancing the discovery of new treatments for metabolic disease, reducing the incidence of drug-induced liver injury, and developing new strategies for liver regeneration and transplantation.

**2344** Characterization of the Local Extracellular Action Potential Signal for Use in Cardiac Safety Evaluation

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The comprehensive in vitro Proarrhythmia (CiPA) Assay is the result of a public-private collaboration to improve the assessment of Proarrhythmic risk using *in silico* and cell-based *in vitro* assays. A central component of the cell-based track of the CIPA assay is the evaluation of stem cell derived cardiomyocyte electrophysiology using the field potential acquired from microelectrode array (MEA) recordings. Although the FP signal acquired from MEA assays has performed exceedingly well in CiPA Phase I and Phase II studies, there are several advantages to the FP signal shape. Namely, many scientists are trained in the context of the cardiac action potential signal, and wish to interpret FP data from the CM-MEA assay in the same way. Also, while repolarization irregularities, such as EADs, are clearly present in the FP signal, they can be difficult to detect in automated analysis. Here, we characterize a new signal type, termed the local extracellular action potential (LEAP), acquired from extracellular microelectrodes for use in CIPA-like cardiac safety assays. The LEAP signal occurs through increased coupling of the cardiomyocyte syncytium to the microelectrode, resulting in a 5-20mV signal with an action potential-like morphology. The LEAP signal was further characterized pharmacologically with E-4031, a potent potassium blocker, nifedipine, an L-type calcium channel blocker, and verapamil, a multi-ion channel blocker affecting potassium and calcium channels. Nifedipine and verapamil caused a dose-dependent decrease in the duration of the LEAP signal and E-4031 caused a dose-dependent increase in LEAP duration, all of which is consistent with previous field potential or action potential assays. Also, E-4031 produced EADs at higher concentrations. Finally, previous action potential experiments using dog perikine fibers to evaluate cardiac liabilities for tolterodine and terodiline from the market due to proarrhythmic risk.

**2345** Comparison of the Proarrhythmia Liability of Standard and Functionally Mature Human iPSC-Derived Cardiomyocytes

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Human iPSC-derived cardiomyocytes (hiPSC-CMs) display a repertoire of ionic currents and channels, which makes them a relevant model for safety/toxicity assessment. However, standard hiPSC-CMs resemble fetal cardiomyocytes and may not have the appropriate composition of ionic currents and channels, appear to be more sensitive to hERG channel blockade, and even show early after depolarization (EAD)-like response after treatment of compounds, which typically have low risk for torsades de pointes (TdP) (Qu et al., 2015). In order to address these limitations we wanted to investigate if improvements in the maturation status of hiPSC-CMs could increase the extent of predictivity of hiPSC-CMs assay for drug proarrhythmia liability. We utilized long-term electrical pacing provided by the xCELLigence RTCA CardioECR system to enhance the maturation status of hiPSC-CM. The contraction and electrophysiology of hiPSC-CMs were monitored by simultaneous measurement of impedance and field potential, respectively. After 15 days of continuous electrical pacing, paced cardiomyocytes displayed more mature characteristics compared to non-paced cells, as demonstrated by two factors: 1) the inherent negative impedance amplitude-frequency relationship of hiPSC-CMs was reversed after pacing, 2) paced hiPSC-CMs displayed better organization of calcium handling proteins, as well as increased expression of genes that are critically involved in the cardiomyocyte maturation process after chronic pacing. A panel of ion channel modulators, including hERG channel blocker and mixed channel modulators, were tested using the functionally mature/enhanced hiPSC-CMs. Our data clearly show that the response of the functionally enhanced/mature cardiomyocytes (paced) to the ion channel modulators, especially multi-ion channel modulators, are more comparable to the clinical outcome than the control non-paced hiPSC-CMs. In summary, our data suggest that maturation status of hiPSC-CMs can be improved after employment of chronic electrical pacing, resulting in enhanced predictivity for proarrhythmia liability of drugs.

**2346** A Novel Maintenance Medium Enables Long-Term Applications for Both Human Primary Hepatocytes and Human Pluripotent Stem Cell-Derived Hepatocytes in Conventional 2D Cultures

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Human primary hepatocytes (hphep) are the current gold standard for drug development and toxicity assays *in vitro*. However, their rapid loss of viability in conventional 2D cultures limits their utility. In contrast, human pluripotent stem (hPS) cell-derived hepatocytes have a potential to become a more predictive *in vitro* model system for these applications if they possess a relevant window and function. To address this problem, we developed a novel hepatocyte maintenance media which enables culturing of both hhep and hiPS cell-derived hepatocytes (hiPS-HEP) with viable cells and stable activity of key enzymes for several weeks. Cryopreserved hiPS-HEP cultured in our medium express essential genes involved in drug metabolism, such as CYPs, phase II enzymes, and transporters. Importantly, they also demonstrate mature and functional features as shown by multiple analyses including immuno-stainings and functional assays such as albumin secretion and CYP activity assays. Additionally, this extended culture time enables long-term experiments, such as chronic compound exposure. Here, we performed a proof-of-concept chronic toxicity test with compound exposure for up to 14 days. In this study, our hiPS-HEP responded expectedly to known hepatotoxins, demonstrating their utility for chronic toxicity studies. To determine if similar improvements in viability and functionality were possible with hheps, we also reformulated the novel maintenance medium for hhep. The novel culture medium clearly showed stable morphology, viability, and functionality of hhep for four weeks post-thawing. We observed, functional, stable CYP activities and albumin secretion during the four week-period. Additionally, hhep cultured for four weeks showed inducible expression of CYP1A2, 2B6, 2C9, and 3A4. Taken together, our novel medium prevents the typical rapid loss of functionality and viability of hhep *in conventional* 2D cultures. Also, this medium allows long term culture of cryopreserved hiPS-HEP, from multiple lines, with preserved functionality. This increased longevity and functionality of hepatocytes in 2D cultures significantly advances the applications and uses of *in vitro* hepatocyte models.

**2347** A Cell-Based BoNT Potency Assay Using Electrophysiological Activity Measured with Microelectrode Arrays


The standard for measuring botulinum neurotoxin (BoNT activity) has been the mouse L5D0 (mLD50) bioassay. Although the mLD50 bioassay is highly sensitive, there have been efforts to replace this with an *in vitro* assay which will cost less to perform, provide a more controlled testing environment, reduce the inherent variability associated with animal bioassays, and reduce the use of animals. In support of this effort, we have developed an *in vitro* neuronal-cell based assay to evaluate BoNT potency which will include key steps in BoNT toxicity. These steps include cellular binding, uptake, translocation, and proteolytic cleavage of the target SNARE proteins involved in vesicular fusion, which can be evaluated by measuring electrophysiological changes. The goal of this effort was to show a dose dependent change in electrophysiological activity within rat primary neuronal cells after exposure to pure BoNT serotype A (BoNT/A) or BoNT/A in complex. A cell-based BoNT potency assay was conducted by exposing rat primary neuronal cells to BoNT/A and using a microelectrode array (MEA) output to measure electrophys-
iodological activity. Using fresh and frozen primary rat neurons exposed to pure BoNT/A concentrations ranging from 0.5 to 500 pM, statistically significant, dose dependent reductions in electrophysiological activity were observed. Exposing cells to BoNT/A in complex also showed statistically significant reductions in electrophysiological activity, but this was not explored to the same extent as pure BoNT/A, and a dose dependent reduction was not established. This cell-based BoNT/A potency assay was capable of detecting BoNT toxicity at concentrations as low as 0.5 pM in 4-5 replicates of up to 5 treatment conditions simultaneously on a single plate. Although the assay was developed using BoNT/A, it is likely capable of detecting toxicity from other BoNT serotypes and even other neurotoxins or neurotrophic chemicals. This assay appears to have the necessary sensitivity and throughput to replace the mLD50 assay.

**2348 Validation of a Co-Culture Cell Model for Identifying Angiogenesis Inhibitors on a High-Content and High-Throughput Screening Platform**

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Angiogenesis is a fundamental developmental and physiological process of forming new blood vessels that is required for organ growth and repair. Recent studies have also drawn attention to the role of angiogenesis inhibitors that are closely related to cardiovascular toxicity. In vitro angiogenesis models for analyzing tube formation serve as useful tools to study these processes. However, current in vitro co-culture models using primary cells have limitations in usefulness and consistency. Therefore, in the present study, an in vitro co-culture assay system was optimized in a 1,536-well format for high-throughput screening using human terminal differentiated HUVEC (hTERT)-immortalized mesenchymal stem cells and aortic endothelial cells. The National Center for Advancing Translational Science (NCATS) Pharmaceutical Collection (NPC) library containing 2,816 drugs was evaluated using this in vitro co-culture assay system. The screen, 35 potential inhibitors (IC50 ≤ 1 µM) were identified, followed by 15 weaker inhibitors (IC50 ≈ 10 µM). Moreover, many known angiogenesis inhibitors were identified such as topotecan, docetaxel, and bortezomib. Several potential novel angiogenesis inhibitors were also identified from this study including thimerosal and podophyllotoxin. Some of these compounds were shown to be involved in the hypoxia-inducible factor-1-alpha (HIF-1α) and the nuclear factor-kappa B (NF-κB) pathways. In addition, many inhibitors, such as sunitinib, sorafenib, and vatalanib are closely related to increased cardiovascular risk. The co-culture model developed by using hTERT-immortalized cell lines described in this study provides a consistent and robust in vitro system for anti-angiogenic compound screening.

**2349 Establishment of In Vitro Cytotoxicity Assay Using Primary Monkey Cardiomyocytes**

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Cynomolgus monkey is one of the most commonly used animal species as a nonhuman primate in the toxicology field, but cytotoxicity assay using their primary cardiomyocytes have not been established. The aim of the current study is to establish in vitro cytotoxicity assay platform using primary monkey cardiomyocytes. Primary cardiomyocytes were isolated from newborn fetal hearts and cultured at different gestation day (from day 39 to 90). The cells were collected using commercially available isolation kit. Briefly, the whole fetal heart was digested by trypsin at 4°C for overnight. The cells were digested in collagenase solution at 300 units/ml for 45 minutes at 37°C, followed by being centrifuged at 100 x g for 10 minutes. Under the present condition, the percentage of living cells was more than 80% from all fetuses. Importantly, spontaneous contraction was observed only in the cells from gestation day at 63 or earlier. Transcriptome analysis of the isolated cells using microarray indicated that the cells have essential components of cardiac function such as myosins, α-actin, cardiac troponins, and calcium-released molecules, consistent with those in rat cardiomyocytes. Next, we evaluated the sensitivity of the monkey cardiomyocytes to doxorubicin. Exposure of monkey cardiomyocytes to doxorubicin at 300 nM or more for 72 hours increased LDH release and decreased ATP levels, but the sensitivity of monkey cardiomyocytes to doxorubicin was weaker than that of rat cardiomyocytes. The mechanism underlying the differential sensitivity to doxorubicin between monkey and rat was further characterized by microarray. The analysis revealed that doxorubicin predominantly increased several key genes involved in endoplasmic reticulum stress pathway in monkey cardiomyocytes compared to those in rats. In conclusion, primary monkey cardiomyocytes with spontaneous beating activity showed similar functional and transcriptional profiles to rat cardiomyocytes. Furthermore, monkey cardiomyocytes showed differential sensitivity to doxorubicin compared to rat cardiomyocytes through the upregulation of several key genes involved in endoplasmic reticulum stress pathway. The monkey cardiomyocytes would be valuable in investigating interspecies differences in drug-induced cardiotoxicity and its mechanism.

**2350 Human-Derived Cardiomyocytes for Testing Cardiotoxicity of Industrial Chemicals of Concern**


Cardiac toxicity, especially via sensitizing (or priming) the heart for a subsequent arrhythmia, has been reported for some industrial chemicals of concern (iCOC) including halogenated hydrocarbons and acrolein (an unsaturated aldehyde). Other iCOC are potential cardiocytosins via their modes of action (most commonly oxidative stress and/or calcium dysregulation). The applicability of a cardiomyocyte in vitro assay for screening and correctly identifying cardiotoxic iCOC was tested using several known reference compounds (sotalol, isoproterenol, and propafenone), known cardiotoxic compounds (digoxin, haloperidol, rotenone, and carbonyl cyanide 3-chlorohydrazone (CCCP)), active iCOC acrolein, chlorohydrin, acrylonitrile and phenol. Cardiomyocytes were maintained in culture for 9-11 days prior to testing, and a beating syn- chrony was confirmed prior to testing. The cells were pretreated with a fluorescent calcium-sensitive dye (Ex:Em: 485/535) so that the oscillation behavior of individual calcium could be measured spectrophotometrically. The cardiomyocyte response to the compounds was rapid with the compounds being observed after a 10 minute exposure. A dose dependent decrease in beat frequency was observed for acrolein and phenol while minimal changes in beat parameters were observed for chlorohydrin and acrylonitrile. Additionally, the intermediate doses of acrolein showed a decrease in the number of beats per minute (BPM) that preceded the drop in BPM observed at the higher doses. The increased BPM occurred between 10 and 200 µM acrolein. The cardiomyocyte response to phenol was a cessation of beating at approximately 1000 µM. Neither chlorohydrin nor acrylonitrile was cardiotoxic at concentrations as high as 2 mM. Rapid toxicity evaluations of iCOC and substances intended for military applications are key for timely environmental health and occupational safety recommendations. Based on the data presented here, the utility of an in vitro functional cardiotoxicity assay for the Army Public Health Center (APHC) screening level toxicity assessments on iCOCs was demonstrated. Further testing of this system for characterizing acrolein induced cardiac sensitization is ongoing.

**2351 Cardiovascular Toxicity Screening of Polychlorinated Biphenyls and Their Major Metabolites**

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 Xenobiotic metabolism is complex and determines the physiologic fate and toxicological properties of drugs and environmental chemicals. Accounting for bioactivation and detoxification processes remains among the most challenging aspects of predictive in vitro toxicity. While co-culture systems with metabolically active components are currently being explored, the significance of the metabolic profiles remains unknown. Considering the physiological relevance of novel organotypic culture models and their high-throughput screening capacity, we hypothesize that direct characterization of chemicals and their major metabolites using multidimensional chemical-biological profiling is a sensible alternative. In this study, we tested 25 polychlorinated biphenyls (PCB 3, 11, 52, 95, 126, 136, 153) and their relevant metabolites (hydroxylated, methoxylated, sulfated, and quinone) for concentration-dependent (10 nM-100 µM) acute effects in iPS-derived human cardiomyocytes and endothelial cells. acute effects in iPS-derived human cardiomyocytes and endothelial cells. cardiac sensitization is ongoing.
fation and methoxylation of PCB resulted in diminished bioactivity. This study demonstrates a strategy for assessing metabolism in \textit{in vitro} screening and provides information from organotypic culture systems for human health assessments of PCBs and their metabolites. This work was supported by NIEHS grants P42 ES013661 and P42 ES027707A.

While the adverse outcome pathway (AOP) concept has been a key aspect of human cancer risk assessment for some time, it was recently adopted by the OECD as a pragmatic tool which may facilitate transition of chemical safety assessment from measurement of apical endpoints in animals to toxicity prediction based on mechanistic information. The concept is that identification of key events and systematic mapping of AOPs for a given hazard endpoint can form the basis for the development of alternative tests as part of an integrated testing strategy to eventually replace conventional guideline studies. With the overall aim to further develop the database of integrated animal- and human-based approaches to assessment of repeated dose systemic toxicity, we developed and critically evaluated AOPs for proximal tubule injury initiated by (1) covalent protein binding (2) lysosomal overload and (3) inhibition of mtDNA polymerase $\gamma$, as these present fairly well-defined mechanisms by which certain chemicals/drugs cause nephrotoxicity. The usefulness of AOPs provide a mechanistic framework for the development of \textit{in vitro} bioactivity assays that cover key events across these AOPs and that can be integrated with quantitative \textit{in vitro} to \textit{in vivo} extrapolation and exposure modelling to derive quantitative risk estimates. The selected and validated assays were used for high-content analysis of model nephrotoxins in rat and human proximal tubule cells to establish the quantitative relationships between upstream and downstream key events.

Assessing the acute toxic potential of a substance is necessary to determine the potential effects of accidental or deliberate short-term exposure. There are several approaches available, and few in \textit{silico} models, to predict acute oral toxicity. Until recently, a paucity of experimental \textit{in vivo} acute toxicity data was available for model development and evaluation. Here, a large acute oral toxicity dataset totaling 15,698 unique chemicals was compiled from different sources including the Organization for Economic Corporations and Development’s eChemPortal, the National Library of Medicine’s (NLM) Hazardous Substances Data Bank, NLM’s ChemIDplus via the Toxicity Estimation Software Tool, the European Union Joint Research Centre’s AcutoxBase and the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Method’s Pesticide Active Ingredients Database. Many of the LD50 values originated from limit tests which estimate an LD50 value as being above/below a specific threshold, typically 2000 mg/kg or 5000 mg/kg. These limit tests present challenges for model development since they provide less information than an explicitly quantified LD50 value. To overcome this limitation, three approaches were used to model acute oral toxicity using ToxCast/Tox21 activities as biological descriptors and ToxPrints and physico-chemical properties as chemical descriptors. All models were developed and evaluated using 80% data as training set and 20% data as an external test set. The first approach was a global random forest model, and below a LD50 of 5000 mg/kg. The balanced accuracy of the model was 0.76 and 0.35, respectively. The third was a set of 10 cluster-based local

**2352** Mapping Adverse Outcome Pathways for Kidney Injury: Opportunities for Development of Mechanism-Based Animal-Sparing Approaches to Assessment of Nephrotoxicity

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**2353** Metabolome Analysis \textit{In Vitro} in NRK-52e Cells: A Potential Tool for Investigation of Mode-of-Action of Nephrotoxicants

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**2354** Variability of LD50 Values from Rat Oral Acute Toxicity Studies: Implications for Alternative Model Development

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Alternative models developed for estimating acute systemic toxicity are generally evaluated using \textit{in vivo} LD50 values. However, \textit{in vitro} acute systemic toxicity studies can produce variable results, even when conducted according to accepted test guidelines. This variability can make a fair assessment of alternative models extremely challenging. To characterize the variability of \textit{in vivo} acute systemic toxicity data, we examined a large compilation of LD50 values reported in oral rat acute toxicity studies. Data were obtained from multiple curated databases including the NLM’s Hazardous Substances Data Bank and ChemIDplus, the OECD’s eChemPortal, and the JRC’s AcutoxBase. The resulting dataset comprised a total of 2,120 rat oral LD50 values representing 15,698 chemicals. A subset of chemicals was identified in at least three independent rat oral acute toxicity studies to be used to assess variability. Of this subset, 20% (234 chemicals) had at least one study generating an “extreme” LD50 value (i.e., falling outside 1.5 times the interquartile range of the LD50 distribution for that chemical). Furthermore, 30 chemicals had LD50 values ranging across at least two orders of magnitude, with some of these chemicals having LD50 values ranging across at least three orders of magnitude. This degree of variability can confound hazard categorization: LD50 values from 47 chemicals fell into at least three different Globally Harmonized System (GHS) oral toxicity labeling categories, and values from 10 chemicals fell into at least three EPA hazard categories. These findings underscore the importance of considering an appropriate margin of uncertainty when using \textit{in vivo} oral acute toxicity data to assess the performance of alternative methods and provide a reference dataset to ensure that appropriately representative LD50 data are routinely used for the development and validation of alternatives models. This project does not necessarily reflect US EPA policy and was funded in whole or in part with federal funds from the NIEHS, NIH under Contract No. HHSN273201500010C.
random forest models built using the k-means algorithm for deriving the clusters. The RMSEs and R² for the test sets ranged from 0.52-0.83 and 0.20-0.48, respectively. Overall, the local cluster-based models performed better than the global models. This abstract does not necessarily reflect US EPA policy and was funded in part with federal funds from the NIEHS, NIH under Contract No. HHSN273201500010C.

**2356 Single versus Repeated-Exposure Analysis for In Vitro Protein Hazard Characterization with Human Polarized Intestinal Epithelial Cell Line Monolayers**

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Recent studies suggest that human derived intestinal epithelial cell (IEC) lines cultured as polarized monolayers on permeable Transwell® filters are effective at differentiating between hazardous and non-hazardous proteins. This experimental platform is based on a single exposure of IEC monolayers to a test protein followed by assessment of whether IEC monolayers display evidence of loss in barrier integrity or cell viability after overnight exposure. In this study, Caco-2 and T84 IEC polarized monolayers were evaluated for barrier integrity and cytotoxicity following a single overnight exposure to hazardous or non-hazardous proteins and compared to IECs exposed nine times over the span of 29 days to the same protein. The objective was to determine whether repeated exposures altered responses observed following a single exposure. Hazardous proteins included *Clostridium difficile* toxin A (ToxA), Streptolysin O (SLO), Wheat Germ Agglutinin (WGA), and *Phaseolus vulgaris* haemagglutinin-E (PHA-E). Non-hazardous proteins included bovine serum albumin (BSA), porcine serum albumin (PSA), peroxidase, and fibrinogen (Fbn). In general, evidence of diminished barrier integrity and/or cell viability following exposure to hazardous proteins was more pronounced in magnitude when IECs were subjected to multiple rather than single exposures. In some cases, an effect on IEC monolayers was observed only when the repeated exposure protocol was chosen. Non-hazardous proteins failed to elicit any effects following either single or repeated exposures. Results from these studies further support the utility of using cultured human IEC polarized monolayers to differentiate between hazardous and non-hazardous proteins and suggest that a repeated exposure protocol may reveal a greater magnitude of response when compared to a single exposure protocol.

**2357 Detecting GI Toxicity Earlier Than in Dog: Developing an In Vitro Assay to Predict Clinical Diarrhea**

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GI toxicity is a common adverse event that limits pharmaceutical development across diverse therapy areas. Preclinical testing in dog (but not rat) shows good predictivity (Olson et al., 2000), however, the cost and throughput prevents routine testing needed to optimize GI safety during drug discovery. In vitro models have been developed to elucidate the role of molecules with lower risk and/or to develop dosing schedules that mitigate the risks. Traditional 2D in vitro cell cultures approaches using immortalized human colorectal Caco-2 cells have been successful for routine assessment of ADME properties. Here, the utility for predicting GI toxicity was assessed with 2D Caco-2 cultures and 3D GI microtissue prepared using the MatTek SureClick™ technology. Derived from primary human ileum and cultured on transwells, the microtissues develop structural features resembling villi, replicate diverse intestinal epithelial cell phenotypes, and replicate barrier function. Since, impaired epithelial barrier function is associated with clinical diarrhea, microtissue transepithelial electrical resistance (TEER) was evaluated to correlate with clinical incidence of diarrhea. Clinical correlation was assessed with compounds selected based on differing incidences of diarrhea; this included 17 drugs with low (<3%) and with 15 high (>40%) incidence. Two independent microtissue experiments conducted under blinded conditions revealed predictive accuracy of 84% and 81% compare to 72% with Caco-2. Microtissue sensitivity was 80% and 67% which was superior to similar experiments with Caco-2 cells (36%). The label-free nature of TEER readings enable daily measurement. Time course data showed low tissue-to-tissue variance as well as excellent day-to-day stability for at least 21 days. Follow-on experiments confirmed the ability to assess the kinetics of on-set and recovery. These data indicate that human 3D GI microtissue delivers excellent predictivity for high vs. low incidence clinical diarrhea and should provide a platform useful for lead optimization and candidate drug dose schedule exploration.

**2358 Gut-on-a-Chip: Toward a More Physiological and Predictive Human Intestinal Barrier Model**


A majority of the screening and predictive models do not reflect properly the physiology of the human intestinal tract and predictions remain unreliable by lacking the in vivo morphology and physiological circumstances. The often used Caco-2 cell models are not well suited to investigate the different processes involved in general gut health or toxicity aspects. Therefore, realistic models resembling the human in vivo situation are needed, for instance ex vivo models that successfully applied human intestinal tissue into the InTESTine™ two-compartmental disposable device. This set-up is suitable for standard 6- or 24-well plate format and allows the study of (regional) drug absorption, intestinal wall metabolism, mucus interactions and pathological and physiological effects. With this device we have extended our InTESTine platform by successfully using human intestinal tissue for repeated exposure protocol. This model has been applied as a reliable tool for the assessment of processes that determine human intestinal permeability, (long-term) toxicity and pathological effects.

**2359 Multicellular Human Bronchial Models Exposed to Diesel Exhaust Particles Induce Inflammation, Oxidative Stress, and Macrophage Polarization**

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Human airway epithelium forms the first line of defense in lung, and is a primary target of diesel exhaust particles (DEPs). Macrophages, with phagocytosis as main feature and inflammation as well as immunity as secondary features, have the ability to remove DEPs from the lung. We have reported that both bronchial epithelial cells and macrophages are equipped with Toll-like receptors (TLRs). In this study, we exposed a multicellular model consisting of primary bronchial epithelial cells (PBEC) and macrophages (MQ) to a diesel exhaust particle (DEP) during 6 days and measured changes in cell viability, cytokine release, and mRNA expression. PBEC cultured at ALI were co-cultured with MQ (PBEC-ALI/MQ), DEPs and MQ were separated by a liquid interface (ALI) with DEPs. We aim to identify the effects of DEPs in macro- or multicellular airway models. PBEC cultured at ALI were co-cultured without and with MQ (PBEC-ALI/MQ, respectively) and exposed to 63.5µg/cm² DEPs aerosols via Xpose™. Control exposures to clean air were performed. After exposure, the models were incubated for 24h. The secretion of CXCL8 and IL-6 in basal medium were measured by ELISA. The mRNA levels of CXCL8, CXCL12 to CXCL19, NFKB, TNFα, IL-10, IL-13, IL-4, and IL-13, MRC1, MRC2, and RETNLA were analyzed by qRT-PCR. The surface expression of CD14/CD204 were detected by FACS. Cell viability and apoptosis rate were analyzed with LDH-assay and FACS. In PBEC-ALI, the secreted level of CXCL8, CXCL12, expression of inflammatory markers (CXCL8, TNFα), oxidative stress markers (NFKB, HO1, GPx) were significantly induced by DEPs exposure. However, after DEPs exposure, mRNA expression of these markers (CXCL8, IL6, NFKB, HO1) were reduced in PBEC-ALI/MQ compared to PBEC-ALI. After sham exposure, the surface expression of CD14 and CD204 on PBEC in PBEC-ALI/MQ was significantly attenuated compared to PBEC-ALI. Exposure to DEPs increased surface expression of CD14 and CD204 on PBEC in PBEC-ALI/MQ. DEPs exposure resulted in similar expression pattern of CD14 and CD204 on PBEC-ALI/MQ. In PBEC-ALI/MQ, DEPs exposure increased M2 macrophage markers (IL10, IL4, IL13, MRC1, MRC2) transcription. DEPs induced an inflammatory and...
oxidative stress response in PBEC-ALI models which was attenuated in the presentence of MQ. In both PBEC and MQ, DEP's exposure increased TLR2 but decreased TLR4 surface expression. The combination of DEPs exposure and co-culture with PBEC drove the polarization of MQ to M2 phenotype.

2360 Metabolomics Approach for Toxicity Screening of Volatile Substances
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In 2007 the National Research Council envisioned the need for inexpensive, high throughput, cell-based toxicity testing methods relevant to human health. While there has been throughput in vitro screening approaches, these have largely addressed these problems by using robotics. However, the challenge is that many of these chemicals are volatile and not amenable to robotic liquid handling applications. We assembled an in vitro cell culture apparatus capable of screening volatile chemicals for toxicity with potential for miniaturization for higher throughput. BEAS-2B lung cells were grown in an enclosed culture apparatus under air-liquid interface (ALI) conditions, and exposed to a small subset of xenobiotics in 5% CO2. ALI conditions allows direct contact of cells with a gas xenobiotic, as well as release of endogenously-produced gaseous molecules without interference by medium on the apical surface. To identify potential xenobiotic-induced perturbations in cell homeostasis, we monitored for alterations of endogenously-produced gaseous molecules in air directly above the cells, termed “headspace”. Alterations in specific endogenously-produced gaseous molecules in headspace is indicative of xenobiotic-induced perturbations of specific cellular processes. Furthermore, endogenously-generated volatile organic compounds (VOCs) may be monitored in a nonspecific, discovery manner to determine whether cell processes are perturbed by xenobiotic exposure, potentially producing specific patterns of changes. Preliminary results indicate the novel cell culture system is capable of detecting gaseous products of specific enzyme pathways such as CYP2A6. Additionally, numerous VOCs were detected in the headspace of cells grown in the novel cell culture apparatus. We believe our novel cell culture apparatus, once refined and validated, will allow for screening of both volatile and non-volatile xenobiocs by measuring cellular responses detected as alterations in gaseous molecules released by cells. This abstract may not reflect official USEPA policy.

2361 Cell Line-Based In Vitro Models of Normal and Chronic Bronchitis-Like Airway Mucosa to Study Toxic Potential of Aerosolized Palladium Nanoparticles
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Physiologically-relevant cell line-based models of human airway mucosa are needed for assessment of nanoparticle-mediated pulmonary toxicity. We have developed a platform that can be used to test aerosol (PN) doses emitted from the catalytic converters in vehicles. We aimed at developing in vitro airway models to assess the toxic potential of PN in normal and chronic bronchitis-like mucosa. Bronchial mucosa models cultured at air-liquid interface were developed using epithelial cells (16HBE) and fibroblasts (MRC-5). Further both normal and chronic bronchitis-like models (IL-13 treatment) with increased number of goblet cells were used. Using XposeALI™ the models were exposed to three different doses of aerosolized PN ranging between 3.2 and 5.0 μg/cm² with clean air as control. After 24 hours incubation the expression of inflammatory (IL-6, CXCL8, TNFa, NFkB), oxidative stress (HO1, SOD3, GPx, GSTA1), and tissue injury/repair (MMP9/TIMP1) markers were assessed using qRT-PCR. Secretion of CXCL-8 and MMP-9 were measured with ELISA. P<0.05 was considered as significant. Significant (P<0.05) increased expression of CXCL8, TNFa, IL6, and NFkB were observed at highest dose of PN in chronic bronchitis-like models, whereas in normal mucosa models, a maximum effect on CXCL8, TNFa, and NFkB expression were observed at medium PN dose. In normal mucosa models, SOD3 showed clear dose response of PN, while GSTA1 and GPx expression were significantly increased (P<0.05) only at lowest dose of PN. HO1 decreased significantly (P<0.05) after exposure to the two highest doses of PN in normal mucosa models. Secretion of CXCL-8 increased dose-dependently in dose-response curves following exposure to PN. Dose-response curves followed exposure to PN. Our normal and chronic bronchitis-like mucosa models combined with exposure to aerosolized PN using XposeALI™ exposure system closely mimic cell-particle interactions observed under in vivo physiological condition in the lung. Further, the observed results (inflammatory and oxidative stress response) in both normal and chronic bronchitis-like mucosa models implicate strongly a relevance of these in vitro findings for human health risk assessment.

2362 Applying Concepts from Adverse Outcome Pathways to Assessment of Airway Irritants
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Airway irritant exposures in the workplace cause rhinorrhea, cough, and bronchospasms, and are associated with intrinsic asthma, non-al-lergic rhinitis, and related chronic inflammatory conditions. Our objective is to apply the in vitro approaches to assess volatile organic chemicals (VOCs) for their potency to cause adverse outcomes. To this end, we have conducted a study to assess the induction of airway irritation from VOCs. The VOCs were identified through a literature search for chemicals that are able to produce airway irritation. The effects were measured in vitro using the rat isolated perfused lung (IPL) model and in vivo using human lung epithelial cells under air-liquid-conditions with the P.R.I.T.-ALL Technology. The objective was to identify VOCs that can mediate airway irritation and to develop a cost-effective screening system to identify chemicals likely to be airway irritants. We hypothesize that this can be done using in vitro measurement of molecular initiating events (MIEs) upstream of sensory irritation responses, similar in principle to the use of adverse outcome pathways. We reviewed literature relevant to sensory irritation responses and used a modified adverse outcome pathway method to evaluate a key event framework beginning with MIEs. Our assessment identified (1) activation of transient receptor potential and acid-sensing ion channels, (2) lipid peroxidation, and (3) pro-inflammatory changes as MIES upstream of sensory irritation. Downstream events include depolarization of nociceptive neurons and epithelial inflammation, both of which are events mediating airway irritation responses. Based on this framework, we have identified potential endpoints that can be evaluated in cultured human airway cells. A predictive assay suite based on multiple increased dose-distributions would allow for the identification of chemicals of low concern for which derivation of OELs from oral toxicity data may be allowable, and may ultimately lead to methods to reduce animal testing for sensory irritants. This would improve the efficiency of risk assessments for these chemicals of low concern (and perhaps ultimately other chemicals) without compromising health protection.

2363 Acute Inhalation Toxicity In Vitro and Ex Vivo Test Battery Prior to Regulatory OECD 403 Studies
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New crop protection agents are usually tested for acute inhalation toxicity in vitro according to OECD Test Guideline 403. In order to reduce the amount of animals and test compound needed, this project aimed at the implementation of ex vivo and in vitro screening tests that can be used to estimate the acute inhalation toxicity prior to regulatory OECD 403 studies. Therefore, two reference substances (mancozeb and deet) were selected for the identification of chemicals of low concern. A combination of four test substances resulted in dose-dependent alterations of inflammatory and oxidative stress response and establishment of dose response curves. In both assays mancozeb showed a significantly lower toxic potential than chlorothalonil and thus confirms the findings of the in vivo studies. In the next step both assays will be used to predict the acute toxic potential of further substances with known and unknown toxic potential to broaden the data base and verify the reliability of this test battery.
Exposure, in occupational scenarios or at consumer levels, to chemicals such as acid anhydrides or fragrances can lead to the development of respiratory allergies. At present, no in vivo method is available for the prediction of respiratory sensitization potential of chemicals. To mimic the alveolar-capillary barrier we developed a 3D in vitro model grown at the air-liquid interface including alveolar type II epithelial cells (AS49), endothelial cells (EA.hy926), macrophage-like cells (PMA differentiated THP-1), and dendritic-like cells (DC) (non-differentiated THP-1). The model was exposed to the ALI to trimellitic anhydride (TMA) and phthalic anhydrid (PA), which are chemical respiratory sensitizers, to acrolein (Acr - respiratory irritant) and to house dust mite (HDM - protein allergen). The model was exposed to concentrations leading to a reduction of 25% cell viability or, whenever no cell viability reduction was observable, to the highest possible concentration. Several parameters were evaluated after exposure of the model to the different compounds, including surface markers of activation for DC (e.g., CD54, OX40L, TSLPR, etc.) and cytokines (e.g., MCP-1, IL-10, RANTES, etc.). In addition, a panel of genes related to respiratory inflammation and DC activation was analyzed by qRT-PCR. Exposure to PA and TMA led to the upregulation of CD54 and TSLPR on DC cells as compared to Acr or vehicle controls. HDM induced upregulation of the surface marker OX40L, which was unchanged after treatment with respiratory chemical sensitizers, while it resulted in downregulation with respiratory irritants. Taken together, surface markers for DC activation, gene expression, and cytokines constitute a panel of endpoints that allows prediction of respiratory sensitization potential. In conclusion, the presented in vitro model represents a useful tool for the prediction, in vitro, of respiratory sensitization, allowing discrimination between respiratory sensitizers, either chemicals or protein sensitizers, and respiratory irritants.

When combining multiphase aerosol (particulate and vapor) delivery and exposure with in vitro assays, knowledge of the delivered dose and its time course is critical to interpreting and extrapolating results. In this study the particle dosimetry of aerosol exposure of cells was measured using monodisperse fluorescent particles (0.5, 1.1, 2.1, & 3.2 µm in diameter) in each of the 48 wells of the Vitrocell AMES 48 exposure system (in vitro exposure system). Three different flowrates at each of the 48 wells were evaluated (5, 10, and 20 cc/min) in three replicate experiments. Fluorescent particle distribution across each well was photographed using fluorescent microscopy and counted using image analysis software. Particle losses throughout the in vitro exposure system were quantified by repeatedly washing each part with aqueous surfactant and counting the collected fluorescent particles using either flow-cytometry or a hemacytometer with fluorescent microscopy. Results indicate that overall deposition efficiency is below 1% for each well regardless of flowrate for each particle diameter tested. For each particle diameter there were characteristic deposition patterns across the wells. Depending on the particle diameter, different distances from the perimeter were completely particle free. These particle free areas should be considered when conducting in vitro studies in the in vitro exposure system to ensure that cellular responses are integrated only from cells that are directly exposed to particles. For multiphase aerosols, such as e-vapor aerosols, combination of our particle dosimetry results with predictions of vapor deposition using computational fluid dynamic techniques would provide a more complete picture of cellular exposure that occurs in the in vitro exposure system.
The current assumption for assessing carcinogenic risk of polycyclic aromatic hydrocarbons (PAHs) is that they function through a common mechanism of action; however, recent studies demonstrate that PAHs can act through unique mechanisms potentially contributing to cancer outcomes in a non-additive manner. In this study, we assessed potential differences in mechanism of action for PAHs in a primary human 3D bronchial epithelial culture (HBEC) model based on short-term bio-signatures that could be extrapolated from global transcriptional profiles. PAHs were divided into the groups of differentially expressed genes between PAHs and DMSO controls. Analysis of global gene expression and significantly enriched pathways between the AIR mixture, DBC, and BAP, while BAA, CTE, and BAA enriched a subset of unique pathways. Principal components analysis of global gene expression and significantly enriched pathways found groupings of lower potency PAHs (BAA, CTE, CTE, lower dose BAP) and grouping of BAP, DBC, and AM. Pathways were filtered to those significantly enriched with carcinogenic PAHs to classify the carcinogenic potential of PAHs and mixtures. These data suggest that bioactivity signatures from 3D HBEC can be used to identify mechanisms linked to carcinogenic risk of PAHs in humans. By profiling the short-term transcriptomic signatures of PAHs and mixtures of known components, we aim to develop a predictive model to assess carcinogenic potential of PAHs and PAH mixtures.

Cigarette smoking is a complex mixture of over 4,000 chemicals that is comprised of particulate and gaseous phases. The use of dosimetry (mass) plays a key role in understanding the exposure of cigarette smoke within in vitro systems. The goal of this study was to evaluate the utility of multiple dosimetry techniques in determining the amount of whole cigarette smoke delivered by a VITROCELL® VCT® smoking robot into two different exposure modules of the VITROCELL® system (4- and 12-well (12 mm)). Eight University of Kentucky 3R4F cigarettes were smoked under International Organization for Standardization (ISO), or Health Canada Intense (HCI) regimes, and assessed over a range of diluting airflows. Dosimetric tools utilized during exposure experiments were quartz crystal microbalances (QCM), laser photometers (area under the curve (AUC)), and fluorescence measurements (Ex/Em 355/485) of smoke particulate matter captured in DMSO. For each experiment, the first well contained a QCM and the second and third wells contained a set volume of DMSO. An in-line laser photometer was positioned between the smoke dilution system and the first well of the exposure module. QCMs were equilibrated to room temperature in the module until a stable baseline was achieved and the photometers were harmonized to each other prior to the study. Concentration of the captured particulate matter was determined by a standard curve generated for each module and type of smoke. Additionally, three endpoints (QCM - gravimetric mass, photometric - AUC, and laser photometer) can be used as tools to measure whole smoke dosimetry in real time and post experimental analysis.
The Nrf2 transcription factor controls expression of enzymes involved in defense against electrophilic and oxidative damage. In the current work, an organotypic model of human airway epithelium containing a luciferase reporter for Nrf2 activation was developed. The reporter model was characterized with 12 reference chemicals, whole tobacco smoke (WTS) and electronic cigarette (e cig) vapor. Primary human tracheobronchial epithelial cells (NHBE) from 2 donors were transduced with a lentiviral Nrf2 luciferase reporter. Stably transduced cells were expanded and cryopreserved to produce large pools of reporter-expressing cells which were then utilized to produce differentiated organotypic airway epithelial models. Organotypic structure and barrier properties of reporter models were found to be similar to untransduced models, as determined by histological evaluation and barrier assessment. The airway reporter models were exposed to test chemicals by apical application of solutions containing up to 0.6% DMSO. A smoking machine exposed the models to WTS or e cig vapor. Luciferase activity was evaluated using a commercial kit and a microplate luminometer. Toxicity was evaluated by LDH release. Dose response experiments were performed to determine a range that spanned non-toxic to moderately toxic concentrations. Test chemicals included: isothiocyanates (sulforaphane), oxidants (H2O2, menadione) and electrophilic chemicals (acrolein, iodoacetamide, nitrobenzyl bromide, cinnamaldehyde, dihydrochalcone, 2-butylnitrosoquinalone), parthenolide, quercetin, and cyclophosphamide (precursor of menadione) and electrophilic chemicals (acrolein, iodoacetamide, nitrobenzyl bromide, cinnamaldehyde, dihydrochalcone, 2-butylnitrosoquinalone). Only slight Nrf2 activity was observed following treatment with sulforaphane, a reversible thiol binding chemical. H2O2 and menadione produced only weak activity across the entire span of doses. However, strongly electrophilic chemicals (acrolein, iodoacetamide, nitrobenzyl bromide, cinnamaldehyde, dihydrochalcone, 2-butylnitrosoquinalone) elicited strong induction of Nrf2. WTS also induced strong Nrf2 activation. E cig vapor produced no to weak activity. The results demonstrate that the Nrf2 airway reporter model is a highly sensitive detector of reactive electrophilic chemicals or mixtures including WTS. The model may prove useful for safety evaluation of new generation nicotine delivery products.
isotopic labeling strategy by incorporating cells with a sterol upstream of DHCR7 metabolism, C27-Lanosterol, into the cholesterol biosynthetic pathway. It was determined by calculating the ratio of C27-DHCR7 to C27-CHOL that control cells exposed to HPD had significantly decreased DHCR7 activity, while having no effect in HD cells. These findings suggest that HD neuroprogenitors are unaffected to chemical inhibition of DHCR7. Supported by NIEHS T32 ES000267 (PW, ABB), ES016931 (ABB), and ES024133 (NP).

### 2376 Carbon Monoxide (CO) Poisoning in Cats

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Carbon monoxide is a colorless, odorless gas, the inhalation of which can be fatal. There is only one report on CO poisoning in cats in the literature. Two adult Singapurra brown ticked cats were submitted to the San Bernardino branch of CAHFS for necropsy. These animals had been found dead on 13th. The presentation along with their two decades owned. At necropsy, gross lesions were similar in both cats and consisted of multifocally large and irregular, bright red spots on the skin of the abdomen and the inner surface of ear pinna, bright red muscles and blood. The carcasses, and tissues fixed in formalin retained the bright red discoloration for up to two weeks. Microscopic lesions were also similar in both cats and included diffuse pulmonary congestion and edema, and multifocal intense basophilia of cardiomyocytes. The latter was seen mostly affecting whole fibers but it was occasionally affecting only a portion of the fiber, with a clear transverse line of demarcation from the red to the pale color. Rarely, discrete areas of hypercontraction bands were seen in individual cardiomyocytes. Based on the clinical history, gross and microscopic changes, cyanide or carbon monoxide poisoning was suspected, and frozen muscle and blood from the two animals were submitted for toxicologic analysis. The muscle samples were homogenized by DLC (PCB 126, the non-DLC PCB 153, and a mixture of PCB 126 and 153, which produced cancer and other non-neoplastic lesions of the liver and lung. In contrast, a general reduction in TL was observed with PCB 153 (non-DLC) treated rats that exhibited minimal toxicity. Telomere shortening may be an early indicator of carcinogenicity that occurs following two years of exposure to DLCs. Supported in part by the intramural research program of NCI/NIH.

### 2377 Telomeres as a Potential Target for the Chronic Toxicity of Polychlorinated Biphenyls (PCBs)

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Telomeres are DNA-protein complexes found at the ends of chromosomes that help protect the genome from degradation and interchromosomal fusion. Telomere length (TL) can be affected by various factors, including age and increased oxidative stress, and has been associated with higher risks for several types of cancer. The NTP conducted two year studies exposing animals to various doses of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), the dioxin-like compound (DLC) PCB 126, the non-DLC PCB 153, and a mixture of PCB 126 and PCB 153 (https://ntp.niehs.nih.gov/results/pubs/longterm/reports/longterm/index.html). Relative telomere length (RTL) is a biomarker that may be associated with neoplastic and/or non-neoplastic responses observed with chronic exposures to PCBs. DNA was isolated from liver and lung tissue obtained from the NTP studies and RTL was assessed by quantitative PCR. Relative to time-matched vehicle controls, increases in liver TL between 3% and 10% were seen in rats given various doses of TCDD for 13 weeks, while TL increased by similar ratios in rats dosed for 52 weeks. Similarly, the RTL increased between 7% and 13% in the liver of rats receiving PCB 126 for 13 weeks and increased 17% to 26% following 52 weeks of exposure. Rats dosed with the mixture of PCB 126 and PCB 153 saw increases in RTL in both the liver and lung tissues. Following 13 weeks of exposure to the mixture of PCB 126 and 153, TL increased 9% to 11% in the liver and 12% to 16% in the lung. After 52 weeks of exposure to the mixture, liver TL increased 13% to 34%, while lung TL increased 6% to 28%. In contrast, RTL decreased from 5% to 9% in the liver of rats receiving PCB 153 alone for 13 weeks. The association between RTL and test compound is congruent-specific and associated with the varying toxicological activity of PCB congeners. An increase in RTL was observed in rats treated with TCDD, PCB 126 and the mixture of PCB 126 and 153, which produced cancer and other non-neoplastic lesions of the liver and lung. In contrast, a general reduction in TL was observed with PCB 153 (non-DLC) treated rats that exhibited minimal toxicity. Telomere shortening may be an early indicator of carcinogenicity that occurs following two years of exposure to DLCs. Supported in part by the intramural research program of NCI/NIH.

### 2378 Impaired Learning and Memory in Rats after Subchronic Inhalation Exposure to an Indoor School Air Mixture of PCBs

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Polychlorinated biphenyls (PCBs) are persistent, bioaccumulative, toxic pollutants, which are still present in the ambient environment and indoor buildings despite their production being ceased since the 1970s. Exposure to PCBs, especially during fetal and childhood developmental period, can affect IQ, learning and memory abilities. In this study we exposed female Sprague-Dawley rats to a PCB School air mixture of East City indoor school air in order to evaluate the effects of PCBs on learning and memory. Female Sprague-Dawley rats were exposed simultaneously to either SAM vapor (n = 8) or clean air (n = 8) through a nose-only exposure system. The exposure was 4 h/day, 7 days/week for 4 weeks. Positive control rats (n = 6) for assessing neurotoxic behavior were treated with 1-bromopropane (1-BP, 800 mg/kg bw, by gavage) for 12 days. Sentinel rats (n = 2) were kept in vivarium for health surveillance. Morris Water Maze (MWM) was performed for 6 days to evaluate the learning and memory abilities. Oxidative stress in super-natant of brain homogenates was assessed by determination of reactive oxygen and nitrogen species, detection of 4-hydroxynonenal (HNE) protein adducts and formation of malondialdehyde (MDA). Significantly increased escape latency was found in 1-BP group at 4th and 5th day, compared with control group (p < 0.01, p < 0.01 respectively). In contrast, no significant difference was found between negative for SAM group and control group, but from the learning efficiency, SAM group was slightly lower than control. Additionally, significant decreases of time spent in target quadrant and number of crossing platform were observed in 1-BP group, compared with control group (p < 0.01, p < 0.01, respectively); similar decrease of number of crossing platform was found in SAM exposure group (p = 0.01). None of the oxidative stress tests showed a statisti cal significance when SAM group was compared with controls. Results suggest the impairment of memory and learning after SAM exposure might not result from oxidative stress. Further investigation is needed to uncover the mechanism of the learning and memory deficit caused by PCBs.

### 2379 Low-Dose Perfluorooctane Sulfonate (PFOS) Is Associated with Induction of Fatty Acid Uptake Mechanisms in Diet-Induced Non-Alcoholic Fatty Liver Disease (NAFLD)

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Obesity, diabetes, and insulin resistance are all risk factors associated with the development of hepatic steatosis and non-alcoholic fatty liver disease (NAFLD). It is estimated that 20-30% of the population present with NAFLD in the United States alone. The contribution of environmental exposures as risk factors for fatty liver disease has been largely overlooked. Perfluorooctane sulfonate (PFOS) is a widespread environmental toxicant that persists in over 98% of the general population. The aim of this study was to evaluate whether PFOS exposure in combination with a moderately high-fat diet, augmented hepatic lipid content and biomarkers associated with NAFLD. 6-week-old male C57BL/6 mice were co-fed either a 10% kCal low fat diet (LFD) or 45% kcal high fat diet (HFD), with or without 0.0003% PFOS (LFD-PFOS and HFD-PFOS, respectively) for 12 weeks. The HFD increased liver weight by about 30% and body weight by 50% compared to the LFD controls. HFD-PFOS administration increased liver weight by 50% and body weight by 40% when compared to the LFD group. LFD and LFD-PFOS groups had similar body weights and liver weights, with LFD-PFOS trending toward increased liver weight (p < 0.06). The LFD-PFOS and HFD-PFOS groups had higher liver lipid accumulation scores when compared to the LFD controls. Triglycerides increased 50% with HFD and 25% with HFD-PFOS compared to LFD alone. LFD-PFOS decreased liver triglycerides by 35%. Both HFD-PFOS and LFD-PFOS administration trended toward decreased non-esterified free fatty acids (NEFA) compared to the LFD controls, whereas HFD increased NEFA by 35%. A targeted liver gene expression array was conducted to assess the potential mechanism of PFOS induced steatosis within a LFD and HFD diet. Fatty acid uptake
genes, fatty acid translocase (Fat/Ca36) and long-chain fatty acid transport protein 1 (SLC27A1), were significantly induced by PFOS. PFOS treatment also resulted in significantly increased expression of glutathione S-transferase mu 3 (GmSU3) and sterol regulatory element-binding transcription factor 1 (Srebf1). The data suggests that PFOS exposure at an environmentally relevant dose (0.0003%) may have an adverse effect on hepatic lipid accumulation when combined with a LFD. The effect of diet on the distribution and mechanism of PFOS is described herein.

2380 Rat PCB Inhalation Study: Do PCBs Resembling a School Indoor Air Mixture Induce Hematotoxicity in Bone Marrow Cells?

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Polychlorinated biphenyls (PCBs) are ubiquitous in our environment and it is estimated that about 1/3rd of all school buildings in the US have detectable levels of PCBs in indoor air. PCBs are immunotoxic and linked to childhood leukemia, Non-Hodgkin’s lymphoma and hematotoxicity. We previously reported that several PCB congeners and a mixture reduce telomerase activity in cells in culture. We then discovered that a single ip injection of PCB126 significantly reduced telomerase activity and down-regulated TERT gene expression and protein in male Sprague-Dawley rats by 48h/day, 7 days/week by nose-only inhalation to Aroclor 1254 vapor. Subsequent cultivation of these stem cells in soft agar showed an increase in Colony Forming Units (CFU) and a shift of hematopoietic stem/progenitor cells (HSC) differentiation toward the granulocytic direction compared to the untreated control. Our hypothesis is that inhaling a PCB mixture can affect telomerase activity and thereby interfere with proliferation and differentiating of hematopoietic stem/progenitor cells. We exposed female SD rats 4h/day, 7 days/week by nose-only inhalation to Aroclor 1254 vapor which closely resembles a school air mixture from a Chicago school. Then whole blood was drawn and analyzed for GSH/GSSG and Cys/CySS redox homeostasis and DNA damage in form of micronuclei per 1000 immature erythrocytes. Bone marrow nucleated cells were isolated to determine the number of viable and functional progenitor cells, telomerase activity, and TERT expression. No significant differences were seen in the GSH/GSSG, Cys/CySS and micronuclei analysis. However, the PCB inhalation group showed significantly decreased TERT expression and telomerase activity. Also, bone marrow HSC differentiation into the granulocytic and macrophage direction was promoted, as indicated by increased number of CFU-G (granulocytes) and CFU-M (macrophages) colonies. These results suggest that inhalation of PCBs may exhibit hematotoxicity by negatively affecting the immortality activity of telomerase in HSCs and through modification of their differentiation pattern towards the myelocytic pathway. The role of these mechanisms in PCB immunotoxicity needs further investigation. Supported by NIH E01 ES013661.

2381 Characterization of an Immortalized Mouse Pancreatic Stellate Cell Line: Effects of PFOA Exposure

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Pancreatic cancer is the fourth leading cause of cancer death in the United States whose etiology remains largely unknown. PFOA is a chemical widely used in consumer and industrial applications that has been shown to induce pancreatic acinar cell tumors in rodents through a yet to be determined mechanism. Pancreatic cancer is characterized by a dense desmoplastic/stromal reaction, which has been attributed to activation of pancreatic stellate cells (PSCs). PSCs are located in the periacinar spaces and become activated in response to pancreatic injury and inflammation. Once activated, PSCs secrete cytokines and excess amounts of extracellular matrix proteins which leads to fibrosis. We have previously shown that the pancreas is targeted in vivo following PFOA exposure, resulting in the expansion of early neoplastic lesions (pancreatic intraepithelial neoplasia; PanIN), concomitant with the induction of oxidative stress. We hypothesize that PFOA-induced oxidative stress leads to activation of stellate cells which in turn leads to expansion of PanIN and promotion of pancreatic cancer. To determine the effects of PFOA exposure on stellate cell activation, we have generated conditionally immortalized mouse pancreatic stellate cell lines derived from the Immortomouse. The Immortomouse harbors a thermolabile mutant of the SV40 large T antigen under the control of the ubiquitous interferon-γ-inducible promoter (H-2Kb-tsAS8) which is rapidly degraded when cells are switched from permissive (33°C; presence of IFNγ) to nonpermissive conditions (37°C; absence of IFNγ). We have successfully established two immortal pancreatic stellate cell lines (ImSt1 and ImSt2) isolated under permissive culture conditions at 33°C in the presence of IFNγ. Nonpermissive culture conditions led to increased expression of α-smooth muscle actin (αSMA) and perisin, both markers of activated PSCs. In addition, exposure of ImSt1 and ImSt2 to PFOA led to increased mRNA production of cytokines which increase extracellular matrix protein production, such as CTGF and TGFβ1, while conditioned media from PFOA-treated ImSt cells displayed increased levels of TNFα, DLK-1, and MMP3, suggesting that cytokines derived from stellate cells may influence pancreatic cancer progression. Collectively, these results demonstrate that ImSt cells provide a useful model system to assess the effects of environmental chemicals on pancreatic injury and disease.
The Pacific Decadal Oscillation and Contaminant Concentrations in Eggs of Thick-Billed Murres (Uria alioana) and Common Murres (Uria aalge) from the Gulf of Alaska and Bering Sea between 1999 and 2010

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Persistent organic pollutants (POPs) are ubiquitous in the Arctic food web. The Seabird Tissue Archival and Monitoring Project (STAMP) is an ongoing, long-term effort to track trends in environmental quality along the coast of Alaska. It is a collaboration between the U.S. Fish and Wildlife Service Alaska Maritime National Wildlife Refuge (USFWS-AMWR) and the National Institute of Standards and Technology (NIST). Historical records of sea surface temperature in the Pacific Northeast show that temperature appears to oscillate between cold and warm phases. This pattern has been called the Pacific Decadal Oscillation (PDO). Using contaminant measurements made by NIST and PDO measurements made by the University of Washington, we assessed the relationship between chemical concentration in seabird eggs and average PDO, measured from February-May (egg-laying season). Chemicals were log-transformed for statistical analyses. The association between eggs of thick-billed murres and each of 34 POPs congeners was estimated using linear spline regression to accommodate a non-linear relationship, while that between eggs of common murres and POPs was estimated using linear regression, both adjusted for year of measurement. Models used cluster-robust standard errors to account for clustering by seabird colony. In the negative PDO range, the geometric mean ratio of oxychlordane in thick-billed murre eggs was 2.83 per unit increase in PDO (95% CI: 1.98 to 4.02), while in positive values of PDO, the geometric mean ratio was 0.81 (95% CI: 0.39 to 1.65) per unit increase in PDO. In the same species, the ratio of geometric mean of 4,4-DDD and 2,4-DDT (measured together) was 0.29 (95% CI: 0.08 to 1.11) per unit increase in PDO in the negative range, while in the positive range, it was 0.20 (95% CI: 0.06 to 0.73) per unit increase in PDO. Among common murre eggs, the geometric mean ratio for oxychlordane was 1.40 (95% CI: 0.66 to 2.99) per unit increase in PDO. These results suggest a relationship between sea surface temperature changes and the amount of POPs in the Alaskan food web, which has not yet been fully explored.

Quantitative Analysis of Urinary Organophosphate Insecticide Metabolites in Diapered Children in Japan

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Recently, epidemiological studies to examine the relationship between pesticide exposure and neurodevelopmental effects have drawn more attention. Although central nervous system rapidly develops in early childhood, information about pesticide exposure of children who cannot control urination is limited. In this study, we aimed to clarify the exposure levels of organophosphorous pesticide (OP) in urine extracted from disposable diapers. We recruited diapered children participating in Japan Environment and Children’s Study (JECS) at the Aichi Regional Center of JECS. JECS used disposable diapers from 1,073 children (16-23 months old, 548 males and 529 females) from June in 2015 to August in 2016 (Consent rate was 86%). Urine samples were extracted from the diapers. Six urinary dialkylphosphates (DAPs) were analyzed by ultra-performance liquid chromatography with tandem mass spectrometry. This pattern has been called the Pacific Decadal Oscillation (PDO). Using contaminant measurements made by NIST and PDO measurements made by the University of Washington, we assessed the relationship between chemical concentration in seabird eggs and average PDO, measured from February-May (egg-laying season). Chemicals were log-transformed for statistical analyses. The association between eggs of thick-billed murres and each of 34 POPs congeners was estimated using linear spline regression to accommodate a non-linear relationship, while that between eggs of common murres and POPs was estimated using linear regression, both adjusted for year of measurement. Models used cluster-robust standard errors to account for clustering by seabird colony. In the negative PDO range, the geometric mean ratio of oxychlordane in thick-billed murre eggs was 2.83 per unit increase in PDO (95% CI: 1.98 to 4.02), while in positive values of PDO, the geometric mean ratio was 0.81 (95% CI: 0.39 to 1.65) per unit increase in PDO. In the same species, the ratio of geometric mean of 4,4-DDD and 2,4-DDT (measured together) was 0.29 (95% CI: 0.08 to 1.11) per unit increase in PDO in the negative range, while in the positive range, it was 0.20 (95% CI: 0.06 to 0.73) per unit increase in PDO. Among common murre eggs, the geometric mean ratio for oxychlordane was 1.40 (95% CI: 0.66 to 2.99) per unit increase in PDO. These results suggest a relationship between sea surface temperature changes and the amount of POPs in the Alaskan food web, which has not yet been fully explored.

Aflatoxin Exposure in Children of Eastern Province, Kenya

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Aflatoxins (AFs) are fungal metabolites that commonly contaminate food grains such as peanuts and corn. Contamination of corn by AFs is a recognized public health problem in Kenya, which has resulted in over 600 human deaths. In the rural Kenya, like many other developing nations, most children are weaned on corn or peanut related meals. Dietary exposure to AFs can occur in utero, via maternal milk, through weaning foods and throughout an individual’s lifetime. Consistent dietary exposure to AFs has been implicated to be a contributing factor to micronutrient deficiency, immune-suppression and growth impairment in children. In this cross-sectional study, 433 children aged 1-14 were recruited from the Makuani County, in Eastern Province, Kenya and their serum samples were collected and analyzed to determine the extent of AFs exposure. Aflatoxin B1-lysine (AFB1-lysine) adducts being a reliable biomarker for AFs exposure was measured using High Performance Liquid Chromatography with Fluorescence detection. 100% of the samples had detectable levels of AFB1-lysine adducts (>0.4 pg/mg albumin). The AFB1-lysine adduct level was adjusted for albumin concentration before analysis using SAS v.9.4 (Cary, NC). The minimum, maximum and geometric mean (95% confidence interval) of AFB1-lysine adduct levels were determined as 0.74, 901.15 and 20.40 (18.22-22.85) ng/mg albumin respectively. About 11.32% (43/384) samples had AFB1-lysine adduct levels less than 5.0 pg/mg albumin; 19.71% (73/384) serum samples had AFB1-lysine levels ranging from 5.0-9.9 pg/mg albumin; 19.63% (75/384) had 10.0-19.9 pg/mg albumin; 28.41% (109/384) had ≥ 20 pg/mg albumin. Serum samples with over 50 pg of AFB1-lysine adducts per mg albumin. The AFB1-lysine adducts levels were among the highest found in children populations in the literature, which suggest that prevention and intervention strategies for reducing AFs exposure need to be immediately taken in this high risk area, especially in susceptible children populations.
2388 Impacts of Welding Parameters on Workers’ Exposure to Mn Fumes

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Welders’ exposure to fumes has been associated with several types of cancer, as well as respiratory tract and nervous system health effects. Among the numerous metals found in welding fumes, manganese (Mn) remains one of the main contaminants. Chronic exposure to Mn may lead to neurological injuries, such as parkinsonism. Reducing exposure to Mn fumes is therefore crucial to protect workers involved in welding activities. Innovative approaches now tend to focus on the adjustment of welding parameters, in addition to source capture, general ventilation and respiratory personal protective equipment. The aim of this study was to assess the impact of adjustments of certain welding parameters on workers’ exposure to Mn fumes. The assessments were conducted in two welding facilities in Québec that were sampled twice, once before and once after modifying current intensity (using electrodes with a smaller diameter, specifically 1.3 mm instead of 1.6 mm, allowing the reduction of the current intensity) and characteristics of the electrodes (0.202% Mn content instead of 1.62%). Mn fumes were sampled from the personal breathing zones of six workers in the first facility and of two workers in the second. Personal breathing zone samples for workers using Flux Cored Arc Welding (FCAW) to perform the same welding tasks were collected in both facilities, before and after changing the welding parameters. The first results showed mean levels of Mn of 0.41 mg/m³ when the highest current intensity was used and 0.61 mg/m³ when the highest electrode Mn content was used. These levels were respectively two and three times higher than the Québec Occupational Exposure Limit (OEL) of 0.2 mg/m³. The second results showed a significant decrease in workers’ exposure to Mn fumes, with a resulting mean level of 0.25 mg/m³ (1.25 times the OEL) when the lowest current intensity or the lowest electrode Mn content was used. Investigations are currently ongoing by our research group regarding all welding parameters in order to assess their effects on workers’ exposure to Mn fumes.

2389 Evaluating Consumer-Relevant Exposure from Articles for Use in Safety Assessment: A Case Study Using Diapers

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Increasingly, both industry and regulatory agencies are challenged to determine relevant exposure to chemicals in consumer products or articles. In the absence of published methods, it is common for articles (e.g., toys, textiles, hygiene products) to be subjected to extreme conditions (solvents, temperature, homogenization, etc.) that destroy the matrix in an effort to determine anything that can possibly be detected (e.g., metals, microplastics). This constant trend of successive puff parameters could be observed. Each participant smoked all cigarettes with the same puff profile, enabling the creation of a personal smoking topography profile. We are the first to show that detailed data of four random cigarettes per individual are enough to create a personal smoking regime for future machine smoking exposure measurements. Interestingly, the participants’ puff profiles exceed the Health Canada intense parameters used in regulatory machine smoking, underlying the need for appropriate cigarette smoke exposure measurements based on human smoking.

2390 Personal Smoking Topography: Smokers Display a Unique Individual Smoking Profile

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To date, little is known about individual smoking behavior and actual exposure to hazardous smoke components. Additionally, there is a lack of consensus concerning the methodology to determine human smoking behavior. The goal of this human study is to characterize natural human smoking topography, for use in future exposure experiments and to optimize current methodology. A prospective observational human pilot study included five healthy males (25-34 years), used to smoking 13-25 Marlboro cigarettes per day. Habitual smoking behavior was observed in a homelike-apartment for 36 hours. For each smoked cigarette, smoking topography (i.e., puff volume, duration, frequency, flow and inter-puff-interval) was recorded with the CreSSmicro, a portable smoking topography measurement device. Puffing profiles were created by linear regression (least-squares method). Participants smoked cigarettes randomly during the day and showed a significantly different puffing profile when compared to each other. Each participant showed only subtle differences between the single puff parameters per cigarette. When comparing all cigarettes of an individual participant, a constant trend of successive puff parameters could be observed. Each participant smoked all cigarettes with the same puff profile, enabling the creation of a personal smoking topography profile. We are the first to show that detailed data of four random cigarettes per individual are enough to create a personal smoking regime for future machine smoking exposure measurements. Interestingly, the participants’ puff profiles exceed the Health Canada intense parameters used in regulatory machine smoking, underlying the need for appropriate cigarette smoke exposure measurements based on human smoking.

2391 A Pilot Study of Gestational Exposure to Environmental Contaminants Related to Unconventional Natural Gas Exploitation in Communities of Northeastern British Columbia

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The Peace River Valley (Northeastern British Columbia, Canada) is an area of intensive hydraulic fracturing for unconventional natural gas exploitation. Concerns have been raised regarding the potential health effects of contaminants like volatile organic compounds and heavy metals emitted by gas exploitation. Indeed, a recent study conducted in Colorado found associations between density and proximity of natural gas wells and birth defects. In our pilot study, we aimed to evaluate exposure to benzene, toluene and a suite of heavy metals in 29 pregnant women from the Peace River Valley. Enrolled participants collected repeated urine samples over five consecutive days. We measured metabolites of benzene (trans,trans muconic acid (tt-MA), S-phenylmercuric acid (S-PMA)) and toluene (S-benzyl-N-acetylcysteine (S-BMAC)), and heavy metals in urine samples. The median urinary tt-MA level was 3.5 times higher (182 µg/g creatinine) than that from the general Canadian population (48 µg/g creatinine); In Indigenous participants, the median level was 6 times higher (319 µg/g creatinine). Moreover, 5 women had urinary tt-MA levels above the safe exposure guideline (500 µg/g creatinine) put forward by the ACGIH. Median urinary manganese (0.49 µg/L) and vanadium (0.17 µg/L) levels were higher in participants than those from the general Canadian population. Our results suggested elevated gestational exposure to contaminants in our study population compared to the general population. Documenting gestational exposure to environmental chemicals in this region is paramount to assess the health risks associated with unconventional natural gas exploitation and develop exposure mitigation strategies.
Biomonitoring of human exposure to estrogens most frequently focuses on environmental and dietary estrogens, and infrequently includes measures of exposure to potent endogenous estrogens present in serum. Pregnancy is a developmentally sensitive period during which “added” serum estrogenicity exceeding normal intra-individual daily variability is of particular concern. Developing non-invasive biomonitoring methods for estrogens would overcome a key barrier to assessing the potential for biologically significant “added” estrogenicity. We collected repeated measures of serum concentrations of estrone (E1), estradiol (E2), estriol (E3), estetrol (E4), daidzein (DDZ), genistein (GEN) and bisphenol A (BPA) in thirty pregnant women using ultra-performance liquid chromatography coupled with tandem mass spectrometry detection (UPLC-MS/MS) and electrospay ionization (ESI). Serum estrone, estradiol and estriol concentrations varied significantly, with broad ranges across the cohort: 1.61-83.1 nM, 0.09-60.7 nM, and 1.5-36.3 nM respectively. BPA, DDZ and GEN concentrations were 1-5 orders of magnitude lower. The median within-individual coefficients of variation were large, 10.3% for estrone, 9.1% for estradiol, and 9.7% for estriol. The median serum estrone concentration was poorly correlated with gestation period but correlation with gestation period (log concentrations) increased with hydroxylation of the estrogens, tau=0.45 (estradiol), tau=0.58 (estriol) with p values < p = 2.2 x 10^-17. 24-hour urinary elimination of the endogenous estrogens were each strongly correlated with their corresponding serum concentrations, with Pearson’s Correlation Coefficients of 0.83 (E1), 0.82 (E2) and 0.84 (E3). A multivariate regression analysis produced equations for estimating serum concentrations of E1, E2, E3, E4, GEN and DDZ from urinary elimination rates and gestation period, an important step towards non-invasive biomonitoring for assessment of added estrogenicity during pregnancy.
Exposure to elevated levels of heavy metals in residential soils can pose a serious risk to human health. Families living near sources of heavy metal contamination (e.g., coal-fired power plants) and children who often engage in frequent hand-to-mouth activity may be highly susceptible to exposure. Quantification of total metals in soils is necessary to aid in assessing whether residential soils are heavily contaminated with toxic metals thereby posing a significant exposure risk. In this study, 42 residential soils were collected from communities in Stokes County, NC, which is also the home of a coal-fired power plant in Belews Creek, NC. Soil samples were digested using microwave-assisted acid digestion. Inductively-coupled plasma - optical emission spectroscopy was subsequently used to detect and quantify the total concentrations of arsenic (As), cadmium (Cd), lead (Pb), and manganese (Mn) in the soils. Arsenic concentrations ranged from 0.1–43.9 mg As/kg soil. Cadmium concentrations ranged from 0.1–1.6 mg Cd/kg soil. Lead concentrations ranged from 6.8-219.0 mg Pb/kg soil. Manganese concentrations ranged from 26.1-885.3 mg Mn/kg soil. Ninety-five percent of the soil samples fell below North Carolina’s non-cancer child hazard concentration for ingestion of As, Cd, Pb, and Mn (39, 78, 1600, and 1900 mg metal/kg soil, respectively). Among the soil samples collected, those closest to the coal ash impoundments (or the remaining 5%) had arsenic concentrations exceeding the non-cancer child hazard concentration. These data suggest families residing closest to coal ash impoundments may have elevated risk of exposure to toxic metals in soils, thus posing a potential risk to human health that merits additional study.

The occurrence and distribution of heavy metals in excess of natural loads is increasingly a global concern due to the potential risk to ecological receptors and humans via food chain transfer. This study assessed the risk associated with the levels of metals (As, Cr, Cd, Cu, Co, Fe, Mn, Ni, Pb, and Zn) in surficial soils from East Chicago, IN and Columbus Junction, IA since 1979. During the study, we assessed concentrations of trace metals in sidewalk surficial soils in proximity to a cohort in the Airborne Exposure to Semivolatile Organic Pollutants (AESOP) Study which has been following mothers and their children in East Chicago, IN and Columbus Junction, IA since 2008. Surficial soil samples (n=200) from sidewalks were collected in accordance with U.S. EPA Soil Sampling Guidelines (Method #SEDSPROC-300-R1, using a soil sampling core which were then analyzed in situ using X-ray fluorescence spectroscopy (XRF) for Ti, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Pb, Sr, Zr, Mo, Ag, Cd, Sn, Sb, Ba, Hg, and Pb. Concentration of lead in East Chicago was found to be significantly greater than Columbus Junction (p<0.01). Furthermore, lead in soil was found to be greater than the EPA maximum contaminant level (MCL) of 400 ppm in 4% of the samples from East Chicago and Columbus Junction, respectively. Trace metals in clotted blood were analyzed using inductively coupled plasma-mass spectrometry (ICP-MS) using an acid digestion protocol from the CDC (Method 8005). Results from analysis of a subset of clotted blood samples indicate the presence of trace metals in blood of AESOP Study participants in East Chicago. These preliminary results indicate the presence of aforementioned pollutants in East Chicago soil environment and blood and in some cases significantly above the regulatory limits. Ongoing work seeks to better understand the mechanisms responsible for synergistic toxicity resulting from co-exposure to PCBs and trace metals.

The Woolfolk Chemical Works, Inc., located in Fort Valley, GA, was a manufacturing facility where pesticides, insecticides, and herbicides that contained organic and inorganic arsenic compounds were produced during World War II for the US Army. The site was placed on the EPA’s National Priorities List in 1990 because of contaminated groundwater and soils resulting from facility operations. The properties, which had soils contaminated with arsenic above 100 mg/kg, were removed for final disposal at an off-site landfill. Soon after the EPA cleanup, residential homes were built on and around old contamination areas. Concerns for perimeter communities have risen because a drainage ditch from the Spiller Street pipe past the railroad to Big Indian Creek was filled with soil that may have contained contaminated runoff from facility operations which may have not been removed. The objective of this study was to determine if significant levels of arsenic are still present along the perimeter of a Fort Valley community surrounding the Woolfolk Chemical Works, Inc. The study began by collecting soil samples from two control sites and four different perimeter locations from the Fort Valley community. The soil samples were brought to the lab to let dry and then analyzed for the presence of aforementioned pollutants. The objective of this study was to assess concentrations of trace metals in soil samples from the perimeter of a Fort Valley community surrounding the Woolfolk Chemical Works, Inc.

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This is a health-oriented follow-up to a previously reported biomonitoring study of Vietnamese female electronic waste (e-waste) recyclers for metals and halogenated organics. We review potential health aspects of select elevated metals. Forty female Vietnamese e-waste recyclers and 20 Vietnamese non-recyclers were compared. Lead was higher in recyclers than non-recyclers and higher in the Vietnamese e-waste recyclers.
4.1 Three-compartment model

The three-compartment model was applied to evaluate the distribution of phthalates, dl-PCBs, and PBDEs among different demographic categories.

4.1.1 Distribution of Phthalates, DL-PCBs, and PBDEs among Demographic Categories

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Phthalates, dioxin-like polychlorinated biphenyls (dl-PCBs) and structurally similar polybrominated diphenyl ethers (PBDEs) are ubiquitous in environments. Additionally, all three compounds are alleged endocrine disrupting compounds. An alleged consequence of endocrine disruption is obesity. Average concentrations of these compounds were compared among age groups, genders and ethnicities using the National Health and Nutrition Examination Survey (NHANES). ANOVA analyses indicate that males had a significantly higher average blood concentration of a sum of 10 PBDEs in comparison to females. Significant differences of mean urinary phthalate and dl-PCB blood concentrations were detected in comparisons of age groups and ethnicities, with 85+ years and Non-Hispanic Whites having the highest mean values of PBDEs, and 19-30 years and Other Hispanics having the highest mean values of phthalates. Logistic regression analyses were conducted to determine whether an increase in background concentrations of PBDEs, dl-PCBs, and phthalates increased the NHANES survey participants’ odds of being obese. Logistic regression analyses indicate that none of these compounds increased the risk of having an obese body mass index. A comparison of the phthalate levels in the highest vs lowest quartile yielded an odds ratio estimate of 1.7 with a 95% confidence interval ranging from 1.1 to 2.7 for obesity. A comparison of the dl-PCB levels in the highest vs lowest quartile yielded an odds ratio estimate of 1.4 with a 95% confidence interval ranging from 0.6 to 3.3 for obesity. A comparison of the sum of 10 PBDE levels in the highest vs lowest quartile yielded an odds ratio estimate of 1.0 with a 95% confidence interval ranging from 0.6 to 1.7 for obesity. As a result, these data do not support the claim of a significant association between these contaminants, as alleged endocrine disruptors, with obesity.

4.1.2 Potential Risk to Environmental and Human Health by Heavy Metals in a Colombian Rain Forest

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Heavy metals are widespread pollutants in the world. The objective of the present study was to assess the presence of metals in several environmental matrices and its corresponding ecological risk in the Biogeographic Choco at the Colombian Pacific, a rainforest considered one of the most biodiversity-rich areas on the planet, threatened by massive illegal mining. Total mercury (T-Hg) was quantified using a direct Hg analyzer and other 47 elements by ICP-MS. Human exposure to mercury was low, with average and median hair T-Hg levels of 4.9 μg/g and 0.9 μg/g, respectively; although in some gold shops, Hg concentrations were two orders of magnitude greater than those found in reference sites. Mercury concentrations in sediments from Atrato River were moderately low, with minimum variation along the river course. In this compartment, some toxicologically relevant elements such as Ca, Cd, Cr, and Pb had values either respective threshold effect concentration (TEC), whereas Cr and Ni were above their probable effect concentration (PEC). In general, potential ecological risk assessment evidenced most sediment samples from Atrato River are moderately polluted by Cd. Mercury content in fish muscle increased according to the position each species occupies in the food chain. Elements different from Hg were also present in examined fish, with Cs and Rb following a distribution typical of biomagnification processes. However, some species belonging to lower trophic status displayed the greatest concentrations of Co, Ni, Cu, and Sn, in particular Prochilodus magdalenae and Hemiancistrus wilsoni. In conclusion, besides Hg, other metals should be considered in the evaluation of human and environmental health risks in gold mining areas.

4.1.3 Proposition 65 Exposure Assessments of Styrene in Consumer Products

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Styrene is included on California’s Proposition 65 list of chemicals known to the State to cause cancer or reproductive toxicity; it is used widely in the manufacture of consumer goods, such as packaging materials, insulation, and carpet backing, resulting in consumer exposure. The likelihood of exposure and subsequent health effects is the net result of chemical-specific factors (volatility, emitter factors (material composition, density, surface area), exposure factors (dose, duration, and route), and receptor factors (age, inhalation rate), necessitating a diverse approach to exposure assessment. This project evaluated potential exposure to styrene through use of two different consumer products, carpeting and a bicycle helmet shell liner, to determine if exposure is greater than styrene’s Proposition 65 Safe Harbor levels of 0.5 mg/day. For carpeting, we used ConsExpo modeling to estimate styrene air concentrations in home, office, and school settings using the initial, measured styrene concentration, volatility, diffusion (within-material) transfer rate, material-air partition coefficient, mass transfer coefficient, carpet density and surface area, and EPA’s site-specific air volumes and air change rates, together with a published inhalation uptake value and OEHHA’s age-specific body weight and breathing rate values. Maximum styrene exposure was 0.62 mg/day for children in a residential setting. In contrast to the approach for the carpet, we calculated inhalation and dermal exposure to styrene from use of a bicycle helmet shell liner. For inhalation exposure, we used analytical test results to estimate worst-case styrene concentration in the breathing zone and included published inhalation uptake value, manufacturer information on helmet
2406 Human Exposure to Polyhexamethylene Guanidine Phosphate from Humidifiers in Residential Settings: Cause of Serious Lung Disease


Exposure to the humidifier disinfectant, polyhexamethylene guanidine phosphate (PHMG), PHMG in mists generated from ultrasonic humidifiers was studied in a simulation chamber and apartment rooms. PHMG was suspected as a causative agent of lung disease in Korea residences. In the simulation chamber study, the amount of disinfectant discharged from three different ultrasonic humidifiers was measured. Mists generated at 1, 2 and 4 times the recommended amount of disinfectant were sampled with an impinger, and the effect of relative humidity on airborne disinfectant concentration was studied by changing RH from 40-70% to 90-100%. In addition, particle size distribution in mists was measured by SMPS, APS, and Mastersizer. In the apartment study, mists generated from ultrasonic humidifiers were sampled for 6 hrs in small and large rooms during fall and winter. In the simulation study the humidifiers discharged 205±24.6 ml/hr of mist at maximum capacity. Concentrations of airborne disinfectant increased with increasing concentration of disinfectant. RH affected airborne disinfectant concentration in the chamber, with increasing concentration with increasing RH. Below RH 70% no airborne PHMG was detected. PHMG-containing mists generated from ultrasonic humidifiers showed various sizes ranging from 149-157 nm, 690-740 nm and larger than 5.4 µm by SMPS and APS. Surface area mean diameter measured by Mastersizer was 5.39 to 5.72 µm. In the apartment study conducted during the fall, the geometric mean and standard deviation and arithmetic mean and standard deviation of airborne PHMG concentration were 3.22 ± 5.13 µg/m³ and 8.26 ± 12.18 µg/m³. In the winter, GM = GSD and AM = SD of airborne PHMG concentration was 0.21 ± 2.11 µg/m³ and 0.35 ± 0.62 µg/m³. RH and temperature in the apartment rooms for fall and winter were 22.2 ± 1.7°C, 74.5 ± 15.6% and 22.0 ± 2.2°C, 51.1 ± 12.9%. Different RH’s in the fall and winter resulted in very different airborne concentrations of disinfectant in the apartment rooms. Exposure levels and particle size distribution of mists generated from ultrasonic humidifiers in apartments are not sufficient to conclude that PHMG causes lung disease in Korean residences.

2407 Risk of Mycotoxins in Mold-Infested Consumer Products


Despite the findings of learned bodies, there continues to be misunderstanding about risk from indoor fungal growth. The US Consumer Product Safety Commission (CPSC) indicated 12 consumer products were recalled between 2011 and 2016 due to “risk to health because of mold exposure.” CPSC specified mold on these recalled consumer products posed “a risk” of respiratory or other infections in consumers with compromised immune systems, chronic health problems, damaged lungs, or an allergy to mold. Recall notices did not include exposure route, concentration, or how products were intended for use by immunocompromised individuals. We assessed the risk of mycotoxin exposure in nonsensitized individuals using 3 exposure scenarios including: (1) dermal contact of visible mold on products, (2) ingestion of mold spores, and (3) inhalation of mold spores aerosolized during handling of the product. There are no published data available for mold spores or mycotoxin inhalation exposures resulting from handling or using recalled consumer products. We estimated airborne mold spore exposures from recalled consumer products using measurements obtained during excessive handling of an obviously moldy product in a normal home environment. In considering mycotoxin exposure, maximum possible mycotoxin concentrations from breathing mold spores were estimated using published exposure modeling (Kelman et al. 2004) and compared to known effects levels for several mycotoxins. Corresponding margin of exposures (MOEs) for inhalation scenarios, breathed continuously by infants or adults for 24h/d, ranged from 0.003 to 0.010. In addition, the highest mycotoxin concentrations did not exceed the concentration of no toxicological concern (30 ng/m³) as reported by Hardin et al., 2009. Potential dermal and oral exposure from these consumer products were lower than levels associated with mycotoxicoses (human toxicity from molds). It is unlikely that individuals handling these consumer products with mold growth are at risk of exposure to biologically important numbers of mold spores and mycotoxins. Consumer products identified are unlikely to pose an increased risk of adverse health effects immune-compotent populations.

2408 The Plural Chemical Specific IgG Values of Two Small Plants’ Workers Handling Plastic Resins

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When considering plastic allergy and exposure status, most surveys focus on a single primary chemical substance which is handled at a specific plant. However, particularly small plants use several plastic resins according to customers’ requests. At this time, we report the plural chemical-specific IgG values of two small plants’ workers who are handling multiple plastic resins. Workers at plant “A” (N=81) are mainly using urethane resin. “B” plant’s workers (N=39) are handling low heat urethane resin and epoxy resin. Control subjects are the general inhabitants surrounding Kagoshima prefecture in Japan (N = 104). Data including working habits were collected using a self-administered questionnaire. Blood serum was collected and chemical-specific IgG values were measured by a dot blotting assay. The target chemicals were toluene disocyanate (TDI): urethane resin raw material, bisphenol A diglycidyl ether (BADGE): epoxy resin raw material, ethylenediamine (ED): epoxy resin curing agent and formaldehyde (FA): urea formaldehyde resin raw material. Plant A: The TDI-specific IgG values were elevated but not significantly higher compared to control subjects. The FA-specific IgG value was significantly higher than control subjects. B plant: BADGE-specific IgG and ED-specific IgG values were higher than control subjects but not significant. In addition to the primary chemical-specific IgG values of resin production, other chemical specific IgG values were higher in workers compared to controls. It is a significant challenge for industrial physicians to monitor multiple resins and chemical substances at small plants compared to big plants. Therefore, when investigating plastic allergy and plastic resin exposure status, it is important to target plural chemical substances especially at small plants. This study was funded by an Industrial Disease Clinical Research Grant.
Pharmaceuticals and personal care products in water in the environment have become a public concern. Angiotensin II receptor blockers (ARBs) are used widely as antihypertensive drugs and have been detected at high frequency at concentrations less than a few μg/L in urban river water and the effluent of sewage treatment plants. To advance environmental risk assessment of ARBs, it is necessary to estimate the behavior of ARBs in the water environment. Degradation by sunlight is one of the important parameters for the prediction of environmental concentration; however, little is known about the degradation of ARBs by irradiation by sunlight in environmental water. The objective of this research was to clarify the degradation of ARBs in water caused by irradiation using artificial sunlight. The ARBs investigated in this study were candesartan, irbesartan, losartan, olmesartan, and valsartan. The ARBs were dissolved in purified water at a concentration of 100 μg/L. To estimate the degradation of the ARBs near the water surface, the depth of the ARBs solution was set to be shallow as follows: 100 ml of the ARBs solution was put into a beaker (85 mm i.d. × 115 mm) made of quartz, and the water surface was irradiated using an XC-100BSS solar lamp (300 nm < λ < 700 nm, UV-A: 108,000 J/m²/h) at a height greater than 1 m. As a control sample, a beaker containing the ARBs solution was shielded from the light by covering the beaker with aluminum foil and the beaker was incubated in the same way as the non-shielded beaker. An aliquot of the ARBs solutions was taken at incubation times of 0, 1, 2, 4, 8, and 24 h and was analyzed by liquid chromatography tandem mass spectrometry. The half-life of each ARB by irradiation with the artificial sunlight was calculated by first order kinetics. The half-lives of candesartan, irbesartan, losartan, olmesartan, and valsartan were 64, >100, 36, >100, and 44 h, respectively, under the present irradiation conditions. The daily UV-A irradiation in Tokyo, Japan, is about 700,000 J/m², and this daily irradiation is equivalent to 6.5 h of irradiation time under the conditions of this experiment. Therefore, the half-lives of the ARBs are estimated to be more than 6 days near the surface of water. These results suggest that the ARBs investigated in this study are relatively persistent.

Breath analyzers are commonly used to estimate the blood alcohol concentration (BAC) at workplaces and among vehicle drivers. Breath analyzers indirectly, based on a stable partitioning of alcohol between blood and breath (ratio = 2100). However, local exposure to ethanol, eg inhalation of vapors, use of mouthwash etc, may increase the breath alcohol concentration without affecting the BAC leading to an overestimation of the true concentration in blood. In this study we investigated the elimination rate of breath ethanol followed by local exposure by inhalation of vapor and mouth wash to see if they pose a potential risk of a faulty estimation of BAC, particularly related to the Swedish statutory limit of 0.20 mg/g blood. The study was approved by the regional ethical committee and performed after informed consent by the volunteers. Eleven healthy subjects (5 men and 6 women) were exposed to inhalation by 1000 mg/m³ ethanol vapor for 15 min in an exposure chamber. After the exposure, breaths were sampled in Tedlar bags. After a break of 45 min, the subjects rinsed their mouth for 30 seconds with a typical mouth-rinse containing 21% ethanol. Post-exposure breaths were again collected in bags. Capillary blood was sampled from the fingertip before and after each exposure. Ethanol in breath and blood was analyzed by gas chromatography. No or negligible levels (less than 2.7x10⁻⁶ mg/g) of ethanol were detected in blood and breath of the subjects. The decline in breath is mono-exponential, however, the half-life of ethanol in breath (7.7±0.6 min) was shorter than the half-life of ethanol in blood (9.4±1.0 min). The study showed that breath ethanol is overestimated when compared to the blood alcohol concentration.
Crumb rubber (CR) used as an infill in artificial turf has brought public health concerns in recent years. The National Toxicology Program (NTP) is conducting research to improve the understanding of potential human health impacts following exposure to CR. As a part of the NTP research program, a lot of CR prepared by combining material from multiple commercial sources was analyzed using a variety of techniques to generate information on chemical and physical characteristics. Optical and scanning electron microscopy demonstrated that the lot consists of a range of particle sizes (0.1 to 4 mm) and types (dark and light rubber, visible inclusions, fibers). Thermogravimetric analysis revealed that the lot contains a minute fraction of volatile organic compounds (VOCs) and ~8% inorganics by weight. Elemental analysis by inductively coupled plasma with atomic emission spectrometry or mass spectrometry (MS) identified zinc, aluminum, cobalt and other metals and metalloids totaling ~2.9% by weight. Analysis for VOCs by gas chromatography (GC) and MS with head space sampling detected a large number of constituents; 33 compounds were identified totaling ~0.0007% by weight in CR. Extraction of CR with multiple solvents covering different polarities indicated that at 0.6% and ~8% inorganics by weight, respective. The extraction with water and methylene chloride, demonstrating that the majority of the extractable material consists of relatively non-polar organics. Analysis of methylene chloride extract by GC/MS identified 42 compounds with high confidence using authentic standards or reference library spectra, 7 of which were also identified in the VOC analysis, and 60 compounds with lower confidence using reference library spectra. 9 of which were also identified in the VOC analysis. An additional ~200 compounds previously reported to be in CR were investigated but were not detected in the extracts of the current lot. Analysis of solvent extracts of CR by liquid chromatography MS did not reveal any new analytes that were not previously detected by GC/MS. These data demonstrate that VOCs and metals constitute a very small fraction of CR lot.

Public health concern for playing on synthetic turf fields with crumb rubber infill has increased in recent years. Crumb rubber (CR) manufactured from recycled tires contains potential carcinogenic and toxic substances and there is potential for widespread exposure with over 12,000 synthetic turf fields in the United States. The National Toxicology Program (NTP) is conducting research to improve the understanding of potential human exposure and health impacts following CR exposure. CR was obtained from multiple commercial sources, combined into a single lot and size fractionated for use in these studies: 37-170μm for oral gavage, and greater than 170μm for feed and bedding studies. NTP conducted 14-day studies in female B6C3F1/N mice (n=10/group) by oral gavage (0 or 1250 mg/kg/day in corn oil), dose-fed (0 or 50,000 ppm) or by housing on CR mixed-bedding (bedding only or 50%/50% by weight). Plasma and urine were collected to determine internal exposure. Hematology, bone marrow cytology and limited histopathology were evaluated as conventional approaches to assess systemic exposure through evidence of biological effect. There were no effects on survival, food consumption, body weight or organ weights following CR exposure. CR was observed to be cytotoxic to human peripheral lung cancer (A549) cells in vitro and in vivo testing of the material used here, this work will contribute to what is known about potential human exposure to CR constituents resulting from contact with CR used in synthetic turf.

Public health concern for playing on synthetic turf fields with crumb rubber (CR) infill has increased in recent years. CR manufactured from recycled tires contains potential carcinogenic and toxic substances and there is potential for widespread exposure with over 12,000 synthetic turf fields in the United States. The National Toxicology Program (NTP) is conducting research to improve the understanding of potential human exposure and health impacts following CR exposure. The objectives of this study were to determine the leachability and cytotoxicity of CR in vitro using human peripheral lung (HPL-1D) cells and keratinocytes (HaCaT cells) to establish both aqueous and dermal routes of exposure. CR (100 mg/ml) was mixed and incubated in cell type-specific culture media for 3 h, 1, 4 or 7 d at room temperature, 37°C or 60°C. CR-conditioned media was then sterile-filtered and serially-diluted to 50, 25, 12.5 and 6.25 mg/ml for cell exposures. HPL-1D or HaCaT cells were exposed to CR-conditioned media for 24 h or 72 h and cell viability was measured via MTS cell proliferation assay. Cytotoxicity was observed for both cell lines in a concentration-dependent manner following incubation of CR at multiple temperatures and durations. For HPL-1D cells, 100, 50 and 25 mg/ml CR-conditioned media were cytotoxic at 24 and 72 h for all incubation times at 60°C. For HaCaT cells, 100 and 50 mg/ml CR-conditioned media were cytotoxic at 24 and 72 h for CR incubation times of 1, 4 or 7 d (100 mg/ml) and 4 or 7 d (50 mg/ml) at 60°C. For both cell types, cytotoxicity was also observed following CR incubation at 37°C but was most pronounced at 60°C. Untargeted LC-MS was used to characterize the chemical composition of CR-conditioned media. Compounds found to be elevated included 2-mercaptopentanoic acid, which is a known rodent carcinogen used in the vulcanization of rubber. Other compounds of interest included N,N'-diphenylguanidine, 1,2-benzisothiazolone-3-one and multiple phthalates. Studies are in-progress to address the in vitro cytotoxicity of CR using human small
intestinal cells, to reflect oral exposure, and the effects of CR incubation in more physiologically-relevant biofluids. In conjunction with chemical characterization and in vivo testing of CR, this study will contribute to what is known about potential human health effects of playing on synthetic turf fields made from recycled tires.

2417 Benchtop Testing Supporting Feasibility to Conduct In Vivo Studies of Synthetic Turf/Recycled Tire Crumb Rubber

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Over 12,000 synthetic turf fields exist in the US, with up to 1200 added annually. A primary component of synthetic turf is crumb rubber (CR) infill, synthesized from recycled automotive potential carcinogenic and toxic substances. However, the potential for human exposure from playing on these fields is not well understood. As such, likely exposure scenarios in humans that could be translated into exposure routes for in vivo testing were evaluated. Benchtop trials were conducted to evaluate various CR formulations for bedding (indirect contact), feed (indirect ingestion), oral gavage (direct consumption), and dermal (direct contact) exposures. Due to the physical characteristics of CR (irregular sized particles of ground tires that encompass a large size range and composition), physical manipulation methods were performed prior to testing. Milling was not feasible due to the characteristics of rubber (elasticity and thermal properties) and the additional additives employed during the grinding process; therefore, CR was sieved into various particle sizes for evaluation in each exposure scenario. Bedding and feed formulations were prepared at 50:50 (wt:wt) CR:bedding and at 50,000 ppm in feed with various sieved fractions of CR (greater than 170 µm). The formulations were rotated on an orbital shaker for four days to evaluate uniformity and potential for vapor off-gassing (bedding only). Uniformity of the feed formulations was maintained over four days of shaking. While larger CR particles maintained uniformity with the bedding, smaller particles settled to the bottom of the cage. No vapor off-gassing was observed for the bedding. Corn oil gavage formulations were prepared as homogenous suspensions at concentrations up to 200 mg/mL using CR with a particle size no greater than 170 µm. Particle sizes and concentrations greater than 170 µm resulted in blockage of the gavage needle during dispensing. Dermal administration of CR suspensions was determined to not be feasible due to clumping of CR in the vehicle, preventing homogeneous formulations. From this study, three potential human exposure scenarios (incidental exposure, indirect ingestion, and direct consumption) were identified as feasible exposure regimens for in vivo testing.

2418 Cumulative Assessment of Steroid Hormone Receptor-Mediated Activity of Contaminants in Water Samples Using In Vitro Bioassays


Cell-based assays could serve as a useful tool in the regulatory screening toolbox due to their high sensitivity and the ability to assess complex mixtures in which unknown compounds may be present. We have completed three major projects in collaboration with USGS: 1) Chemical Mixtures (surface water), 2) Source and Treated Drinking Water, and 3) Water Reuse. In each study, samples were extracted and assessed using T47D-KBLuc and MDA-kb2 transactivation assays for estrogen receptor (ER) and androgen receptor (AR) activation, respectively. Glucocorticoid (GR) activation was detected using CV-1 cells transduced with hGR and a luciferase reporter construct. In the first two studies, ER-mediated activity was the most frequently detected and was primarily explained by concentrations of estrone. There was limited detection of AR- or GR-mediated activity. For the Water Reuse project we examined the fate and transport of complex mixtures as they travel from a wastewater treatment plant (WWTP) to a drinking water treatment plant (DWTP) across the local drinking water supply (fall, spring, summer). Grab samples were collected upstream of the WWTP, at the WWTP effluent pipe, effluent mixing zone, downstream, DWTP intake, and treated drinking water. Polar organic chemical integrative samplers (POCIS) were deployed for 30 days each season at all locations except WWTP effluent and treated drinking water. The activity was detected at low levels (<0.3 ng E2EQ/L) in most grab samples, but was highest (1.4-28.6 ng E2EQ/L) at the effluent pipe in all seasons. AR and GR activity was only detected in the effluent grab samples (2ng DHTEq/L, 20ng DexEq/L, respectively) and was significantly lower in summer than fall and spring. ER activity in the POCIS samples was highest in the spring at the upstream and mixing zone sites. Importantly, ER activity was below quantifiable limits in the treated drinking water in grab and POCIS samples. No GR activity was detected in the POCIS samples. Overall, cell-based assays and analytical chemistry are complements that allow us to gather more robust data on the possible effects of contaminants in various water sources. This abstract does not necessarily reflect US EPA policy.

2419 Fluctuating Emissions and Availability of Health Reference Values: Implications for Estimating Acute Exposure and Health Risk


Assessing the potential for non-cancer health risks following inhalation of chemicals is important to informing air pollution risk management. Health risks from air pollutants are calculated by comparing exposure estimates from chronic (i.e., an average over one year or greater) or acute (typically 1-hour) durations to chemical- and duration-specific reference values or standards when available. However, modeled estimates of acute chemical exposure concentrations based on emissions data can be uncertain for many air pollutants. This uncertainty is largely due to a lack of hourly emissions data being reported from most industrial sources, compared to the availability of long-term data (e.g., annual emissions). In this study, we analyze some of the rare hourly air pollutant emissions information reported from industrial sources. We examine and discuss differences between these reported hourly emission rates and average hourly emission rates (calculated from annual emission rates) for reduced sulfur compounds from kraft pulp mill operations, and sulfur dioxide and oxides of nitrogen from a large U.S. power plant. We then discuss the additional challenge of assessing health risks based on hourly exposures, particularly the lack of acute reference values for many air pollutants applicable to the general population. The views expressed in this abstract are those of the authors and do not necessarily represent the views or policies of the US EPA.

2420 US EPA’s Non-Targeted Analysis Research Program: Expanding Public Data Resources in Support of Exposure Science


Suspect screening (SSA) and non-targeted analysis (NTA) methods using high-resolution mass spectrometry (HRMS) offer new approaches to efficiently generate exposure data for chemicals in a variety of environmental and biological media. These techniques aid characterization of the exposome and provide critical information on thousands of chemicals in commerce for which exposure data are lacking. EPA is advancing such techniques with workflows (feature extraction, formula generation, structure prediction, spectral matching, chemical confirmation), and tools (databases; models for predicting retention time, functional use, media occurrence, and media concentration; and schemes for ranking features and chemicals) to rapidly identify, prioritize, and quantify novel compounds in high-interest environmental and biological samples. EPA is also leading a Non-Targeted Analysis Collaborative Trial (ENTACT) to evaluate a range of SSA and NTA approaches. Four categories of experiments are underway, with analyses focused on: 1) ten standard chemical mixtures from the EPA’s ToxCast library; 2) extracts of standardized sample matrices (including house dust, human serum, and environmentally deployed silicone paint samples); 3) extracts of standardized sample matrices spiked with known chemical mixtures; and 4) approximately 4600 single chemicals from the ToxCast library. More than 20 laboratories worldwide from academia, government, and private (i.e., vendor) organizations are participating. Each laboratory is using their own SSA/NTA methods, and will submit results to EPA for performance evaluation and public release. A project goal is to produce benchmark methods for sample and data analysis, as well as results reporting, and to identify areas of future research. A further outcome of this work will be to identify which analytical methods are more suitable to detecting specific classes of chemicals in environmental media. Current progress on these varied NTA/SSA projects and initial results will be presented. This abstract does not reflect US EPA policy.
Bioavailability is a crucially important factor for estimating oral inorganic arsenic exposure in human subjects where exposure-response relationships can be better understood. Many studies have been conducted to quantify the potential for human health risk assessment. In the recently published Arsenic in Rice and Rice Products Risk Assessment Report, FDA used an estimate of 70% ~ 90% to quantify a distribution of the bioavailability of inorganic arsenic in rice. Furthermore, some newly published studies with revised design and expanded sample size, including both in vivo and in vitro experiments reported different outcomes from the previous studies considered by FDA. In this study, we conducted a systematic review together with meta-analysis to qualitatively identify the important factors that can significantly impact bioavailability and quantitatively characterize the distribution of bioavailability. A thorough literature search in the PubMed database identified 111 peer-reviewed journal articles using the key words of “arsenic” and “bioavailability” or “bioaccessibility”. 38 out of the 111 papers were selected by removing other irrelevant articles, such as those focused on arsenic bioavailability from soil to crops, and 8 among these 38 included papers provided adequate data for meta-analysis. A preliminary review suggests that, the bioavailability is likely to be correlated with arsenic species, whether its trivalent or pentavalent, and the actual dose of exposure, indicating a higher amount of intake might lead to greater bioavailability, which can range from 50% to 100%. Thus, the more reliably derived distributional estimate of the bioavailability of inorganic arsenic would remarkably improve the exposure assessment in support of human health risk assessment of inorganic arsenic and decision making.

Development of a Bisphenol A (BPA) Exposure Risk Questionnaire and Food Packaging Assessment Tool

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Sponsor: M. Holsapple

Bisphenol A (BPA), an endocrine-disrupting chemical, is widely used in consumer products. The main exposure pathway for BPA is from ingestion of foods contaminated by BPA leaching from polycarbonate plastic food storage containers and epoxy resin-lined cans. Current methods of identifying BPA exposure fail to recognize all sources, with some estimates indicating that only half of the sources are identified. Dietary exposure assessment methods often rely on dietary recalls to identify ingestion sources although dietary recall methods are designed to assess nutrient intake, not to identify environmental chemical exposures. This research aimed to develop and test a) a risk-based questionnaire to identify behaviors that increase the potential for dietary BPA exposure, and b) a food packaging assessment tool (FPAT). The risk-based questionnaire and FPAT methods were developed based on the literature describing the main BPA dietary exposure pathways. The tools were tested with 33 students enrolled in a university-level nutrition science course. Students were instructed on how and when to complete each assessment tool. The risk-based questionnaire, composed of 9 questions designed to identify eating behaviors known to increase BPA exposure, was administered at the same time as the FPAT and a 24-hour dietary recall. 36% of students (n=12) were identified as being at high risk for exposure to BPA according to the risk-based questionnaire scoring matrix. Internal consistency of the questionnaire, tested with Cronbach’s alpha, was 0.57. The FPAT captured potential BPA ingestion exposures by documenting details regarding how food was packaged, stored, prepared, and served. The risk-based questionnaire provided a strong foundation for identifying dietary behavioral patterns and gaps in exposure knowledge. Forty-five percent of students (15/33) did not know that BPA was labeled BPA-free, and 30% of students (10/33) did not know that their plastic bottles for water were BPA-free. The FPAT documented details about potential food exposures currently missed by dietary recalls. The researchers plan to modify the tool based on feedback from students. Planned future applications of the risk-based questionnaire and FPAT are to compare these tools to urinary BPA concentrations to assess their accuracy in identifying exposures.

Environmental pollutants are increasing at an exponential rate and many of them pose a potential health risk. Among these contaminants, pesticides are one hazardous class of contaminants used around the world. Pesticides exposure has been found to be associated with a range of human health problems like immune suppression, hormone disruption, adverse reproductive outcomes, etc. Owing to their xenobiotic nature, Organochlorine pesticides (OCPs) disturb the normal estrogen-progesterone balance, which is important in the maintenance of pregnancy. Preterm delivery (PTD) is the largest cause of perinatal death, neonatal morbidity and mortality and adult illness. Exposure to environmental chemicals and their improper metabolism is one of the factors causing excessive oxidative stress, DNA damage and cytokines imbalance during pregnancy. The root cause of many adverse pregnancy outcomes are not well understood but there is growing evidence that many of them might arise from the complex interaction between genes and environment. Hence, the present was designed to assess the risk of PTD by analyzing blood OCPs level, gene environment interaction between OCPs levels, DNA damage and mRNA expression of inflammatory and antioxidant genes in PTD cases, n=150) and term delivery (controls, n=150). Significantly higher levels of α-HCH, β-HCH and p’p’ DDE were observed in maternal blood and o’p’ DDT and p’p’ DDE in cord blood of PTD cases. The levels of HCH isomers, αHCH, βHCH, γHCH, p’p’ isomer, p’p’ DDE and o’p’ DDT were detected in maternal and cord blood indicating the placental transference of OCPs. The extent of DNA damage was also significantly higher in maternal, and cord blood of PTD cases as compared to controls. It was found that mRNA expression of IL-6 gene is higher and SOD gene was 7.5 folds and CAT gene was 6.95 folds lower in maternal blood of PTD cases as compared to controls. A positive significant linear correlation between the birth weight (kg), period of gestation (POG) and IL-6 ΔCt was found in all subjects. A negative correlation between birth weight, POG and ΔCt of SOD and CAT gene in all subjects were observed. The study concludes that gene-environment interaction is one of the potent risk factor for the etiology of PTD.
Conventional calculations of oral reference doses include uncertainty factors to account for extrapolation uncertainties associated with estimating a human equivalent dose from animal toxicity studies that measure administered dose. Increasingly, internal dose metrics are being used as an alternative method of quantifying the relationship between exposure and health risk. Specifically, changes in concentrations in serum and in target organ tissues are keys to understanding human health toxicity. However, internal dosimetry presents its own uncertainties and requires a careful evaluation of empirical data to support assumptions. In the case of long-chain perfluoroalkyl acids, the relatively long half-life in humans compared with mice has prompted some regulatory agencies to rely on internal dose as a preferred metric for dose-response analysis. Using perfluorooctanoic acid (PFOA) as a case study, we provide a side-by-side assessment of the use of internal and external dose metrics as the basis for calculating the RfD. We reassess some of the key studies for which PFOA kinetic models are based and highlight examples of how evolving understanding of species-specific kinetics and exposure sources impact interpretation of the underlying data. We extend the findings of Worley, Yang, and Fisher (2017) who demonstrated the relevance of non-drinking water exposures in understanding the inter-individual variability in PFOA serum concentrations by showing how this concept is extended to both the serum-to-water ratio and relative source contribution (RSC) assumptions used in derivations of drinking water standards. Using data reported by Emmett et al. (2006) on populations exposed to PFOA in drinking water and populations who were not exposed to this source, we demonstrate how the serum-to-water ratio relevant to quantifying PFOA exposures via the drinking water pathway is approximately 20:1 rather than the frequently cited ratio of 105:1. This approximately five-fold difference coupled with a closer examination of the RSC term has important implications for risk assessment and risk communication for PFOA and other perfluoralkyl acids that are compared with PFOA.

Application of Internal Dosimetry for Perfluoroalkyl Acids and Methods to Assess Uncertainty Factors Used in Risk Assessment

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Biomonitoring data such as concentrations of chemicals in human blood have routinely been used to assess national trends in exposure and to identify communities for which exposures are consistently elevated compared to national averages. Measured concentrations in people are often viewed as more informative exposure metrics than estimates based on exposure, uptake, and kinetic models; however, from a risk assessment perspective, for most chemicals, challenges remain in linking biomonitoring data with statements about the magnitude and likelihood of adverse health effects. Traditionally, reference doses (RfD) and associated human health drinking water threshold levels are calculated from and compared with administered or oral ingestion dose rather than internal dose. However, internal dose measurements and human biomonitoring data are being used more frequently to derive RfDs and drinking water threshold levels. The use of human serum data from biomonitoring studies to calculate a threshold level requires chemical-specific or default assumptions about how a chemical is absorbed, metabolized, distributed, and eliminated. A shift in calculating RfDs towards a focus on internal dosimetry is accompanied by an expectation that we will have greater confidence in the internal dose-response relationship. It is not clear that this is always the case. This presentation will propose a decision making framework for assessing relative uncertainties in dosimetric approaches. Key factors and important data needs will be highlighted, including chemical persistence (biological half-life); frequency, duration, and magnitude of exposure; availability of animal toxicology data that includes internal dose metrics; and an understanding of interspecies differences in metabolic and physiologic processes that affect both dose and route and resultant exposure compounds in drinking water will be presented for which approaches using compartmental models and biokinetic slope factors can be compared with traditional methods based on administered dose.

Detection of Ethylene Glycol in Aqueous Solutions through the Use of Gas Chromatography

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Ethylene glycol is a poisonous compound found in nearly pure form at any auto parts department in the country. Upon ingestion, ethylene glycol can cause death or serious health effects within 24 hours. Liquid foods such as milk and orange juice are a particular safety concern because of transportation of those products in unsecured vehicles. A detection method needs to be developed that will allow milk plants to test for the presence and concentration of ethylene glycol prior to introduction of the milk into the plant environment. The purpose of this study was to determine if cyclohexanol can be used to quantitatively and reliably extract ethylene glycol from aqueous solutions. If successful, this extraction to method could be utilized in liquid foods. Liquid-liquid extraction and quantification through the use of gas chromatography was employed. A set of ethylene glycol standards in water was used to determine the calibration curve for the extraction of ethylene glycol. It was determined that the distribution constant of the extracted ethylene glycol between organic and aqueous solvents was not consistent, because of this, it can be said that cyclohexanol is not a good fit for the extraction of ethylene glycol. The ideal extraction solvent should have a boiling point below 200°C, be immiscible in water, and interact with the hydroxide groups of the ethylene glycol for extraction.

Comparative Estrogenicity of Endogenous, Environmental, and Dietary Estrogens in Pregnant Women II: Accounting for Receptor Competition and Ligand Potency

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The potential for cumulative effects of exposure to multiple exogenous estrogens is a commonly raised concern. The potency for estrogen receptor based mechanisms can be described as comprising two factors: (i) affinity of ligand binding to the receptor, determined by the dissociation constants, and (ii) affinity of the co-activator recruitment to the estrogen receptor-ligand complex, determined by the relative coactiv-
vator affinity (RCA), eventually leading to downstream processes culmi-

nating in an estrogenic response. This goal of this study was to assess

and compare the estrogenicity of a group of seven estrogens, com-

prising endogenous (E1, E2, E3, E4), environmental (BPA), and dietary

(Genistein (GEN), Daidzein (DDZ)). Two related metrics of estrogenicity,
called the fractional receptor occupancy (FRO), and the relative response

(RR), were designed to reflect the above two dimensions of estrogen

potency. FRO is a measure of the effectiveness of an estrogen to bind
to an estrogen receptor (ER), whereas, the RR metric aims to capture the
potency of an estrogen in terms of not only its effectiveness of binding
to an ER but also recruiting a coactivator. To compute these metrics, we
utilized measured total ligand concentrations and available data on ER-α
and ER-β receptor affinity and the corresponding RCAs. The FRO based
estrogenicity was dominated by E1, E2 and E3, which together repre-

sented 84.1-99.9% (median: 99.2%) and 79.9-99.9% (median: 99.3%) of
the total receptor occupancy for ER-α and ER-β receptors, respectively.
The median fraction of receptors occupied by BPA was five orders of
magnitude lower than E1, E2 and E3, and three orders of magnitude
lower than the fetal derived E4 and GEN and DDZ. Likewise, based on
the RR values, E3 was the most potent serum estrogen (median RR
values of 0.746 and 0.794 for ERα and ERβ receptors, respectively. The
median RR values for E2 were 0.243 for ERα and 0.167, for ERβ receptors.
The RR values for the remaining estrogens were consistently less than
0.01 across the two scenarios examined. Moreover, RR values for the
dietary and synthetic estrogens GEN, DDZ and BPA were remarkably
lower. The overall approach proposed in this study represents a general
framework for calculating estrogenicity in serum, which is extendable to
other similar ligand-receptor systems.

Targeted and Non-Targeted Analysis of Serum Pools to Provide Chemical Exposure Data for Exposure Modeling and Chemical Prioritization

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Biomonitoring data can help inform the development and calibration of high-throughput exposure modeling for use in chemical prioritization and
guiding regulation. This project was conducted to evaluate the feasibility of using pooled banked blood samples to generate initial data
on population blood concentrations for compounds not to date routinely
analyzed in biomonitoring efforts such as the National Health and Nutrition Examination Survey (NHANES). Serum samples were obtained from the NIEHS Clinical Research Unit. Serum pools were constructed from 25 individual 1 ml aliquots. Four pools each were constructed
based on samples stratified by age (<45 vs 45 and greater) and male vs. female, for a total of 16 pools. Samples were analyzed in triplicate using GCxGC-TOFMS and LC-qTOFMS analysis (positive and negative mode). An exposure- and risk-based prioritization scheme was used to identify approximately 130 chemicals of interest subjected to additional
processing and evaluation. Targeted GCxGC was used to assess seven
chemicals: triclosan, bisphenol A, dibutyl phthalate (DBP), dimethyl
phthalate (DMP), diethyl phthalate (DEP), di-n-octyl phthalate (DNOP). Although these chemicals are rapidly metabolized, levels exceeding those
in method blanks were detected in most samples tested but not
in method blanks. Among the gender and age stratifications, only DEP showed a significant (p < 0.05) difference between groups, with age ≥45 having a mean of 0.24 (sd 0.06) and age ≤45 having mean 0.17 (0.04). Concentrations of DEP and triclosan in serum were similar to those previously reported in other studies. Non-targeted GC analysis found an average of 355 detections per sample. Females had a significantly higher (p-value 0.00018) number of detections (mean 440, sd 89) than males (mean 270, sd 26). These data can be used to inform development of high-throughput exposure models and design of further explorations of human chemical exposure through analysis of human serum samples. This abstract may not reflect US EPA policy.

Use of Lichens to Evaluate Predictions of Spatial Distribution of Metals by AERMOD in the Ohio River Valley

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Computer models, such as AERMOD, have been used to predict spatial distribution of airborne pollutants from point sources. However, it can be difficult to test these computer models to make sure that they are accurately predicting distribution. In an attempt to test AERMOD, endogenous lichens have been used as biomonitors to map airborne metal deposition in the Mid-Ohio River Valley and compare the results to predictions made by AERMOD for a ferromanganese alloy factory located in Washington County, Ohio. Mapping the lichen data has indicated that the plumes of manganese and chromium released by this point source have a northeast orientation, while AERMOD calculations for the same time period predicted that the plume would have a more northern and lichen dominated trajectory. Transplanted lichens (hypogymnic conditions from a control site were also used in an effort to better understand the rates at which lichens accumulate metals and the impact of wind direction, and precipitation on the spatial deposition of metals from this source. F. caperata collected at a control location was rinsed and placed in plastic mesh packets and transplanted at various distances lasting from one to 6 months, and by 5 months the transplanted lichens were reaching steady state in regards to metal concentrations. By 5 months the metal levels in transplanted lichens were also approximately the same as lichens found at those locations. Lichen samples were acid digested, and metal concentrations (Cr, Cu, Fe, Mn, and Ni) were determined using atomic absorption spectroscopy. Distance from the point source was strongly correlated with accumulation, with the closest transplant site having the highest concentration of Mn (286 µg/g dry weight). Exposures were also conducted each month for a year to compare accumulation of metals in lichens transplanted one kilometer to the north, south, east and west of the point source and relate those levels to wind direction and precipitation during the exposure period. Weather data for the area was obtained from a NOAA weather station located just outside of the Ohio River Valley. Based on prevailing winds from the southwest, we expected the highest metal concentrations to be observed to the north and east of the point source. However, for more than half of the months the highest metal levels were found at the western transplant site. We are currently working to optimize the ability of AERMOD to predict the monthly results that were obtained from the transplanted lichens.

Determining the Health Protective Capability of Analytical Detection Methods for Short Duration Exposures

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Emergency-response decisions require integrating exposure and risk information. Chemical exposure guidelines may be available, and are optimal when based on dose-response data for the relevant duration and address multiple levels of severity. US EPA’s National Homeland Security Research Center (NHSRC) has tools to characterize exposures and risk during temporary reutilization of previously contaminated infrastructure: Provisional Advisory Levels (PALs) and Selected Analytical Methods for Environmental Remediation and Recovery (SAM). Oral and inhalation PALs characterize three tiers of severity (minimal and reversible; more severe, irreversible or escape-impairing; and lethal) for durations of up to 24 hours, 30 days, 90 days and two years. PALs decrease with time and increase with effect severity. SAM recommends optimal analytical methods for a matrix-analyte pair and describes performance. Using SAM, the sufficiency of analytical capability for acrylonitrile, a widely used industrial chemical, relative to the PALs values was evaluated. Oral PALs (expressed as drinking water equivalents for children) ranged as low as 0.064 mg/L. SAM identified EPA Method 524.2 (run time ~ 30 minutes) as the optimal method for acrylonitrile in drinking water (by GC/MS). This method provides a detection limit of 0.00022 mg/L and a limit of quantitation of 0.0009 mg/L, representing sufficient analytical sensitivity to detect concentrations capable of producing even minimal, reversible effects, following a two-year exposure. Inhalation PALs ranged as low as 0.030 mg/m3. SAM identified OSHA Method PV2004 for acrylamide (HPLC/UV) as potentially applicable to acrylonitrile, with a possible detection limit of 0.001 mg/m3. Confidence will be increased when this method can be verified for acrylonitrile and a limit of quantitation is established. When the consequences of exposure to acrylonitrile are needed for exposure durations less than those required to procure, transport and analyze environmental samples (most relevant to the 24-hour exposure duration), field detection capabilities should be evaluated. This process will be applied to other priority chemicals. This abstract may not represent the views and policies of the US EPA/ORD/NHSRC.
**2433 Determination of Temephos and Its Metabolites in Biological Samples**
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Temephos (Tem) is a broad spectrum organophosphorus insecticide mainly used as a larvicide to control mosquitos. Tem is added to drinking water at a dose not exceeding 1 mg/mL. It is an unstable compound in chlorinated water and undergoes oxidation to its respective oxons and other oxidized metabolites. Based on its acute toxicity, Tem is classified in category U. The information about its toxicokinetics and chronic toxicity is very limited. There is no available a routine analytical method to determine oxidized metabolites of Tem in biological samples. The aim of this study was to develop and to validate an analytical method for analyzing metabolites of Tem in biological samples. An HPLC/DAD method to determine at least Tem and six metabolites was developed. The aqueous phase consisted of a 12 mmol/L linear gradient of acetonitrile, and water at a flow of 1 ml/min, a C18 column, and metabolites were monitored at 254 nm. Four metabolites were synthesized and characterized, and two more were commercially obtained, plus the parent compound. Adult male Wistar rats were orally administered for 3 d at a dose of 300 mg/kg/d. Two h after the last dose, animals were sacrificed by cardiac puncture under CO2-induced anesthesia. Serum was obtained from blood and organs removed. Serum and liver samples of non-treated animals were spiked with Tem and metabolites. Tem and its metabolites were extracted with a mix of ethyl acetate:acetonitrile:water at a flow of 1 ml/min, a C18 column, and metabolites ranging from 90 to 120%. The main metabolites detected in serum were Tem-sulfoxide and Tem-dioxo-sulfoxide, in addition to four non-identified metabolites. In liver, the main metabolites were Tem-sulfoxide, thiophenol and thiophenol-sulfoxide. This analytical method may be useful for future analysis of Tem in biological samples and would help in the development of physiologically-based pharmacokinetic models of this pesticide.

**2434 Measurement of Oxidative Stress Biomarker in Saliva: 8-Hydroxyguanine**

Oxidative stress leads to many kinds of diseases. Information about the oxidative status in the body is useful for the prevention of diseases and the mitigation of aging brought on by oxidative stress. To prevent diseases potentiated by oxidative stress, a method for the appropriate assessment of the oxidative stress status is needed. Currently, urinary 8-hydroxydeoxyguanosine (8-OHdG) is widely measured as an oxidative stress biomarker. It would be advantageous if saliva could be used as the sample to measure this oxidative stress biomarker, because saliva is much easier to collect than urine. However, urinary 8-OHdG is hardly detectable as a single peak, even after solid-phase extraction and concentration pretreatments. Salivary 8-OHGua may be a useful biomarker in the human population, in relation to the assessment of the oxidative stress induced by various factors in daily life. Ethics approval: The study was approved by the Ethics Committee of Medical Research, University of Occupational and Environmental Health, Japan.

**2435 Identification of a Sulfur Adduct of 1,4-Naphthoquinone during Reaction with a Polysulfide**
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Atmospheric quinones such as 1,4-naphthoquinone (1,4-NQ) interact readily with protein thiols, resulting in formation of protein adducts involved in cytotoxicity. Reactive sulfur species (e.g., hydrogen sulfide, persulfides and polysulfides) exhibit high nucleophilic property and thus capture electrophiles such as 1,4-NQ. We found that preincubation with sodium tetrasulfide (Na2S4) for 10 min at room temperature, the reaction mixture was applied to an ODS column. While a couple of reaction products were formed, UPLC-MS, FT-ICR-MS and ESI-MS analyses revealed that a sulfur adduct of 1,4-NQ has high purity exhibited a m/z of 361, corresponding to an elemental composition of C20H10O5S. 1H NMR and 1H-1H COZY NMR analysis indicated that the sulfur adduct was identical to 1-[2-(1,4-dioxonaphthalene-2-yl)sulfanyl]-3-hydroxynaphthalene-1,4-dione (1,4-NQ-S-1,4-NQ-OH).

**2436 Mice Lacking the NAD(P)H Quinone Reductase (NQO1) or NQO2 Gene Are More Susceptible than Wild Type Mice to Hyperoxic Lung Injury In Vivo: Protection by β-Naphthoflavone (BNF) Administration**
B. Moorthy, L. Wang, X. Couroucli, K. Lingappan, and W. Jiang. Baylor College of Medicine, Houston, TX.

Supplemental oxygen administration is frequently encountered in the treatment of infants and adults with pulmonary insufficiency. However, hyperoxia contributes to biological damage. NQO1 and NQO2 are phase II detoxifying enzymes that NQO1- and NQO2-null mice are more susceptible to hyperoxic lung injury in vivo, compared to wild-type (WT) mice, and that pretreatment of these mice with the cytochrome P450 inducer β-naphthoflavone (BNF) will rescue this phenotype. Ten- to twelve-week-old wild-type (WT) mice, C57BL/6J strain, NQO1-null, or NQO2-null mice were treated i.p. with vehicle CO or BNF (40 mg/kg), once daily for 4 days, and the animals were either maintained in room air or exposed to hyperoxia for 24-72 h. Lung injury and inflammation was assessed by measuring lung weight/body weight (LW/BW) ratios, histology, and neutrophil recruitment by immunochemistry. Gene expression of CYP1A1, Nrf2 and NQO1/2 genes was determined in lung by real-time RT-PCR. The NQO1-null and NQO2-null mice were more susceptible to oxygen-mediated lung damage and inflammation as evidenced by increased lung weight/BW ratios, lung injury, neutrophil recruitment, and augmented expression of IL-6 and TNF-α, in these animals compared with those in WT mice. There was no significant difference in the extent of lung injury between NQO1- and NQO2-null mice. Pretreatment of WT, NQO1-null, as well as NQO2-null mice with BNF, followed by hyperoxia for 24-72 h, led to significant attenuation of lung injury. BNF treatment led to increased expression of CYP1A1 gene expression in lungs and liver, and of CYP1A2 gene in liver in each of the genotypes. The fact that NQO1- and NQO2-null mice are more susceptible to hyperoxic lung injury suggests that these enzymes are protective against oxygen injury. That BNF-pretreated mice show attenuation of hyperoxic lung injury in WT, NQO1-null, and NQO2-null mice, and the fact that these mice display augmentation of pulmonary CYP1A1 and hepatic CYP1A1/1A2 support the hypothesis the BNF protected against lung injury by the expression of CYP1A enzymes... Future research could lead to the development of novel strategies for prevention and/or treatment of BPD and ALI/ARDS.

**2437 Polysulfide Na2S4 Regulates the 1,4-NQ-Mediated Activation of PTEN/Akt/CREB Signaling in Primary Mouse Hepatocytes**
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Electrophiles can activate redox signal transduction pathways, through actions of effector molecules (e.g., kinases and transcription factors) and sensor proteins with low pKa thiols that are covalently modified. In this study, we investigated whether 1,4-naphthoquinone (1,4-NQ) could affect the phosphatase and tensin homolog (PTEN)-Akt signaling pathway and persulfides/polysulfides could modulate this adaptive response. Simultaneous exposure of primary mouse hepatocytes to sodium tetrasulfide (Na2S4) and 1,4-NQ markedly decreased 1,4-NQ-mediated cell death and S- and cysteinyl- proteins. Modification of cellular PTEN during exposure to 1,4-NQ was also blocked in the presence of Na2S4. 1,4-NQ, at up to 10 µM, increased phosphorylation of Akt and cAMP response element binding protein (CREB) in a concentration dependent manner. However, at higher con-
IL22 Mediates Environmentally Persistent Free Radical Exposure-Exacerbated Influenza Infection

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Particulate matter containing environmentally persistent free radicals (EPFRs) are formed during various combustion processes including thermal remediation of hazardous wastes. Exposure to EPFRs adversely affects respiratory health in infants and is associated with increased morbidity and mortality due to acute lower respiratory tract infections. We reported previously that early-life exposure to EPFRs (1,2-dichlorobenzene adsorbed on CuO/Silica particles at 230 °C; DCB230) damages lung epithelium and suppresses immune responses to influenza (Flu) infection thereby enhancing Flu severity. Interleukin (IL) 22 is important in resolving lung injury following Flu infection in adult mice. In the current study, we determined the effects of EPFR exposure on pulmonary IL22 responses using our neonatal mouse model of Flu infection. RNA expression, protein concentration and immune cells were assessed using RT-qPCR, multiplex assay and flow cytometry, respectively. Exposure to EPFRs resulted in an immediate (0.5-1 day post-exposure; dpe) increase in IL22 expression in the lungs of C57BL/6 neonatal mice; however, IL22 expression was not maintained and failed to increase with subsequent EPFR exposures or Flu infection. This was associated with transiently increased and persistently decreased expression of the inhibitory cytokines IL10 and IL17A, which were earlier shown to activate and induce proliferation of group 3 innate lymphoid cells (ILC3). Because ILC3 are one of the major sources of IL22 and thus play a crucial role in the maintenance of lung epithelium, we also investigated the effects of EPFR exposure on lung ILC3. In congruence with IL22 levels, there was an initial increase in ILC3 frequencies in the lungs of EPFR-exposed neonatal mice that declined to baseline after 1 dpe. Together, these data suggest that exposure to EPFRs results in failure to sustain IL22 levels and an inability to induce IL22 upon Flu infection. Insufficient levels of IL22 may be responsible for aberrant epithelial repair and immune responses, leading to increased Flu severity.

Zinc-Deficient Diet Exacerbates Testicular and Epididymal Damage by Increasing Oxidative Stress and Altering Zinc, Insulin, Testosterone Signaling in Type 2 Diabetic Rat


Proper and planned dietary supplementation can cure or reverse the metabolic syndromes like diabetes. Zinc (Zn) is one of the most important trace elements in the body and is required for insulin secretion and release. Alteration in the Zn levels can cause moderate to severe damage to various organs. Most of the type 2 diabetic patients have altered insulin signaling. Type 2 diabetes induces irreversible damage, which can subsequently pass on to the next generation causing metabolic syndromes in the children. On the other hand Zn is required for proper development and function of the gonads, so the obvious role of Zn in type 2 diabetes-induced germ cell damage requires further exploration. Type 2 diabetes was induced by the combination of high fat diet and a single low dose of streptozotocin (35 mg/kg, i.p.). Animals were confirmed type 2 diabetic by assessing blood glucose, cholesterol and triglyceride levels. Control animals were on normal pellet diet throughout the study, while Zn-deficient diet was given for four consecutive weeks to the diabetic rats which were further kept on high fat diet for 16 weeks. Zn-deficient diet given to diabetic rats further decreased the serum Zn, plasma insulin and serum testosterone levels and increased cholesterol, triglycerides, BUN, %HbA1c and the oxidative stress in testes by decreasing glutathione, catalase and SOD1 levels. It also decreased the levels of GPX5, which is an epididymal secretory protein present in the caput. Further, Zn-deficient diet to diabetic animals decreased Metal response element (MRE)-binding Transcription Factor-1 (MTF-1) levels and SOD1 levels in the caput epididymis. Several of the histopathological alterations in testes, epididymis and sperm head defects were noted in the Zn-deficient group. Apart from these, organs rich in Zn levels like prostate, kidney and liver were also severely affected by the intake of Zn-deficient diet. The present results demonstrated that, any imbalance in the dietary Zn levels can exacerbate the type 2 diabetes induced germ cell and other organ damages.

Role of Cytochrome P450 (CYP) 1A2 in Neonatal Hyperoxic Lung Injury and Its Modulation by the CYP1A Inducer B-Naphthoflavone (BNF)

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Exposure to hyperoxia leads to bronchopulmonary dysplasia (BPD), one of the most common pulmonary morbidities in preterm neonates, which is more prevalent in males than females. We reported recently that adult mice lacking genes for Cyp1a1 or 1a2, and neonatal mice lacking Cyp1a2 are more susceptible to hyperoxic lung injury than wild type mice. In this investigation, we tested the hypothesis that neonatal mice lacking the Cyp1a2 gene will be more sensitive to oxygen-mediated lung injury, and that beta-naphthoflavone (BNF) treatment will rescue this phenotype. Newborn WT or Cyp1a2/-/- mice were treated with BNF (10 mg/kg) or the vehicle corn oil (CO) i.p, from postnatal day (PND) 2 to 8 once every other day, while being maintained in room air or hyperoxia (85% O2) for 14 days. Hyperoxia exposure led to alveolar simplification and arrest in angiogenesis in both genotypes. No significant differences were observed between WT and Cyp1a2/-/- mice. Cyp1a2/-/- female mice had better preservation of pulmonary angiogenesis at PND15 compared to similarly exposed males. BNF treatment attenuated lung injury and inflammation in both genotypes, and this was accompanied by a significant induction of hepatic and maternal CYP1A1 expression in WT but not in Cyp1a2/-/- mice. Under hyperoxic conditions, BNF treatment induced Nqo1 mRNA levels in WT and Cyp1a2/-/- mice compared to corn oil-treated mice. Newborn mice lacking the gene for Cyp1a2 did not have increased susceptibility to hyperoxic lung injury compared to the WT. Nevertheless, the ISCs of lung from Cyp1a2-/ females lacking the gene for Cyp1a2 were more susceptible to hyperoxia-induced lung injury than WT mice. Under normal breathing conditions, BNF treatment attenuated the expression of several pro-inflammatory genes in both genotypes.

Pro-Oxidant and Antioxidant Properties of Polycyclic Aromatic Hydrocarbons and Extractable Organic Matter from Particulate Matter <2.5 μM


We investigated the kinetics of reactive oxygen species (ROS) production and its link to antioxidant response in human embryonic lung fibroblasts (HEL) and human alveolar basal epithelial cells (A549) treated with extractable organic matter (EOM) from PM2.5 and polycyclic aromatic hydrocarbons (benzo[a]pyrene, BaP; 3-nitrobenzanthrone, 3-NBA). We analyzed ROS levels, total antioxidant capacity (TAC), lipid peroxidation (15-F2t-IsoP) and total protein carbonyls (15-F2t-IsoP) in HEL and A549 cells exposed to EOMs. Exposure to EOMs increased ROS levels, total antioxidant capacity (TAC), lipid peroxidation (15-F2t-IsoP) and total protein carbonyls (15-F2t-IsoP) in HEL and A549 cells exposed to EOMs. Exposure to EOMs increased ROS levels, total antioxidant capacity (TAC), lipid peroxidation (15-F2t-IsoP) and total protein carbonyls (15-F2t-IsoP) in HEL and A549 cells exposed to EOMs. Exposure to EOMs increased ROS levels, total antioxidant capacity (TAC), lipid peroxidation (15-F2t-IsoP) and total protein carbonyls (15-F2t-IsoP) in HEL and A549 cells exposed to EOMs. Exposure to EOMs increased ROS levels, total antioxidant capacity (TAC), lipid peroxidation (15-F2t-IsoP) and total protein carbonyls (15-F2t-IsoP) in HEL and A549 cells exposed to EOMs.
after 24h exposure of HEL and 4h treatment of A549. In summary, our data suggest the ability of individual PAHs and EOMs to impact ROS generation, induce antioxidant mechanisms, and affect lipid peroxidation levels. However, the extent of these effects depends on the cell line used for the tests. Supported by Czech Science Foundation (16-14631S).

2442 Air Pollutant 1,2-Naphthoquinone Inhibits Glycolysis through Peroxide-Mediated Protein Inactivation in Human Bronchial Epithelial Cells

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Ambient particulate matter (PM) exposure is a leading cause of morbidity and mortality worldwide. PM-linked health outcomes are thought to be mediated by oxidative stress mechanisms, but the specific redox events involved have not been well-characterized. PM-associated quinones, such as 1,2-naphthoquinone (1,2-NQ), can contribute to cellular oxidative stress through reactive oxygen species (ROS) generation by redox cycling and by direct addition to proteins. We have previously shown that 1,2-NQ induces production of the ROS hydrogen peroxide (H2O2), a potent signaling molecule that oxidizes proteins to regulate their function. We specifically identified the glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as a target of H2O2 oxidation induced by 1,2-NQ. GAPDH inactivation in human bronchial epithelial cells has been hypothesized that this modification could cause a functional inactivation of the enzyme. Glycolysis is essential for many key cellular functions such as ATP production. GAPDH inactivation by 1,2-NQ leading to inhibition of glycolysis would therefore greatly impair cellular function and growth. Here, we examined the functional effects of 1,2-NQ on glycolysis in the human bronchial epithelial cell line, BEAS-2B. We found that environmentally-relevant doses of 1,2-NQ rapidly inhibited glycolytic function as measured using extracellular flux analyses. Overexpression of catalase, an enzyme that scavenges H2O2, reversed the glycolytic inhibition caused by 1,2-NQ, suggesting an underlying H2O2-dependent mechanism to inactivate glycolytic proteins rather than addition. To our knowledge, this is the first report that exposure to low doses of an environmental toxicant results in a functional inhibition in glycolysis through peroxide-dependent mechanisms. These results suggest novel approaches to cancer therapies, as cancer cells are highly reliant on glycolysis for energy production. This study informs our mechanistic understanding of PM-induced human health effects. This abstract of a proposed presentation does not necessarily reflect US EPA policy.

2443 Mammalian Target of Rapamycin (mTOR) Regulates Mitochondrial Metabolism and Redox Homeostasis in Astrocytes

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Astrocytes play an essential role in the brain as they regulate both neuronal excitability and homeostasis. Glutathione (GSH) efflux from astrocytes provides precursors for neurons to synthesize GSH. However the mechanisms and signaling pathways by which astrocytes maintain GSH homeostasis are unknown. In this work, we report the role of the mammalian target of rapamycin (mTOR) in the regulation in GSH homeostasis in primary cultures of rat cortical astrocytes. Inhibition of de novo GSH synthesis with buthionine sulfoximine (BSO; 500 µM) induces GSH depletion. Interestingly, GSH depletion was not paralleled by oxidative stress suggesting an active flux of GSH. GSH depletion was independent from the activity of the multidrug resistance-associated protein 1 (MRP1/ABCC1) as evidenced by the lack of effect of the MRP1 inhibitor MK571 (50 µM). GSH-depletion induced by BSO was reversible after 12 h of BSO withdrawal. Inhibition of mTOR (Torin 250 nM) and phosphatidylinositol 3-kinase (LY294002, 10µM) signaling, as well as mitochondrial pyruvate transport (UK5099, 5 µM) and free fatty acid oxidation (Etomoxir, 200 µM) depleted GSH content in astrocytes, and prevented the recovery of the intracellular GSH pool after BSO withdrawal. Our results suggest that mTOR/PKB signaling and mitochondrial central carbon metabolism (pyruvate and free fatty acid oxidation) regulate GSH homeostasis in astrocytes.

2444 A Sensitive and Cost-Effective Fluorescence-Based Pyrogallol Red Assay for H2O2: Applied to Detect H2O2 Mobility Between Culture Wells


A novel fluorescence-based assay to quantify hydrogen peroxide levels in biological samples was developed with the relatively inexpensive and non-toxic reagents pyrogallol red (PGR) and horseradish peroxidase (HRP). These characteristics of PGR, as well as its ability to be readily oxidized by various species and its absorbance maxima at 540 nm at physiological pH, have led to its use in a number of assays to quantitate levels of small molecule oxidants and antioxidants, metal ions, and catalase. When the reaction is catalyzed, such as by HRP, PGR is oxidized by H2O2. We find that the linear range of H2O2 detection by this method is limited to the low µM range. Given its structural similarity to other anionic xanthene dyes, such as fluorescein, we hypothesized PGR would also have fluorescent properties, which might extend its linear range of H2O2 detection. We find that the reduced and oxidized forms of PGR both fluoresce, with distinct excitation and emission spectra, depending on pH. The linear range of H2O2 detection is significantly extended by fluorescence detection. A rapid 96-well plate assay was developed. Controls are included for 1) specifically detecting H2O2 by subtracting out any changes observed after treating samples with catalase and 2) accounting for any nucleophilic quenching of H2O2 (such as by glutathione) by pretreatment with the electrophile N-ethylmaleimide. This assay was then utilized to determine whether and to what extent H2O2 released in neighboring wells in a multi-well cell culture plate, due to volatilization from a treated well. We find that detectable amounts of H2O2 are found in wells that neighbor a treated well.

2445 Age-Dependent Oxidation of Cysteine/Cystine Redox State (Eh[Cys/CySS]) Is Mediated by Decreased SLC7A11 Expression in Mouse Lung Fibroblasts


Progressive oxidation is associated with aging and may contribute to increased susceptibility to environmental insults. The redox state of human plasma, defined by the concentrations of cysteine (Cys) and cystine (CySS), becomes more oxidized as we age. Recently, we showed that fibroblasts isolated from the lungs of young and old mice retained this differential phenotype; old cells produced and maintained a more oxidizing extracellular redox potential (Eh[Cys/CySS]) than young cells. Microarray analysis identified down-regulation of SLC7A11, the light subunit of the CySS/glutamate transporter, as a potential mediator of age-related oxidation in these cells. The purpose of the present study was to investigate the mechanistic link between Slc7a11 expression and extracellular Eh[Cys/CySS]. Sulforaphane treatment or transient transfection with a plasmid encoding Slc7a11 was used to increase expression levels of Slc7a11 in old fibroblasts, and sulforaphane treatment or siRNA-mediated knock down was used to decrease activity and expression of Slc7a11 in young fibroblasts. L-buthionine-sulfoximine (BSO) was used to inhibit cellular GSH synthesis. Slc7a11 mRNA levels were measured by qPCR, concentrations of Cys, CySS, GSH, GSSG and CySSG were measured by HPLC, and their redox potentials were calculated from the Nernst equation. The results showed that both extracellular Eh[Cys/CySS] and Eh[GSH/GSSG] were more oxidized in old cells than in young cells. Up-regulation of Slc7a11 resulted in a more reducing extracellular Eh[Cys/CySS], whereas down-regulation produced a more oxidizing Eh[Cys/CySS]. Up-regulation via sulforaphane increased total GSH and restored Eh[GSH/GSSG], whereas up-regulation via overexpression plasmid had no effect on these parameters. Neither siRNA-mediated knock down of Slc7a11 nor sulforaphane inhibition of Slc7a11 activity influenced total GSH. Inhibition of GSH synthesis with BSO had no effect on the ability of cells to restore their extracellular redox potential in response to an oxidative challenge. In conclusion, our study reveals Slc7a11 is the key regulator of extracellular Eh[Cys/CySS], and its effects are not dependent on intracellular GSH synthesis.
Bioenergetic Determinants of Cellular Resistance to Ozone-Induced Intracellular Redox Changes

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Exposure to air pollution is a persistent and widespread global public health problem. As a principal component of air pollution, ozone (O<sub>3</sub>) is a highly reactive oxidant gas known to induce adverse health outcomes such as pulmonary inflammation and functional decrements in the lungs of exposed individuals. Importantly, oxidative injury is commonly cited as a key mechanistic feature in the toxic action of O<sub>3</sub>. We have previously demonstrated O<sub>3</sub> to significantly alter the ratio of oxidized to reduced glutathione (glutathione redox potential, E<sub>redox</sub>) of human aortic endothelial cells (HAEC) exposed to 0.15-0.5 ppm O<sub>3</sub>, thus linking O<sub>3</sub> exposure to a specific intracellular redox outcome. Maintenance of the reducing potential for redox active biomolecules such as glutathione is critical for cellular health in an antioxidant defense against oxidative stress, and depends on the availability of reducing equivalents produced through bioenergetic processes. In the present study, we used a live-cell imaging approach to assess cellular susceptibility to oxidative stress by examining the bioenergetic role of glucose availability on intracellular E<sub>redox</sub> recovery following O<sub>3</sub> exposure. Glucose-deprived BEAS-2B cells expressing the E<sub>redox</sub> sensor roGFP2 were monitored in real time during exposure to 0.5 ppm O<sub>3</sub> in a glucose-free exposure medium. As with previous exposures, O<sub>3</sub> induced a substantial increase in the E<sub>redox</sub>. The addition of glucose to the exposure medium during a recovery period following the O<sub>3</sub> exposure resulted in a rapid restoration of the E<sub>redox</sub> to levels near those observed in the established pre-exposure period. A glutaredoxin1-linked version of roGFP2 showed the same pattern of responsiveness to O<sub>3</sub> and glucose-induced recovery with enhanced rate kinetics across the entire exposure period. These results suggest that the availability of reducing equivalents produced from glucose metabolism, as measured by E<sub>redox</sub>, is critical to the maintenance of redox homeostasis and antioxidant defense. Together, these findings provide insight into the mechanisms through which HAEC mitigate environmental oxidative stress. This abstract of a proposed presentation does not necessarily reflect US EPA policy.

Nrf2-Dependent Antioxidative Action of an Anti-Arthritic Drug, Auranofin: The Possible Repositioning for Oxidative Stress-Related Diseases

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The Nrf2 pathway confers biological defense against oxidative stress and redox-reactive xenobiotics. It is anticipated that the activation of the pathway will have applications in therapies and prevention for oxidative stress-related diseases. However, it is not easy to activate this pathway safely, because Nrf2-activating compounds themselves are highly reactive and toxic, and the toxicity may be enhanced under specific conditions. For example, we previously found that sulforaphane, a plant-derived Nrf2 activator, exerted Nrf2-independent toxic effects in combination with sodium arsenite. To circumvent the toxicity problems that have been disturbing smooth commercialization of this therapeutic strategy, we herein aimed to find a safe Nrf2 activator from the lineups of already-approved drugs. For this purpose, we used a zebrafish model, taking advantage of its usefulness in the in vivo assessment of biological actions of chemicals. Protective actions of two Nrf2 activators were evaluated: auranofin and dimethyl fumarate, which are approved therapies for rheumatoid arthritis and multiple sclerosis, respectively. After 12-hour pretreatment with these drugs, zebrafish larvae (4 days post fertilization) were exposed to oxidative stressors and subsequent survival rates were investigated. Although the protection by dimethyl fumarate was unclear, auranofin conferred strong protection against oxidative stress. Only 5.0% of zebrafish larvae survived 24-hour exposure to 2 µM auranofin. Survival rates were 71.6% when 2 µM auranofin was pre-treated. This protective effect was diminished in Nrf2 mutant strain, confirming that the action of auranofin was dependent on the activation of the Nrf2 pathway. In addition, auranofin pre-treatment attenuated toxicity of arsenite. Survival after 24-hour exposure to 1.5 mM sodium arsenite was 14.8%, while 2 µM auranofin pre-treatment raised the rate to 53.8%. This protective effect was also shown to be Nrf2-dependent by the genetic analysis. Importantly, auranofin did not show any combinatorial toxicity with arsenite, at least during 120-hour test period. These results clearly demonstrate that auranofin activates the Nrf2 pathway and confers strong protection against oxidative stress in a relatively safe manner.

Repositioning of this drug for oxidative stress-related diseases will provide a promising therapy.

Cinnamic Aldehyde as a Potential Therapeutic for Preventing Neointimal Hyperplasia in Diabetes


Diabetes Mellitus (DM) accelerates the rate of atherosclerosis. Balloon angioplasty is a common surgical intervention for treating athero-sclerotic disease. However, angioplasty often fails from vessel re-occlusion (restenosis) secondary to neointimal hyperplasia. Neointimal hyperplasia is the result of vascular smooth muscle cells (VSMC) proliferation and migration into the intima, a process driven by reactive species. Systemic redox dysfunction in DM leads to higher restenosis rates. Activation of the Nrf2-KEAP1 antioxidant defense pathway inhibits neointimal hyperplasia in animal models of vascular disease. Therefore, we hypothesize that a Nrf2 activator, cinnamic aldehyde (CA), will prevent neointimal hyperplasia by inhibiting VSMC migration and proliferation. VSMC were isolated and propagated from the aorta of male Zucker Diabetic Fatty (ZDF) rats. Cell viability was measured by MTT assay and flow cytometry. Migration was analyzed using the scratch assay. Nrf2 pathway activation was assessed by western blot and confocal microscopy. The balloon carotid injury model was performed in male ZDF rats after onset of DM. CA (100 µM) in Pluronic F127 (100 µl) or vehicle alone was applied to the perivascular surface of the carotid after injury. Arteries were harvested after 2 weeks for neointimal hyperplasia assessment, or after 3 days for BrdU incorporation and Nrf2 localization. CA inhibited VSMC proliferation by MTT (EC<sub>50</sub> = 23.9±19.5 µM). By flow cytometry, CA caused a 42% decrease in total cell number without changing percent viability (95.7%) or inducing apoptosis. CA inhibited platelet derived growth factor-induced migration by 42%. CA induced Nrf2 translocation to the nucleus and pathway activation both in vitro and in vivo. CA inhibited proliferation in vivo with a 47% reduction in the vessel proliferative index. Most importantly, CA inhibited neointimal hyperplasia with a 47% reduction in intimal area (P<0.025) compared to injury alone. CA effectively inhibited neointimal hyperplasia in our rat model of DM. Therefore, localized Nrf2 activation may provide a novel therapeutic target for inhibiting restenosis and improving surgery outcomes.

Ethanol Induces the Generation of Reactive Oxygen Species through NAPDH Oxidases (NOXs) and Mitochondria to Suppress Early Murine Osteoblast Differentiation In Vitro

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Bone marrow mesenchymal stromal cells (BM-MSCs) can differentiate into osteoblasts and adipocytes. Maturation of osteoblasts is associated with increased expression of factors such as alkaline phosphatase and osteopontin. NADPH oxidase (NOX) enzymes (e.g. NOX4 and NOX2), are expressed at different phases of osteoblast differentiation and produce reactive oxygen species (ROS). Both NOX4 and NOX2 are up-regulated by ethanol and are hypothesized to increase oxidative stress and suppress osteoblast maturation while upregulating bone-resorbing osteoclast differentiation. Mitochondria provide a significant source of ROS but the role of ethanol in mitochondria-induced oxidative stress in bone is not well established. These studies herein used BM-MSCs to model osteoblast and adipocyte differentiation in vitro to address the role of varied ROS sources in response to ethanol. BM-MSCs from WT and NOX4<sup>-/-</sup> C57Bl/6J mice were cultured in the presence of 50 µM l-asorbate phosphate to stimulate osteoblast differentiation, and treated with 0 or 50 mM ethanol. BM-MSCs from WT and NOX4<sup>-/-</sup> mice were exposed to ethanol and the mitochondrial antioxidant mitoTEMPO. At 14 days, fibroblast colonies and osteoblast differentiation were evaluated using hematoxylin and alkaline phosphatase staining, respectively. NOX4 deletion and mitoTEMPO co-treatment, but not NOX2 suppression (via deletion of p47phox), protected against ethanol's suppression of alkaline phosphatase-expression and Nrf2 localization. CA inhibited VSMC proliferation by MTT co-treatment, but not NOX2 suppression (via deletion of p47phox), protected against ethanol's suppression of alkaline phosphatase-expression and Nrf2 localization. CA inhibited VSMC proliferation by MTT and flow cytometry. Migration was analyzed using the scratch assay. Nrf2 pathway activation was assessed by western blot and confocal microscopy. The balloon carotid injury model was performed in male ZDF rats after onset of DM. CA (100 µM) in Pluronic F127 (100 µl) or vehicle alone was applied to the perivascular surface of the carotid after injury. Arteries were harvested after 2 weeks for neointimal hyperplasia assessment, or after 3 days for BrdU incorporation and Nrf2 localization. CA inhibited VSMC proliferation by MTT (EC<sub>50</sub> = 23.9±19.5 µM). By flow cytometry, CA caused a 42% decrease in total cell number without changing percent viability (95.7%) or inducing apoptosis. CA inhibited platelet derived growth factor-induced migration by 42%. CA induced Nrf2 translocation to the nucleus and pathway activation both in vitro and in vivo. CA inhibited proliferation in vivo with a 47% reduction in the vessel proliferative index. Most importantly, CA inhibited neointimal hyperplasia with a 47% reduction in intimal area (P<0.025) compared to injury alone. CA effectively inhibited neointimal hyperplasia in our rat model of DM. Therefore, localized Nrf2 activation may provide a novel therapeutic target for inhibiting restenosis and improving surgery outcomes.

Chronic ethanol abuse increases susceptibility to acute lung injury. Previous studies suggested that ethanol acts through one or more nicotinic acetylcholine receptor (nAChR) subtypes to induce expression of fibronectin, an extracellular matrix protein implicated in lung injury and disrepair. Culturing fibroblasts in media with an oxidized cysteine/cystine redox potential (Eₜ-Cys/CySS mimics the effect of ethanol, suggesting a potential mechanism by which ethanol activates these receptors. The purpose of the present studies was to investigate redox regulation of the α4 nAChR subtype in response to ethanol. Primary lung fibroblasts were isolated from α4 nAChR knock out and wild type mice. NIH 3T3 fibroblasts in which wild type or α4 nAChR was stably knocked down were used to express mutants of α4 nAChR. Fibronectin and CySS metabolizing genes were measured by real time-PCR. Intracellular and extracellular Cys, CySS, glutathione (GSH) and glutathione disulfide (GSSG) were measured by HPLC. Redox potentials (Eₜ) were calculated from the Nerst equation. Fibronectin mRNA levels were increased by 60 mM ethanol or 0 mM oxidized redox media. Lung fibroblasts from α4 nAChR knock out mice also had defects in CySS metabolism and distribution. Expression of the CySS transporter Slc7a11 was low, extracellular CySS accumulated, intracellular GSH levels dropped, and lower amounts of Cys and GSH were exported in cultures of α4 nAChR knock fibroblasts. Extracellular Eₜ-Cys/CySS of knock out fibroblast cultures was more oxidized than wild types, and total GSH concentrations in the conditioned media were lower. These results showed Cys residues on the extracellular surface of α4 nAChR mediated cellular responses to ethanol in primary mouse lung fibroblasts. Furthermore, these studies provide evidence for a feedback loop, wherein activation of α4 nAChR by extracellular oxidation up-regulates intracellular production and export of Cys and GSH, providing a means to restore the redox state of α4 nAChR and limit signaling.

2451 Evaluation of Positive Controls for In Vitro Assays of Oxidative Stress

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Increases in oxidative stress plays a critical role in the carcinogenic process as well as many other toxicities. The evaluation of this study compared the dose-response relationship and time course for effects on cell viability, mitochondrial membrane potential, and oxidative stress in in HepaRG and HaCaT cells. Diquat, antimycin, menadione, tert-butyl hydroperoxide (TBHP) and etoposide were evaluated as positive controls in these assays. Potassium chloride and sucrose were used as negative controls. Cells were exposed for 1 or 24 hours, after which CellTiterGlo, ROSGlo, and JC-10 assays were performed and dose-response curves generated. These chemicals displayed unique dose- and time-response relationships. There was little difference in the response of the HepaRG and HaCaT cells to the test chemicals for all assays. Both antimycin and etoposide induced oxidative stress at one hour, but not at 24 hours of exposure. Induction of ROS by menadione was similar between the two-time points. Both diquat and TBHP induced greater ROS at 24 hours and at lower concentrations compared to one hour exposures. Decreases in cell viability, as measured by CellTiter-Glo occurred at one hour for antimycin and menadione but not with the other test chemicals. At 24 hours of exposure decreases in cell viability were observed for all chemicals except etoposide, KCl, and sucrose. At 1 and 24 hours antimycin, menadione, and diquat altered the MMP assay in both cell types, while it took 24 hours for TBHP to alter the MMP assay. While antimycin induced ROS, decreases in cell viability and alterations in MMP occurred at lower concentrations. Changes in viability, MMP, and ROS occurred at similar concentrations for menadione and diquat; however for diquat, the ROS and viability changes required 24 hours of exposure. These studies suggest that when evaluating ROS using in vitro assays, multiple time points and positive controls may be necessary due to the differences in potential mechanisms of oxidative stress induced by the chemicals under evaluation.

2452 Effects of Oxidants on Human Superoxide Dismutase (SOD)

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Amyotrophic lateral sclerosis (ALS) is a progressive neurological disease, characterized by the gradual degeneration and death of motor neurons. In some dominant familial amyotrophic lateral sclerosis (FALS) pedigrees, mutations have been linked to a genetic defect for Cu, Zn-binding in superoxide dismutase (SOD). SOD catalyzes the dismutation of superoxide radical anion to hydrogen peroxide (H₂O₂) and oxygen (O₂). The modern view is that mutations in SOD1, the gene for Cu, Zn superoxide dismutase (CuZn-SOD), may be implicated in FALS. It is thought that the free radical generating activity by CuZn-SOD in the FALS mutant is enhanced relative wild-type human SOD possibly due to a decrease in the Kₘ value for H₂O₂. Previous reports also indicate that CuZn-SOD is capable of using high concentrations of H₂O₂ as a substrate to form hydroxyl radicals. In the present studies, we determined the treatment of human SOD with low concentrations of H₂O₂ (1-100 µM) in the absence of superoxide anion, SOD catalyzes the formation of hydroxyl radicals from H₂O₂. For these studies, we used a highly sensitive probe, diodeoxytrephephosphate (TPT), as a hydroxyl radical trap to quantify the formation of the free radical. TPT reacts with hydroxyl radicals to form 2-hydroxyterephthlate (2-OHTA)-H₂O₂ in a 1:1 ratio. 2-OHTA can be detected and quantified using a microplate reader with fluorescence detection (excitation and emission wavelengths, 310 and 425 nm). We found that SOD readily generates hydroxyl radicals from H₂O₂. Hydroxyl radical formation was time and concentration-dependent, concentrations of H₂O₂ used ranged from 1-100 µM. Hydroxyl radical formation was inhibited by the antioxidant, dimethyl sulfide. Based on these results, we conclude that human SOD is capable of catalyzing the formation of hydroxyl radicals from H₂O₂. In future experiments, we will use high performance liquid chromatography to determine if treatment of human SOD with lower, physiologically relevant concentrations of H₂O₂ also catalyzes the formation of hydroxyl radicals. This may be an area of further study to better understand the progressive nature of FALS and how SOD may be implicated in the disease process.

2453 Fracking Sand Dust Elicits ROS and Pro-Inflammatory Cytokines from Murine Macrophage Cells

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Hydraulic fracturing is used in the majority of natural gas wells across the United States. Water, sand, and chemicals are delivered at high pressure to drilled wells to cause fractures in the shale formations, allowing for the release of natural gas. Fracking sand, comprised mainly of silica dioxide (SiO₂), along with water and chemicals, is used to keep these fissures open. Silicosis is a pulmonary disease that affects workers exposed to inhaled silica and is characterized by inflammation and fibrosis, causing a decrease in lung capacity. Fracking sand dust (FSD) is generated during preparation of fracting fluid for injection. In this study, murine macrophage cells (RAW 264.7) were used to investigate whether pro-inflammatory signals are associated with inhaled FSD (<10 µm). We hypothesized that soluble and insoluble components in the FSD would each play a unique role in initiating pro-inflammatory responses and cytotoxicity. FSD was washed in PBS two separate times, 5 d each time, allowing for any soluble material to be released. On the 10th day of washing, sand that was twice washed was re-suspended in PBS (10 mg/ml) so that comparisons could be made to a freshly prepared, unwashed mixture. Production of the hydroxyl radical (OH), measured with electron paramagnetic resonance (EPR), was the highest in unwashed sand, followed by PBS from the 5 d and 10 d washes. Unwashed FSD sand also generated the most intracellular reactive oxygen species and the response was significantly larger than that obtained from FSD re-suspended after two consecutive washes. Cells were treated with a 1.2 and 1:10 dilution of stock solution. Compared to PBS controls, the viability of RAW 264.7 cells decreased by 40% following exposure to FSD that was washed and re-suspended after 10 days, whereas unwashed sand decreased viability by 30% over a 24 h period. Finally, production of the pro-inflammatory cytokines TNFα, IL-1β, and IL-6 were measured using ELISA. While IL-1β and IL-6 production decreased with washing, TNFα production remained elevated. Our results indicate that FSD is cytotoxic to RAW 264.7 cells, as evidenced by decreases in viability, and stimulates intracellular ROS and OH production. The substantial differences in the production of cytokines stimulated by the soluble and insoluble components of FSD warrants future studies of the pro-inflammatory effects of the dust.
Diarhoea is one of the most common health conditions affecting people globally. However, less developed countries are most susceptible. Therefore, identification of new source of antidiarrhea drugs becomes one of the most prominent focuses in modern research. This study was aimed at evaluating the antidiarrhoea activity of methanol stem extract and solvent fractions of Terminalia catappa using two different animal models. Phytochemical screening was qualitatively performed on the methanol extract using standard method, followed by partitioning to get the chloroform and aqueous fractions. Antidiarrhoea effect was evaluated by stooling test using castor oil-induced mice and charcoal transit assay using rats. 100mg/kg, 200mg/kg, and 400mg/kg of the methanol extract and 80mg/kg and 50mg/kg of solvent fractions were used for both antidiarrhoea studies, while loperamide (10mg/kg) and atropine (0.6mg/kg) were administered as positive control, distilled water (2ml/kg) and normal saline (2ml/kg) were administered as negative control, respectively, for both animal models. However, the presence of phenol, alkaloid, flavonoid, tannins, terpenoids, steroids, and cardiac glycoside was observed. The plant extracts (methanol, aqueous, and chloroform) showed pronounced and dose-dependent antidiarrhoeal activity. Oral doses of 100, 200, and 400mg/kg of methanol extract showed percentage inhibition of 37.9%, 46%, and 100%, respectively. 80 and 50mg/kg of aqueous and chloroform fractions showed percentage inhibition of 14.7% and 9.3%, respectively, and were comparable to that of the reference drug, loperamide (10mg/kg). The methanol extract (100 and 200mg/kg) produced a decrease in intestinal transit (28.5% and 35.3%, respectively); the aqueous and chloroform fractions (80 and 50mg/kg) also showed decrease in intestinal transit (24.6% and 29.5%) and were comparable to atropine (0.6mg/kg). The results showed that the extract and fractions of T. catappa has a significant antidiarrhoeal activity, which supports its use in traditional herbal medicine practice.

Thiol-Containing Compounds Attenuate Oxidative Stress and Neuronal Hyperexcitability In Vitro

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Chemical agents such as industrial chemicals, pesticides, and chemical warfare agents can induce uncontrolled seizure activity (neuronal hyperexcitability). Oxidative stress has been implicated as a pathogenic factor in the etiology of seizures and epilepsy. However, whether and how cellular redox status modulates neuronal hyperexcitability is unclear. We hypothesized that the modulation of cell redox status with thiol-containing compounds would decrease oxidative stress, and attenuate neuronal hyperexcitability in vitro. 3,3-dimercaptopropionic acid (DMP), a thiol-containing compound showed percentage inhibition of 14.7% and 9.3%, respectively, and were comparable to that of the reference drug, loperamide (10mg/kg). The methanol extract (100 and 200mg/kg) produced a decrease in intestinal transit (28.5% and 35.3%, respectively); the aqueous and chloroform fractions (80 and 50mg/kg) also showed decrease in intestinal transit (24.6% and 29.5%) and were comparable to atropine (0.6mg/kg). The results showed that the extract and fractions of T. catappa has a significant antidiarrhoeal activity, which supports its use in traditional herbal medicine practice.

Mechanistic Role of Human NQO1 Promoter Polymorphisms in Oxygen-Mediated Toxicity to Human Lung Cells

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Oxidative stress following exposure to high concentrations of inspired oxygen is a known risk factor in the pathogenesis of acute lung injury and bronchopulmonary dysplasia (BPD). NAD(P)H quinone oxidoreductase 1 (NQO1) is a phase II/antioxidant enzyme involved in the formation of reactive oxygen species. Previous reports have demonstrated that the A/C single nucleotide polymorphism (SNP) at -1221 of the NQO1 promoter resulted in decreased in vitro transcription of luciferase in room air, and following exposure to hyperoxic conditions. However, little is known regarding the functionality or role of SNPs in oxygen toxicity. In this investigation, we tested the hypothesis that H441 human lung cells transfected with plasmids containing the A/C SNP at -1221 in the NQO1 promoter will demonstrate less protection against oxidative stress, and attenuate neuronal hyperexcitability compared to cells transfected with wild type promoter constructs. Human NQO1 with a CMV, wild type (WT) or -1221 SNP-containing promoter was subcloned into the pcDNA3.1 plasmid and confirmed by DNA sequencing. Overexpression of NQO1 protein was confirmed by western blotting using H441 human lung cells transfected with NQO1-containing plasmids. Cell viability assays and cellular apoptosis assays were performed following incubation of control and NQO1 transfected lung cells in room air or 80% oxygen conditions for 24 hours. In another set of experiments human lung cells transfected with plasmids containing NQO1 were pretreated with BNF or sulforaphane prior to exposure to hyperoxia. Our results showed that cell viability was enhanced by transfection of cells containing the WT NQO1 promoter compared to the SNP-containing NQO1 promoter under hyperoxic conditions. Transfection with the WT NQO1 promoter, but not the SNP-containing NQO1 promoter was protective against hyperoxia-induced cell death. Additionally, Pretreatment with BNF or sulforaphane decreased cell death under hyperoxic conditions in cells transfected with the WT NQO1 or the SNP-containing promoter, but the extent of protection was lesser in cells treated with the latter. These results suggest that NQO1 expression driven by the -1221 C NQO1 promoter decreases cell oxygen toxicity compared to the WT promoter. Further studies could lead to development of rational strategies for the prevention and/or treatment of BPD in premature infants.

Suppression of Nonsense-Mediated MiRNA Decay under Environmental Stresses

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Nonsense-mediated mRNA decay (NMD) is a cellular mechanism eliminating mRNAs that harbor premature translation termination codons (PTCs). It is known that several stress-induced genes are NMD targets because of upstream open reading frame (uORF), alternative mRNA splicing that introduces nonsense codons, or uncharacterized mechanisms. The inhibition of NMD stabilizes these mRNAs and aug-
ments the cellular stress responses. Here we investigated the effect of environmental stresses such as methylmercury (MeHg) exposure and endoplasmic reticulum (ER) stress (thapsigargin (TPTG)) on the activity of NMD using mouse myogenic cells, rat cerebral cortical neuronal cells (CNC) and astroglial cells (AGC). NMD suppression, evidenced by upregulation of SlincR1 or GASS mRNA harboring PTC and phosphorylation of UPF1, was observed in MeHg treated myogenic cells and CNC whereas NMD suppression was weak in MeHg-treated AGC. In order to know the causative factor of stress-induced NMD suppression, the role of phospho-eIF2α/ATF4 pathway, a key pathway involved in adaptation to stresses, was investigated. Knockdown studies demonstrated that phosphorylation of eIF2α/ATF4 was an upstream factor of NMD suppression used under mild ER stress whereas ATF4 expression was not, suggesting that phosphorylation of eIF2α plays a role in the induction of NMD suppression. Further we investigated the effect of NMD suppression on cellular MeHg content. MeHg can be transported to cells as MeHg-cysteine complex via an anti-cystic transporter or transported through an ATP-binding cassette (ABC) transporter such as ABCC4 which functions the efflux of glutathione conjugates. We previously reported that integrated stress responses change the expression of these membrane transporters. Here we demonstrate that NMD suppression and ATF4 expression under mild ER stress affect the intracellular mercury content using synthetic siRNA-mediated knockdown study.

2458 Phosphorylation of eIF2α Promotes Cell Survival in Response to Benzo(a)pyrene Exposure

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Translational control is a mechanism which allows quick cellular adjustments promoting cell survival during stress situations such as hypoxia, starvation, and irradiation. Currently, the relationship between translational control and xenobiotic stress is poorly understood. In this study, the importance of the unfolded protein response (UPR) for cellular adaptation to the environmental carcinogen benzo(a)pyrene (BaP) was determined. Activation of the UPR results in phosphorylation of eukaryotic translation initiation factor 2 alpha (eIF2α) via the kinase PERK and subsequently affects global and gene specific mRNA translation. Mouse embryonic fibroblasts (MEFs) carrying the wild-type (WT) eIF2α allele or a severe to alanine mutant allele (SSA) were used in this study. SSA- MEFs cannot phosphorylate eIF2α and are therefore impaired in regulating mRNA translation upon UPR activation. We found that SSA- MEFs are 2.8 fold more sensitive to 10 μM BaP treatment compared with WT-MEFs in clonogenic assays. Furthermore, BaP exposure reduced cell proliferation to a larger extent in SSA-MEFs compared to WT-MEFs. To explain the differences in cell survival between SSA- and WT-MEFs DNA damage after BaP exposure was measured with the COMET assay. DNA damage in SSA-MEFs was increased after BaP exposure compared to WT-MEFs, suggesting that SSA-MEFs either accumulate more DNA damage or are impaired in DNA damage response upon exposure. We found no evidence of ROS formation after BaP exposure, nor did co-treatment with antioxidants rescue SSA-MEFs from BaP-induced toxicity. Overall, our study demonstrates the importance of signaling through eIF2α in the response to the classical chemical carcinogen BaP and suggests a connection between eIF2α signaling and the DNA damage response.

2459 The Role of a Novel Long Noncoding RNA in the Regulation of Sox9b and Its Contribution to TCDD-Induced Toxicity Endpoints


In zebrafish, sox9b has been determined to be one of the most-reduced transcripts upon activation of the aryl hydrocarbon receptor (AHR) by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). We identified a novel long noncoding RNA named sox9b long intergenic non-coding RNA (slincR) located adjacent to sox9b, and previously demonstrated that slincR expression increases in an AHR2-dependent manner in response to multiple strong AHR ligands. SlincR is required for proper expression of sox9b and its known downstream targets, and reduced slincR expression significantly affects some aspects (i.e., neuromuscular and somitogenesis of elongation) of development. In the current study, we performed a comprehensive capture hybridization analysis of RNA targets (CHART) RT-qPCR to determine if slincR binds to the sox9b promoter, and found that slincR is enriched at the 5' untranslated region (UTR) of the sox9b locus in 48hpf DMSO and TCDD-exposed zebrafish. To understand slincR’s role in the TCDD-induced toxicity pathway, we performed RNA sequencing and clustered gene ontology enrichment analysis on 48hpf control and slincR morphants exposed to 0.1% DMSO or 1ng/mL TCDD. We identified significant enrichment in processes related to skeletal and cartilage development unique to TCDD-exposed control morphants, and angiogenesis and vasculature development unique to TCDD-exposed slincR morphants. To further investigate slincR’s role in TCDD-induced toxicity phenotypes, we evaluated the cartilage of TCDD-exposed 72hpf zebrafish, and found a significant difference in the structure of the craniofacial cartilage in slincR morphants compared to control morphants. Additionally, a blood hemorrhage screen showed that TCDD-exposed slincR morphants at 48hpf had a lower proportion of hemorrhaging compared to TCDD-exposed control morphants. We also mined unpublished and published data from 16 PAHs, and found 6 PAHs caused a significant increase in both cyt1a and slincR expression, of which 3 are from the EPA’s priority PAH list. Collectively, our results suggest that slincR regulates sox9b expression by binding to the 5'UTR of the sox9b locus, regulates cartilage development, contributes significantly to TCDD-induced angiogenesis, and is upregulated by multiple environmentally relevant PAHs. This study was supported by the SRF Grant P42 ES016465, NIEHS Core Center Grant P30 E000210, and NIHES Training Grant T32 ES007060.

2460 Hepatocyte-Specific Deletion of Tiparp Increases Sensitivity to Dioxin-Induced Hepatotoxicity and Lethality

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The aryl hydrocarbon receptor (AHR) mediates diverse cellular responses to numerous phytochemicals, metabolites and environmental contaminants, such as dioxin (2,3,7,8-tetrachlorodibenzo-p-dioxin; TCDD). Dioxin causes a range of toxic responses in laboratory rodents, including thymic atrophy, hepatotoxicity and a lethal wasting syndrome. Although the mechanisms of dioxin-toxicity remain unknown, AHR signaling in hepatocytes is necessary for dioxin-induced liver toxicity. We have previously reported that loss of Tiparp, an ATP-dependent poly(ADP-ribose) polymerase (TIPARP/PARP7/ARTD14), an AHR target gene and mono-ADP-ribosyltransferase, increases the sensitivity of mice to dioxin-induced toxicities. To test the hypothesis that Tiparp is a negative regulator of AHR signaling in hepatocytes, we generated Tiparpfl/fl mice where exon 3 of Tiparp was flanked by loxP sites. Using Cre-lox technology, we created hepatocyte-specific Tiparp (Tiparpfl/flCreα/α) and full knockout mice (Tiparpfl/flCreα/α; referred to as Tiparp1/1) by crossing with mice expressing Albumin Cre or CMV Cre, respectively. Tiparpfl/1 mice given a single injection of 10 μg/kg dioxin did not survive beyond day 7 and 9, respectively, whereas Tiparp null mice treated with 100 μg/kg did not survive beyond day 3. All Tiparp1/1 mice survived the 30-day treatment. Tiparpfl/1Creα/α or Tiparp1/1 mice treated with 10 μg/kg dioxin displayed increased hepatotoxicity and hepatotoxicity as indicated by increased alanine aminotransferase activity compared with similarly treated wildtype mice. Tiparpfl/1Creα/α or Tiparp1/1Creα/α mice exhibited increased Ahr signaling denoted by increased expression of dioxin-induced gene expression. The ability of Tiparp to mono-ADP-ribosylate and repress AHR activity was also found to be conserved among dioxin-sensitive and insensitive species. Taken together, these data illustrate that Tiparp is a key negative regulator or repressor of AHR activity and its specific loss in hepatocytes is sufficient to increase the sensitivity to dioxin-induced hepatotoxicity and lethality. The data also reveal that the AHR-Tiparp axis is conserved among species.

2461 Nrf2 Regulates Redox-Responsive Transcription of HIF1A

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Reactive oxygen species (ROS) are important signaling molecules for the cell, however, if left unchecked, ROS can lead to sustained damage of cellular macromolecules and cell death. Fortunately, cells have evolved mechanisms to combat this potentially harmful condition. Nrf2 is a transcription factor that regulates the cellular response to oxidative stress. Once activated by ROS, NRF2 translocates to the nucleus and upregulates the transcription of dozens of potential target genes. Recently we found evidence that Hypoxia Inducible Factor 1α (encoded by HIF1α) transcription is controlled by NRF2 and ROS. Like NRF2, HIF1α is also a redox-responsive transcription factor, only it controls expression of an arsenal of proteins in response to hypoxia. We found that treatment with ROS inducing compounds (menadione and tert-butyl hydroperoxide) leads to significant increases in HIF1α expression in three cell types (HEPG, MCF7, and MDA-MB-231). For all three cell types, MDA-MB-231 cells treated with the EC50 of menadione had significantly increased rel-
The polymorphic nature of the human 3'IGHRR is polymorphic due to an invariant sequence (IS) mouse studies these enhancers are thought to collectively play a role. Each 3'IGHRR contains three enhancers (hs3, hs1.2, hs4). Based on production of immunoglobulins (Ig) is partially controlled by the Ig heavy chain gene (IgH). The IgH gene consists of two regulatory regions or transcription units. Each 3'IGHRR contains three enhancers (hs3, hs1.2, hs4). Based on mouse studies these enhancers are thought to collectively play a role in the regulation of IgH expression and class switch recombination to different Ig isotypes (IgG1-4, IgA1-2, IgM, IgE). The hs1.2 enhancer within the human 3'IGHRR is polymorphic due to an invariant sequence (IS) that is repeated one to four times in tandem. The polymorphic nature of the hs1.2 enhancer is of interest due to its association with human autoimmune disorders and its sensitivity to xenobogenous chemicals, including 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Previous work in mouse models has shown TCDD-induced inhibition of the hs1.2 enhancer and 3'IgHRR, correlating with inhibition of secreted Ig. However, TCDD has been shown to activate the human hs1.2 enhancer and produce different effects on Ig secretion; i.e., decreased IgG and increased IgE. These results highlight the potential species differences in hs1.2 activity and 3'IgHRR function. Our objective is to understand the role of the hs1.2 polymorphism in the expression and production of different Ig isotypes and sensitivity to TCDD. Using CRISPR/Cas9 gene editing with an altered hs1.2 genotype that corresponded to changes in basal and stimulated IgM and IgG secretion, as well as changes in the different Ig isotypes with corresponding increases or decreases were determined utilizing new nanopore sequencing technology by which large intact amplicons (8-kb) can be sequenced without fragmentation. Implementation of this new sequencing technique has been vital for providing long-range sequencing data that is impossible to achieve with standard sequencing due to the redundant sequences within and flanking the hs1.2 enhancer. Ongoing efforts are focused on utilizing the hs1.2-edited clones to determine the role of the hs1.2 genotype in the sensitivity of each Ig isotype to TCDD. Connection of the functional effects to a specific genotype of the hs1.2 enhancer may influence the sensitivity of Ig expression to TCDD in humans.
cell culture screening model identified novel genes that are relevant for neuronal and astrocyte homeostatic adjustment to cellular energy loss. The established cellular screening model is also applicable for testing genetic variability outcome paradigms under various environmental or chemical exposure settings.

2466 LncRNA MALAT1 Regulates Xenobiotics-Induced Toxicity in Pancreatic Islets and Insulin Resistance

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The metasis-associated lung adenocarcinoma transcript 1 (MALAT1) is a long noncoding RNA and its overexpression is associated with the development of many types of malignancy. MALAT1 null mice show no overt phenotype. However, in transcriptome analysis of MALAT1 null hepatocytes and pancreatic islets we found significant upregulation of antioxidant related genes including nuclear factor-erythroid 2 p45-related factor 2 (Nrf2), Nqo1, Sod1, Nos2 with significant reduction in reactive oxygen species (ROS) as indicated by the suppression of carbonylation level in proteins from hepatocyte and islets. Nrf2 is a master regulator of the antioxidant genes and we performed LncRNA pulldown assay using biotinylated antisense oligonucleotides against MALAT1 and found MALAT1 interacted with Nrf2, suggesting Nrf2 is transcriptionally regulated by MALAT1. Exposure to excessive ROS has been shown to cause insulin resistance through activation of c-Jun N-terminal kinase (JNK) which leads to inhibition of insulin receptor substrate 1 (IRS-1) and insulin-induced phosphorylation of Akt. We found MALAT1 ablation suppressed JNK activity with concomitant insulin-induced activation of IRS-1 and phosphorylation of Akt suggesting MALAT1 regulate insulin responses. MALAT1 null mice exhibited favorable insulin-sensing response to fast-refeeding and glucose/insulin challenges and significant increases in insulin secretion in response to glucose challenge in isolated MALAT1 null islets, suggesting an increased insulin sensitivity. Based on the role of ROS in the development of T2DM, we developed a pancreatic islet cell-based assay for screening diabetogenic environmental chemicals. Using this method, we found that bisphenol A (BPA), Benzo[a]pyrene (Bap), and polychlorinated biphenyls (PCBs), could induce high level of ROS suggesting that they may potentially induce damage in pancreatic cells. In summary, we demonstrate that MALAT1 plays an important role in regulating insulin sensitivity and has the potential as a therapeutic target for the treatment of diabetes as well as other diseases caused by excessive exposure to ROS.

2467 Nuclear Receptor 4A1 (NR4A1) Ligands Exhibit Antidiabetic Activity in Pancreatic β-Cells

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Pancreatic β-cells play a key role in maintaining cellular homeostasis by sensing glucose levels and secreting insulin to maintain normoglycemia but in type 2 diabetes the β-cell function is compromised. In this study we used INS-1 rat β-pancreatic cells to investigate the antidiabetic activity of NR4A1 ligands. Real-time PCR and western blot analysis of INS-1 β-cells lysates show that NR4A1, NR4A2, and NR4A3 are expressed in these cells at the mRNA and protein level. RNA interference (RNAi) experiments show that knockdown of NR4A1 in INS-1 β-cells resulted in a >35% decrease in proliferation; whereas knockdown of NR4A2 (Nurr1) or NR4A3 (NurI) resulted in a 20% and <5% decrease in proliferation of INS-1 β-cells. In previous studies we identified 1,1-bis(3’-indolyl)-1-(p-hydroxyphenyl) methane (C-DIM8/DIM-C-pPhOH) as an NR4A1 ligand that exhibit selective NR4A1 modulator activities in cancer and non-cancer cell lines. C-DIM8 and related NR4A1 ligands decreased proliferation of INS-1 β-cells and thus resembled the responses observed after knockdown of the receptor. Incubation of INS-1 β-cells in low (3.3mM) and high (22.2mM) glucose resulted in an increase in secretion of insulin from these cells and co-treatments with C-DIM8 and related compounds further increased insulin secretion only in the cells maintained on high glucose. The role of NR4A1 and its ligands in modulation of stress-induced responses, mitochondrial respiration, and insulin secretion in INS-1 β-cells and other β-cell lines, the applications of bis-indole-derived NR4A1 ligands as a novel class of antidiabetic drugs will be discussed.

2468 Benzo(a)pyrene Regulates Gene Expression by Modifying mRNA Translation


Controlling mRNA translation initiation is an important mechanism for modulating protein expression, especially under stressful conditions that require rapid responses. Still, the relevance of translational control for the response to xenobiotics has been poorly addressed. Therefore, the goal of this study was to analyze the contributions of changes in mRNA translation to differential gene expression on a genome-wide scale. Primary rat hepatocytes were exposed to 1 and 10 μM benzo(a)pyrene for 1 and 24 hours. At each time point, mRNAs were isolated using sucrose gradients to obtain 4 fractions with increasing translation rates. Next, RNA sequencing was performed on these 4 fractions as well as on the total mRNA for each time point to determine gene-specific changes in mRNA translation. From these data, we determined both transcriptional changes (mRNA abundance) and translational changes (translated mRNA) in response to BaP. Surprisingly, 1h exposure to 10 μM BaP only resulted in differential gene expression for 4 genes due to changes in mRNA abundance and for 6 genes due to differential translation. While after 24h exposure to BaP, 190 genes were altered with respect to mRNA abundance (FDR<0.05) and +2000 genes were differentially translated. Based on this analysis we were able to distinguish 3 categories of genes; (i) genes with concordant changes in mRNA abundance and mRNA translation, (ii) genes with altered mRNA abundance only and (iii) genes with changes in mRNA translation only. GO term analysis showed that genes in the “mRNA translation only” category were involved in specific biological processes including translation, apoptosis and oxidation-reduction process. Genes from the “concordant” group were mainly associated with BaP metabolism and anti-oxidant response. To test the robustness of these findings we validated 3 genes from each category by means of qPCR. All 9 genes tested revealed similar expression changes. We are currently investigating the basis for this selective translation after BaP exposure. This includes the presence of upstream open reading frames within the 5’translated region of these genes. In conclusion, our data reveal that gene expression changes in response to BaP occur more on the levels of mRNA translational than on the level of mRNA abundance.

2469 Identifying Mitochondria Cell Signaling Networks by Functional Profiling

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In order to maintain homeostasis and safeguard cellular fitness, permanent communication between the mitochondria and the nucleus, through tightly regulated signaling pathways, is indispensible. This ensures synchronizing the mitochondrial function with the ever-changing cellular requirements. Mitochondrial biogenesis is influenced by a myriad of intrinsic and extrinsic factors, where a complex network of pathways dictates the response. Such pathways include the anterograde, nucleus to mitochondria, and the retrograde, mitochondria to nucleus, communications. While the anterograde communications are more studied, little is known about the retrograde pathways. In our study we aim to explore mitochondrial-nuclear signaling centers using functional profiling on Saccharomyces cerevisiae, in an attempt to identify key components involved in responding to certain mitochondrial stressors. We individually targeted the mitochondrial electron transport chain complexes III, IV, and V by using complex-specific inhibitors, formic acid, and oligomycin, respectively. We screened a pool of ~4,600 different yeast mutants using the 20% inhibitory concentration (IC20s) of the mentioned compounds in YPL (2% lactate) media for three time points (5, 10, and 15 generations). Each yeast mutant is tagged with a unique barcode sequence that can be used to identify and quantify the abundance of the mutant by next-generation sequencing. By comparing barcode reads of vehicle controls to treated samples, we identified genes involved in the biological response to the mitochondrial dysfunction that play a major role in the capability of the yeast cell to survive the stress. Based on the results we speculate that the retrograde pathways are involved in the response to the stress produced by oligomycin since knockouts of genes involved in these pathways show increased sensitivity to oligomycin. On the other hand, deleting components of the EGO complex leads to oligomycin resistance while increasing sensitivity to formic acid. We also observed that the RIM pathway is involved in response to complex III inhibition but additional work should be done to confirm this link and understand how it works. Our work revealed stressor-specific differences in signaling requirements, suggesting a nuanced response to mitochondrial dysfunction and communication between the mitochondria and the nuclear genome.
2470 Circadian Regulation of AhR Induced Cyp1a1 Gene Expression Is Dependent upon P53 Binding Activity

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The circadian clock plays a key role in coordinating many biological processes including physiological processes and cellular metabolism and mitosis. Despite the fact that circadian rhythms are associated with critical biological processes, very little is known about their mechanism in carcinogen metabolism, endocrine disruptor activity and mammary carcinogenesis. Furthermore, recent studies have linked disruption of circadian rhythms in breast cancer cell lines to increased breast cancer risk, suggesting that, when working alternative shifts may have a higher risk of breast cancer. We have found that time of day, as well as individual circadian clock components, uniquely regulate processes governing polyaromatic hydrocarbon (PAH) metabolism in the mouse mammary gland and breast cancer cell lines. We have shown in vitro and in vivo that the pattern of PER1, BMAL1, and AhR-induced Cyp1a1 gene expression and PAH metabolism is similar to BaP-induced Cyp1a1 and Cyp1b1 gene expression. Despite time dependent regulation of Cyp1a1 gene expression, we found no change in AhR binding over time. Here we show that circadian regulation of p53 binding activity is responsible for time-dependent differences in Cyp1a1 and/or AhR induced target genes. Therefore, we hypothesize that a link between the circadian clock, specifically PER2 and p53, plays an essential role in PAH metabolism.

2471 Finding the Source of AhRR Expression in Whole Blood

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The responsome is the collection of all physiological responses that can be influenced by the multitude of chemical and environmental stimuli to which humans are exposed. Understanding the responsome can be used to identify chemical exposures and determine their effects on human health. MiRNA can be used in this manner to serve as a biomarker of chemical exposure in human populations. We had previously proposed to define the human responsome though changes in gene expression caused by activation of the aryl hydrocarbon receptor (AhR) pathway in the blood of cigarette smokers, who chronically expose themselves to high levels of polycyclic aromatic hydrocarbons. We found that the aryl hydrocarbon receptor repressor (AhRR) gene was significantly upregulated in the two independent populations of cigarette smokers. In order to improve the sensitivity of the assay, we set out to determine if there was a specific cell type in the whole blood that was responding to the chemical insult. We created a model of AhRR induction in the mouse using beta-naphthoflavone (BNF), a nontoxic AhR agonist that more closely imitates PAHs than the classic AhR agonist 2,3,7,8-tetrachlorodibenzo-p-dioxin. Using our model of AhRR induction by BNF and magnetic cell separation and other techniques, we have determined that the AhRR induction occurs in a subset of the leukocyte fraction of the whole blood.

2472 Activation of PXR Modulates the Expression of Long Noncoding RNAs in Mouse Liver

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Long non-coding RNAs (lncRNAs) are novel regulators of cell physiology, including gene transcription and translation. Dysregulation of lncRNAs by environmental chemicals, such as heavy metals, solvents, and polycyclic aromatic hydrocarbons, modulate the expression of xenobiotic biotransformation related genes and may serve as novel biomarkers for exposure. However, little is known regarding the transcriptional regulation of lncRNAs. The pregnane X receptor (PXR/NR1I2) is a critical regulator of p53 binding activity, and may serve as important biomarkers for oxidative stress. Therefore, lncRNAs regulated by PXR activation and that they may be important regulators of xenobiotic metabolism.

PXR-DNA binding. Surprisingly, the most frequent PXR-DNA binding motif was direct repeat with a 1bp spacer (DR-1), but not DR-4 as was previously reported. Among lncRNAs induced by PCN, PXR was predicted to co-localize with hepatocyte nuclear factor 4 alpha (Hnf4α), nuclear receptor subfamily 2 group member 2 (Nr2z2/Tak1), Nr2f1 (Coup-Tf1), Nr2f6 (Coup-Tf3), peroxisome proliferator activated receptor gamma, and retinoid X receptors. There was limited overlap of PXR binding with the epigenetic mark for transcriptional activation (histone-H3K4-di-methylation, H3K4Me2), but no overlap with the epigenetic marks for transcriptional silencing (H3K27Me3 and DNA methylation). Among differentially expressed lncRNAs, 264 were in proximity of PCGS, and gene ontology and differentiation analysis using STRING showed an enrichment for response to oxygen-containing compounds. This study was among the first to demonstrate that lncRNAs are regulated by PXR activation and that they may be important regulators of xenobiotic metabolism.

2473 Myomir Regulation of Pulmonary Response to Smoking and Diet-Induced Obesity

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Smoking and obesity are two of the most prevalent risk factors for human morbidity and mortality. We previously observed a combined effect of mainstream cigarette smoke and diet-induced obesity on markers of inflammation and oxidative stress in the lung of exposed mice. Recent literature suggests an important role for miRNAs in regulating acute inflammation in the lung associated with toxicity and chronic inflammation associated with lung disease and obesity. To better understand the specific role of miRNAs in response to both risk factors (individually and in combination), the expression levels of miRNAs in the lung of regular weight and diet-induced obese mice exposed to mainstream cigarette smoke were evaluated by RNA sequencing. A family of miRNAs, known as myomirs, was significantly lower (p<0.05) elevated in obese mice compared to regular weight mice and was subsequently repressed (q<0.05) in obese mice after exposure to cigarette smoke. Differential expression of myomir miR-133a/b, miR-1a, miR-206 by obesity and smoke was confirmed by quantitative PCR. Predicted gene targets of significantly altered miRNAs were integrated with expressed and quantitatively expressed mRNA gene expression to determine the functional consequences of miRNA regulation in mouse lung. Myomir regulation was associated with significant (p<0.05) alteration of inflammatory and immune response pathways in mouse lung suggesting they are important regulators of inflammation in the lung in response to environmental insult. Previously, myomirs have been associated with inflammatory myopathies and adipogenesis and were measured in plasma of COPD patients. These studies are the first to identify the role of miRNAs are regulators of inflammation and oxidative stress mechanisms in obese mice that may make them more vulnerable to environmental lung toxicants.

2474 Akt Involvement in p21 Activation Induced by Mitomycin C and Its Analog


Cancer with normal p53 activity is commonly treated with Mitomycin C (MC), an anti-tumor drug that treats a specific type of cancer, whose pharmacological mechanism has been studied thoroughly. MC is a well-known DNA alkylating agent, where alkylation occurs both monofunctionally and bifunctionally. When cells are treated with MC, the main DNA inter-strand crosslink (ICL) is generated. However, little is known regarding the transcriptional regulation of ICL. The pregnant X receptor (PXR/NR1I2) is a critical xenobiotic-sensing nuclear receptor that regulates the expression of many drug-processing genes. To test our hypothesis that ICLs are regulated by PXR in concert with PXR-targeted protein-coding genes (PCGs), adult male C57BL/6 mice were treated with corn oil or the PXR agonist PCN (n=3), and RNA from the livers were subjected to RNA-Seq using poly-A tail and TruSeq v3 chemistry (Illumina HiSeq2000). Data analysis and visualization was performed using R and the edgeR package. This p21 activation triggered by MC and DMC is p53 independent. The purpose of this study is to elucidate the signaling pathway that facilitates p21 activation in MCF-7 and K562 cells. The Akt signaling pathway plays an important role on p21 regulation by phosphorylating p21 at threonine 145 (T145), resulting in the cytoplasmic localization of p21 and its degradation. This study focused on revealing how Akt is involved in MC- and DMC- triggered p21 activation. The results exhibited that MC and DMC inhibited Akt activation in MCF-7 cells, but not in K562 cells. When the p53 expression of MCF-7 cells was knocked down by p53 shRNA, the inhibition of the Akt activation in MCF-7 cells was alleviated. This implied that the deactivation of Akt caused by MC and DMC was p53 dependent. When MCF-7 cells were pre-treated with the Akt acti-
High throughput in vitro toxicity testing of multitudinous chemicals across any number of biological endpoints allows for rapidly assessing human and ecosystem health impacts, thus reducing resources associated with traditional animal testing. For application in risk assessment, it is necessary to compare chemical concentrations sufficient to produce bioactivity in vitro with those capable of perturbing a relevant in vivo target, as determined by exposure factors and pharmacokinetic (PK) properties. This work presents a variety of computational approaches demonstrating how uncertainty in exposure and PK properties can influence application of in vitro dose-response data in risk assessment. First, qualitative screening of chemicals with in vitro acetylcholinesterase inhibition activity was achieved through in silico prediction of ADMET properties to assess potential for reaching the target enzyme. This analysis resulted in a 33% reduction in the number of candidates, and the remaining compounds were further ranked based on quantitative estimates of in vivo bioactivities derived using a PK/pharmacodynamic (PD) model. Next, with sufficient exposure and PK data, external points of departure were predicted for six thyroid peroxidase inhibitors using a physiologically based PK/PD model. It was found that investigating exposure, potency, or PK individually is insufficient for evaluating chemical risk. Finally, potential metabolites were predicted for chemicals shown to be inactive across eighteen estrogenic assays and validated in vitro assays, and ER binding activity for these potential metabolites was predicted using a quantitative structure activity relationship model. Of the 1,400 inactive parents investigated, 20% were found to have at least one metabolite predicted to be active. The integration of in vitro testing with computational approaches will help to improve confidence in evaluation of chemical risk by identifying those chemicals requiring no further testing and those inactive parents capable of producing potentially active metabolites. Disclaimer: The views expressed in this abstract are those of the authors and do not represent Agency policy or endorsement.
Novel Typing System of Bacteria by Their Intrinsic Ability to Utilize Amino Acids

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Amino acids (AAs) are critical substances for nitrogen and carbon metabolism in bacteria. Different bacteria may have distinctive abilities in utilizing amino acids, depending on their genetic and biochemical differences. Therefore, the intrinsic ability for AAs consumption presents a novel mechanism to distinguish bacteria species by characteristic AAs consumption pattern. More than 50 bacterial strains, including isolates of Pasteurella multocida and Escherichia coli, Pasteurella multocida, Salmonella enterica and Pseudomonas aeruginosa, were evaluated for their intrinsic ability to consume 20 essential AA individually. The bacteria were cultured in minimum-nutritional media containing 3 mM of each AA for 18 hours and the supernatant was analyzed for the remaining AA concentration by flow-injection analysis. In a first phase, a subset of in vivo assays statistically associated with a specific in vivo outcome was selected and curated. Results of these assays were then used as descriptors of a machine learning model predicting the outcome of interest. In a second phase, QSR models were developed to predict each in vivo assay selected. Finally, the two types of models were linked to predict the in vivo outcome for a new chemical structure. QSR models allowed the prediction of in vivo bioactivities and these predictions were then used as input of the in vivo outcome predictive model. In general, each in vivo outcome of interest led to its own predictive model since the in vivo assays were chosen according to this outcome. This global approach relies on different choices for several key aspects (machine learning algorithms, bioassays selection methods, qualitative vs quantitative data, biological interpretation, etc.), which were assessed based on the first results obtained using public data. The evaluation of the approach was done using a reference dataset consisting of 418 compounds for which bioactivity data and chemical structures were available to predict hepatotoxicity observed in rat carcinogenicity studies.
Differentiation and subtyping of bacteria can be done by many approaches. In the present study, we demonstrated a novel bacterial differentiation system solely based on their amino acid (AA) consumption characteristics. The bacteria were cultured in minimum-nutritional medium containing single AA and a flow-injection analysis system emploting electrochemical detection was used to quantitate the AA consumption by bacteria. The results suggested that differential AA utilization profile presents a new approach to differentiate between (1) toxigenic vs non-toxigenic strains of Pasteurella multocida, (2) type A vs type D P. multocida, (3) different serovars of Salmonella enterica subsp. enterica, and (4) methicillin-resistant Staphylococcus aureus (MSSA) vs methicillin-resistant S. aureus (MRSA). The consumption of valine and methionine showed striking contrast between P. multocida toxin-producing and non-producing strains. P. multocida type A and type D strains utilized isoleucine, tryptophan, tyrosine, arginine and histidine in the opposite manner. Furthermore, five serotypes of Salmonella serovars and MSSA and MRSA as well, exhibited different AAs consumption preferences. In summary, different bacteria subtypes/groups may consume AAs differently and these characteristics were significant enough to afford the method as a new bacteria subtyping system. Further studies are warranted and may prove that the AA utilization profile has a great potential to be an alternative tool for subtyping/grouping of bacteria that are not conveniently identified with traditional methods.

Measurements of In Vitro Neural Network Activity Are Influenced by the Number of Electrodes in Micro-Electrode Arrays

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Multi-well micro-electrode array (mwMEA) recordings are used to screen compounds for the potential to disrupt neural network function. Conventional MEA formats use 60-64 electrodes to detect and record activity within a neural network, but mwMEA plates have different well configurations containing varying numbers of electrodes (i.e., 12-well = 64 electrodes/well, 48-well = 16 electrodes/well, 96-well = 8 electrodes/well). It is unclear how measures of neural network activity may be influenced by the number of electrodes used to collect the data. Therefore, the goal of this study was to determine how network activity parameters changed depending on the number of electrodes used to measure network function. The present study used 12-well mwMEA plates (n=2) to measure two network parameters: mean firing rate (MFR) and network spikes (NwSpk). Rat primary cortical cells express stable spontaneous electrical activity after 12-15 days in vitro (DIV) in the form of individual spikes and bursts. On DIV 14 or 34 initial baseline (BL) data were recorded and MFR and NwSpk, number of electrodes (#AE), and concentration were calculated and analyzed using an in-house analysis program. Next, BL recordings were further analyzed by using an R-script to randomly eliminate data from 4 to 60 electrodes, in sets of 4. This R-script analysis was repeated 10 times, in efforts to increase accuracy of the computational analysis. For BL recordings, MFR was not influenced by the number of electrodes used to determine network parameters. By contrast, although NwSpk was also constant across different numbers of electrodes, it become inconsistent once data from more than 48 electrodes were eliminated. Post BL, cortical cells received either lindane (1μM) or verapamil (10μM) to determine if fewer electrodes alters the detection of chemically induced network changes. Although lindane increased and verapamil decreased MFR as expected, there was no correlation with numbers of electrodes. However, NwSpk showed a similar response as untreated cultures. These results indicate that investigators should carefully consider the mwMEA format selected when designing experiments, particularly if network parameters will be evaluated. This abstract does not reflect the policies of the US EPA.

Intrinsic Amino Acid Consumption as a New Approach for Bacterial Differentiation and Subtyping

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Computational Integration of Human Genetic and Toxicological Data to Evaluate Adverse Outcome Pathway-Specific Susceptibility

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There is a need for approaches to define human variability and susceptibility in response to environmental chemical exposure. Direct estimation of the genetic contribution to variability in susceptibility to environmental chemicals is only possible in special cases where there is an observed association between exposure and effect (e.g., genotype and phenotype information). The availability of genetic and toxicological data sources makes it possible to indirectly estimate the relative contribution of genetic variability to differential human susceptibility. We developed a computational workflow that integrates genetic and toxicological resources. This approach implements the adverse outcome pathway (AOP) framework in order to integrate molecular targets associated with AOPs with functional genomic annotations and population allele frequencies. Resources include the EPA internal Adverse Outcome Database (AOP-DB), and publicly available resources, such as the AOP-wiki, Ensembl genomic annotations, expression quantitative trait loci identified by the GTEx consortium, and 1000 Genomes Project. With this information it is possible to formulate predictions of genetic susceptibility built upon established toxicological and genetic knowledge that are specific to an adverse outcome. The computational workflow was developed in R and built around the Ensembl database interfaces (REST API and biomaRt R package). It downloads, integrates, and analyzes the data from different data sources using a computational pipeline that is based on the AOP framework. The computational workflow integrates both genetic and toxicological information, from which predictions of genetic susceptibility are derived. The tool is available as an online portal at https://www.epa.gov/toxtools/aopdb. The tool provides predictions of genetic susceptibility that are specific to an adverse outcome. The tool is available as an online portal at https://www.epa.gov/toxtools/aopdb. The tool provides predictions of genetic susceptibility that are specific to an adverse outcome. The tool is available as an online portal at https://www.epa.gov/toxtools/aopdb. The tool provides predictions of genetic susceptibility that are specific to an adverse outcome. The tool is available as an online portal at https://www.epa.gov/toxtools/aopdb. The tool provides predictions of genetic susceptibility that are specific to an adverse outcome.
Applying a Tiered Risk-Based Approach to Prioritizing Thousands of Chemicals for Further Evaluation: A Comparison of Current High-Throughput Computational Approaches

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Current computational technologies offer novel opportunities to help in the prioritization of chemicals for toxicity testing. Here, we present a tiered risk-based approach using an initial triage based on the ratio between high-throughput exposure estimates and thresholds of toxicological concern (TOCs), followed by automated read-across to identify suitable analogues for identifying potential endpoints and potency estimates. To demonstrate the applicability of TTCs for the initial triage, 300+ ToxCast chemicals were processed as follows: 1) oral equivalent doses (OEDs) were calculated based on ToxCast bioactivity measurements and available metabolism data for estimating in vivo clearance, 2) TTC values were determined using the Cramer classification system, 3) OEDs and TTCs were then compared with available ExpoCast exposure estimates to determine their respective activity/exposure ratios (AERs). This evaluation demonstrated that TTCs could serve as a reasonable basis for further prioritizing compounds. The TTC approach was then applied to the Collaborative Estrogen Receptor Activity Prediction Project (CERAPP) database, a set of ~45,000 chemicals. An in-house read-across tool was used to identify suitable analogues for the top prioritized chemicals based on available experimental and predicted data. Potential analogue identification was guided by categorizing the CERAPP compounds into structural classes (e.g., carbamates) using SMARTS. This resulted in 17 structural classes with at least 13 chemicals for which predicted and measured bioactivity data were publicly available. We also identified an optimal combination of chemical descriptors that were predictive of bioactivity across specific endpoints (e.g., estrogen receptor, skin sensitization). We then interrogated the impact that various combinations of descriptors may have on identifying regions of chemical space that might be amenable to read-across. Our endpoint specific read-across approach employed supervised machine learning for optimal analogue selection for AER-ranked compounds. This study demonstrated the utility of exploiting computational approaches as part of a tiered risk-based approach to prioritize thousands of chemicals. This abstract does not necessarily reflect US EPA policy.

Automated Analyses of Dendritic Morphology in Rodent Primary Neuronal Cell Cultures


Altered dendritic morphology is associated with neurodevelopmental disorders (NDDs); thus, primary neuronal cell cultures are a useful in vitro model for studying NDD pathogenesis. However, analyzing dendritic morphology is challenging due to the structural complexity of dendrites, particularly when grown at high density. Low-efficiency transfection for microtubule-associated-protein-2B to label a small percentage of neurons overcomes some difficulties; however, image analysis remains challenging as it involves manually tracing processes. Alternatively, dendritic morphology is assessed using Sholl analysis where the user chooses individual neurons, defines a center point and thresholds manually. Automated software superimposes concentric rings around the center point, determines the number of dendritic intersections at each ring, and generates a distribution plot. Both methods are time-intensive and suffer from thresholding bias. Therefore, we developed new software, which has been added to the previously published Omnisphero software, for automated identification and thresholding of neurons, analysis of neurite mass, total neurite length, number of primary processes, average neurite length, number of branching points, number of terminal tips, and Sholl analysis. First, neurons are identified and binarized, at which point the researcher is able to manually annotate neurons of interest or delete identified neurons. This allows analysis of specific neuronal subpopulations, like pyramidal neurons, which will be a valuable asset for machine learning approaches. Second, only selected objects are analyzed. This method eliminates bias associated with tracing and thresholding, decreases evaluation time and provides an interactive user interface, allowing the user to manually judge and refine the analysis. The increased rate of analysis also enables an increased number of neurons to be analyzed, thereby increasing statistical power. Manual vs. automated analysis of sex differences in murine neurons results in the same statistical effects in Sholl analysis. Automated analysis additionally identified significant effects in number of branching points and neurite length. These data suggest the potential for this software to increase sensitivity and decrease bias in morphometric analyses of dendritic arborization. Supported by NIEHS (RO1 ES014901; PJL, T32 ES007059-55), NICHD (F32 HD088016; KPK).

Structural Changes of Androgen Receptor Ligand-Binding Domain Due to Antagonist Binding Elucidated by Long-Time Molecular Dynamic Simulations

H. Hong. US FDA/NCTR, Jefferson, AR. Sponsor: W. Tong

Androgen receptor (AR) is one of the important receptors for assessing endocrine-disrupting potential of chemicals. Ligands binding to AR induces conformational changes in AR-LBD (ligand-binding domain) that affect the binding of co-regulator proteins and DNA to AR. Three-dimensional (3D) structures of AR-antagonist complexes are helpful to understanding of androgenic activity of chemicals using various computational techniques. Unfortunately, wild-type(WT)-AR-LBD complex with an antagonist is not available in the protein data bank. Hence, we applied molecular docking and MD simulations to identify the important residues involved in the structural changes due to antagonist binding. Molecular docking was carried out to find a suitable binding orientation of the antagonist (bicalutamide) in the ligand-binding pocket (LBP) of AR-LBD. The complexes of WT-AR-LBD with bicalutamide, WT-AR-LBD with agonist (R1881), and mutant-AR-LBD with bicalutamide obtained from molecular docking were optimized through long time, 1 micro-seconds (μs), MD simulations to identify the conformational changes in LBP and activation function 2 (AF2) site of AR. The results revealed that the binding of WT-AR-antagonist in WT-AR-LBD moved residues in H12 (Phe222, Met226, and Ile230) and Arg726 outward when compared with WT-AR-LBD-agonist. This displacement of residues in H12 moved the helix outward and Arg726 and distorted AF2 site which plays a major role in binding of co-regulators. The structural changes elucidated in our study could be helpful to gain a structural insight of WT-AR-LBD-antagonist and are expected to facilitate development of in silico predictive models for assessment of endocrine-disrupting potential of chemicals. Disclaimer: This abstract is not a formal dissemination of information by US FDA and does not represent agency position or policy.

A Targeted Approach toward Enhancing Salmonella Mutagenicity QSAR Models for Regulatory Use

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The International Conference on Harmonisation (ICH) M7 guidelines describe the use of (quantitative) structure-activity relationship (q(2)SAR) models to assess the mutagenic potential of drugs in the early stages of drug discovery and development. As the number of new and generic drug products increases, there is a need for optimal predictive performance and utility, models need to be continually updated with new data to ensure coverage for new drug substances entering the regulatory pipeline. Additionally, limitations in predictive performance caused by inadequate structural representation in selected areas of chemical space should be addressed. A targeted approach to enhancing a training set ensures that the existing performance of a model is maintained for well-represented areas of chemical space, while areas with few or no structural examples are improved upon. To this end, data were harvested from published literature (n=326), FDA approval packages for drugs approved between 2009 and 2017 (n=159), Center for Food Safety and Applied Nutrition databases (n=111), the Japanese Ministry of Health, Labor and Welfare databases (n=501), and through data sharing efforts (n=53). After removing duplicates, mixtures, salts, and inadequately tested chemicals, 521 compounds were selected to support the current FDA Center for Drug Evaluation and Research training set. Through real-time application of the Salmonella mutagenicity models for internal assessments, 17 sub-structural features were identified as un- or underrepresented and 315 compounds containing functional groups such as internal iminal dialdehydes and hindered epoxides were added to the training set from the harvested dataset. Additionally, an external validation set of 442 drug impurities representing proprietary pharmaceutical space was constructed from FDA submissions. This dataset was used in conjunction with other benchmark datasets (n=6,040) to confirm that data gaps were filled while high predictive performance was retained by the model. This effort represents a major enhancement to (QSAR) models that are recommended for use under ICH M7, leading to improved patient safety through greater predictive accuracy and applicability when assessing the mutagenic potential of drug impurities.
2492 A Machine Learning Model for Prediction of Compound Mode-of-Action from Toxicogenomics


Genome-wide gene expression (transcriptomics) studies provide an attractive means of evaluating chemical toxicity because of the rapidity with which they can depict the profiles of genes affected by a compound, transcriptomic studies can yield essential information on mode of action. Furthermore, transcriptional responses are predictive of the dose that will lead to cell and tissue toxicity; chemicals that cause positive responses in classical apical endpoints in vivo (i.e., the two-year rodent bioassay) evoke gene expression changes at corresponding exposure levels. Here, we present a computational model for relating chemical modes of action based on the expression responses they evoke in primary rat hepatocytes. The model is trained using a subset of the 170 compounds tested in the TG-GATEs library, many of which are “reference” agents with well-defined modes of action. In some cases, compounds with similar modes of action demonstrated shared patterns of enriched transcriptomic pathways in Gene Ontology. For example, the similarity index (here, a modified Jaccard index) is 0.56 between PPARalpha agonists fenofibrate and Wy-14,643. In contrast, the average similarity index was 0.20 between randomly chosen compounds. To understand whether artificial intelligence could aid in the classification of compound mode of action, we trained seven distinct machine learning models on compounds representing four modes of action: PPARalpha agonism, CAR agonism, estrogen receptor binding, and genotoxicity. Samples with different exposure times and concentrations were treated as independent. As inputs, we used quantitative gene expression changes and enriched Gene Ontology pathways. Model performance was evaluated using 10-fold cross validation. The two top performing models were Logistic Regression (mean accuracy: 0.96) and Gaussian Naive Bayes (mean accuracy: 0.89). In nearly every case, models based on continuous gene expression changes outperformed models based on Gene Ontology pathways. We are currently evaluating the utility of these predictive models with independent validation data sets from 2D and 3D in vitro models and in vivo liver studies. By leveraging recent developments in computational and bioinformatic tools, we expect this approach to improve early decision-making about chemical safety.

2494 Pathway Analysis and Mode-of-Action Prediction Based on Computational Modeling of High-Throughput Toxicogenomics

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The utility of transcriptomic data for interpreting cellular modes-of-action (MOA) after exposure to chemicals has been established for over a decade. It is conventionally performed by whole transcriptome gene expression tools such as microarray and next-generation sequencing (RNA-seq). However, for the toxicity assessment and testing prioritization of chemicals, there is a pressing need for efficient alternatives such as high-throughput transcriptomics (HTT). The goal of this study was to develop suitable HTT computational models for evaluating the diverse cellular modes of action of environmental, pathological, and agricultural chemicals. As a proof of concept, we used Genometry’s L1000 platform to demonstrate the possibility for establishing statistical relationships between genes and to infer whole genome transcriptional profiles. Then we developed a qualitative MOA-oriented strategy to predict relevant cellular pathway information and applied it to Affymetrix array data collected from three human cell lines HepG2, MCF7, and A549 exposed to three aromatic amides (misonidazole, fenbucouznole, and 2,4-dichlorophenoxoxacyclic acid) over nine concentrations. To avoid commonly encountered pitfalls from comparing different technologies and to increase prediction accuracy, we built three-class categorical models predicting up-regulated, down-regulated and unchanged genes essential to the cellular MOA. This work was then extended to predict gene expression changes resulting from exposure to heterogeneous chemicals from a wide range of classes. The models were developed using the Open TG-GATEs database providing toxicogenomics data of multiple cell lines for over 900 samples covering 170 compounds. Different machine learning techniques were used to identify a set of landmark genes which can predict differential expression changes of the genomic profile. A 10-fold cross validation balanced accuracy of ~65% was reached compared to a correlation coefficient of 42% with continuous models. The predicted and actual Affymetrix pathway enrichment profiles for HepG2 cell line in response to 0.01 μM Fenbucouznole showed the 22 (out of 39) highest enriched pathways in common. Such models can be used for MOA prediction and pathway analysis to prioritize large libraries of chemicals starting from gene expression data of representative genes.
A Non-Parametric Approach for Selecting Benchmark Responses for In Vitro or Alternative Animal Model Data

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Benchmarks provide a way to compare alternative data to validate standard benchmarks. It is well known that the toxicity response of the genotoxicity endpoint is highly variable. Common approaches to evaluate drug toxicity, such as genotoxicity, are expensive and time-consuming. In this study, we present a comprehensive set of QSAR models built using a previously-reported downsampling method to improve sensitivity while maintaining structural coverage. The set of models consists of effects on male and female fertility in mice and rats, sperm toxicity in mice and rats, newborn behavioral toxicity in mice and rats, rat keratination, and rat and rabbit reproductive toxicity. For this work, an enhanced, high-throughput method for predicting DART endpoints based on chemicals based on chemical structure.

In Europe, increased requirements for pesticide registration and the drive to reduce animal use have increased the use of in silico tools to predict potential genotoxicity of impurities and metabolites. Today, at least one rule-based and one statistical system are required, and emphasis is put on reliability of predictions. In order to choose the most reliable in silico systems, Bayer agrochemical substances were entered into the genotoxicity models in Derek Nexus, Toxtree, TopKat, Leadscope, and Vega, to compare the in silico prediction to the experimental outcomes for each substance. In this study, we present a comprehensive set of QSAR models for DART endpoints based on predominantly negative data are limited or unavailable. An earlier generation of QSAR models was developed for DART endpoints based on predominantly negative training data. The datasets contained only 10-30% positives, yielding models with high specificity and negative predictivity, but low sensitivity. In this study, we present a comprehensive set of QSAR models built using a previously-reported downsampling method to improve sensitivity while maintaining structural coverage. The set of models consists of effects on male and female fertility in mice and rats, sperm toxicity in mice and rats, newborn behavioral toxicity in mice and rats, rat keratination, and rat and rabbit reproductive toxicity. For this work, an enhanced, high-throughput method for predicting DART endpoints based on chemicals based on chemical structure.

Reliability of In Silico Genotoxicity Predictive Software for Bayer CropScience Chemistry

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In Europe, increased requirements for pesticide registration and the drive to reduce animal use have increased the use of in silico tools to predict potential genotoxicity of impurities and metabolites. Today, at least one rule-based and one statistical system are required, and emphasis is put on reliability of predictions. In order to choose the most reliable in silico systems, Bayer agrochemical substances were entered into the genotoxicity models in Derek Nexus, Toxtree, TopKat, Leadscope, and Vega, to compare the in silico prediction to the experimental outcomes for each substance. In this study, we present a comprehensive set of QSAR models built using a previously-reported downsampling method to improve sensitivity while maintaining structural coverage. The set of models consists of effects on male and female fertility in mice and rats, sperm toxicity in mice and rats, newborn behavioral toxicity in mice and rats, rat keratination, and rat and rabbit reproductive toxicity. For this work, an enhanced, high-throughput method for predicting DART endpoints based on chemicals based on chemical structure.

New QSAR Models for Developmental and Reproductive Toxicity with Enhanced Sensitivity

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Drugs entering the regulatory pipeline may require non-clinical evaluation of their potential to cause developmental and reproductive toxicity (DART), which is performed through a standard battery of in vivo assays described in International Conference on Harmonisation (ICH) guidance. These in vivo assays include the assessment of male and female reproductive toxicity in rodents and behavioral abnormalities and fetal dysmorphogenesis in teratology studies. They are intended to provide an assessment of the potential effects on human fertility, reproductive viability, as well as effects on the fetus in female patients exposed to the drug through clinical use. Quantitative structure-activity relationship (QSAR) models may be used as early screening tools during drug development and to support regulatory safety decisions. As QSAR data are limited or unavailable. An earlier generation of QSAR models was developed for DART endpoints based on predominantly negative training data. The datasets contained only 10-30% positives, yielding models with high specificity and negative predictivity, but low sensitivity. In this study, we present a comprehensive set of QSAR models built using a previously-reported downsampling method to improve sensitivity while maintaining structural coverage. The set of models consists of effects on male and female fertility in mice and rats, sperm toxicity in mice and rats, newborn behavioral toxicity in mice and rats, rat keratination, and rat and rabbit reproductive toxicity.
In recent years, transcriptomics has become a powerful tool in predictive toxicology and contributed much to our understanding of the molecular and cellular mechanisms of chemical toxicities. However, even with the modern highly parallel assay systems, large-scale experimental acquisition of transcriptomic data is still costly. Thus, it is an appropriate target for the development of a computational structure-activity relationship (QSAR) modeling that has been used to predict a wide range of chemical-induced biological responses. However, QSAR has not been utilized to model chemical-induced genome-wide gene expression changes until very recently, owing to the complexity of training and evaluating a very large number of models. To address this issue, we examined the performance of variable nearest neighbor (v-NN) method that uses information of near neighbors conforming to the principle that similar structures have similar activities. Using a dataset of gene expression signatures of 13,150 compounds derived from cell-based measurements in the NIH Library of Integrated Network-based Cellular Signatures program, we were able to make predictions for 62% of the compounds in a 10-fold cross validation test, with a correlation coefficient of 0.61 between the predicted and experimentally derived signatures—a reproducibility rivaling that of high-throughput gene expression measurements. To evaluate the utility of the predictions, we compared them with experimentally derived signatures in their ability to flag drugs known to cause specific human organ injuries. Overall, the predicted and experimentally derived signatures had similar receiver operating characteristics across three human organ injury models. Detailed analyses of enrichment curves indicate that the gene expression signatures predict toxicological mechanisms by which environmental exposures affect human health. Our results demonstrate that the v-NN method can serve as a practical approach for modeling large-scale, genome-wide, chemical-induced gene expression changes.

The Comparative Toxigenomics Database (CTD; http://ctdbase.org) is a premier public resource that helps illuminate the molecular mechanisms by which environmental exposures affect human health. CTD’s literature-based content is derived from four manually curated modules: toxicogenomic core (chemical-gene interactions), disease core (chemical-gene associations and human andlevant gene-disease associations), experiment (environmental stressor-receptor-event-outcome statements), and our new phenotype module (chemical-induced modulations of cellular and physiological traits). At CTD, we distinguish between phenotypes and diseases, wherein a phenotype refers to a non-disease-term biological event: e.g., decreased cell cycle arrest (phenotype) vs. lung cancer (disease), increased fat cell proliferation (phenotype) vs. obesity (disease), decreased spermatocyte division (phenotype) vs. male infertility (disease), etc. All CTD chemical-phenotype interactions are noted in a structured format using controlled terms for chemicals, phenotypic processes (Gene Ontology), taxon, and anatomical descriptors. These terms are from established and experimentally validated resources that include synonyms and accession identifiers (allowing terms to be computationally processed and mapped to additional vocabularies for database interoperability). To date, we have manually curated over 18,500 scientific articles for this module, producing 105,000 interactions that associate 6,260 chemicals to 1,700 phenotypes for 680 anatomical terms in more than 200 comparative species. Integrating this information with CTD’s extensive chemical-disease content allows novel connections to be inferred between phenotypes and diseases, yielding potential insight into the biological processes of a pre-disease state, before the clinical manifestation of a toxicogenomic disorder. As well, integrating all four CTD modules furnishes unique opportunities to generate computationally predictive adverse outcome pathways (AOPs), linking chemical-gene molecular initiating events, phenotypic key events, diseases, and population-level health outcomes. To our knowledge, this is the first comprehensive set of manually curated, literature-based, contextualized chemical-phenotype data provided to the public.

Image-based phenotypic profiling is a high-throughput screening method which combines automated microscopy and image analysis workflows to measure a large variety of features at the single-cell level. Such methods have been used for functional genomics studies and in the pharmaceutical industry for compound efficacy and toxicity screening. Here, we describe the initial steps in optimization, miniaturization, and application of a microfluidics-based laboratory workflow and image-based phenotypic profiling assay for use as a bioactivity screen for environmental chemicals. MCF7 cells were plated in 384-well plates (2,500 cells / well) using a MultiFlo FX tool. After 24 h, cells were treated with 7 concentrations (0.02-100 μM, n = 3/plate) of 16 reference compounds in a randomized pattern using an Echo 550 acoustic dispenser. After 48 h of treatment, cells were live-stained with MitoTracker (mitochondria), fixed, permeabilized and stained with Hoechst-33342 (nuclei), SYTO14 (nucleoli) and fluorescent conjugates of phalloidin (actin cytoskeleton), wheat germ agglutinin (Go1gi / plasma membrane) and concanavalin A (ER). Images were acquired using a Cellomics ArrayScan XTI and analyzed using CellProfiler software, yielding >1,000 features per cell. Cell level data were MAD normalized using DMSO controls. A screening threshold for effects was established as median +/- 2 units from control. A multiplexed cell viability and apoptosis assay was run in parallel. Examples of effects observed at non-cytotoxic concentrations included: berberine chloride affecting intensity and texture measurements in the mitochondria channel, fenbendazole altering intensity measurements in the DNA channel and tetrabenazine affecting intensity, radial distribution and texture measurements in the mitochondria and Golgi/membrane channel. The measured changes were confirmed by visual inspection of the images. In summary, the described protocol was successfully applied to MCF7 cells using automated liquid handling tools. Testing of diverse compounds yielded distinct patterns of affected parameters below the threshold for cytotoxicity, indicating that the assay can detect toxicant-specific effects and may be used to group (fingerprint) toxicants with similar profiles. Future efforts will include screening of additional chemicals and cell lines. This abstract does not necessarily reflect US EPA policy.

The past decade has seen significant research and regulatory initiatives devoted to the ultimate objective of supplanting in vivo toxicity studies with less extensive in vitro and computational alternatives, especially within the pharmaceutical industry for compound efficacy and toxicity screening. Here, we describe the initial steps in optimization, miniaturization, and application of a microfluidics-based laboratory workflow and image-based phenotypic profiling assay for use as a bioactivity screen for environmental chemicals. MCF7 cells were plated in 384-well plates (2,500 cells / well) using a MultiFlo FX tool. After 24 h, cells were treated with 7 concentrations (0.02-100 μM, n = 3/plate) of 16 reference compounds in a randomized pattern using an Echo 550 acoustic dispenser. After 48 h of treatment, cells were live-stained with MitoTracker (mitochondria), fixed, permeabilized and stained with Hoechst-33342 (nuclei), SYTO14 (nucleoli) and fluorescent conjugates of phalloidin (actin cytoskeleton), wheat germ agglutinin (Go1gi / plasma membrane) and concanavalin A (ER). Images were acquired using a Cellomics ArrayScan XTI and analyzed using CellProfiler software, yielding >1,000 features per cell. Cell level data were MAD normalized using DMSO controls. A screening threshold for effects was established as median +/- 2 units from control. A multiplexed cell viability and apoptosis assay was run in parallel. Examples of effects observed at non-cytotoxic concentrations included: berberine chloride affecting intensity and texture measurements in the mitochondria channel, fenbendazole altering intensity measurements in the DNA channel and tetrabenazine affecting intensity, radial distribution and texture measurements in the mitochondria and Golgi/membrane channel. The measured changes were confirmed by visual inspection of the images. In summary, the described protocol was successfully applied to MCF7 cells using automated liquid handling tools. Testing of diverse compounds yielded distinct patterns of affected parameters below the threshold for cytotoxicity, indicating that the assay can detect toxicant-specific effects and may be used to group (fingerprint) toxicants with similar profiles. Future efforts will include screening of additional chemicals and cell lines. This abstract does not necessarily reflect US EPA policy.
Perchloroethylene organ-specific toxicity has been associated with both oxidative and conjugative metabolism pathways. Previous perc PBPK modeling could accurately predict oxidation but we suggested the need to better characterize glutathione (GSH) conjugation as well as toxicokinetic uncertainty and variability. We updated the previously published “harmonized” perc PBPK model for mice to characterize the uncertainty and variability of perc toxicokinetics, with particular emphasis on modeling GSH conjugation metabolites. The updated PBPK model includes physiologically based sub-models for conjugation metabolites trichlorovinyl glutathione (TCVG), trichlorovinyl cysteine (TCCV), and N-acetyl trichlorovinyl cysteine (NACTCV), and added a brain compartment for perc and GSH conjugates. In vivo perfusion data were used to calibrate these pathways for both oxidative and conjugative metabolites. We successfully predicted across the three strains of mice, with estimated residuals errors of two-fold for the majority of data. Inter-strain variability across the three strains was evident for oxidative metabolism; GSH conjugation data were only available for one strain. Updated PBPK model fills a critical data gap in quantifying the oxidative and conjugative metabolism of perc. Previously compiled mouse kinetic data on perc and TCA in B6C3F1 and Swiss mice was augmented to include data from a recent study in male C57Bl/6J mice that measured perc, TCA, and GSH conjugation metabolites in serum and multiple tissues. A hierarchical Bayesian population approach was used to estimate model parameters and characterize the uncertainty and interstrain variability, implemented using Markov chain Monte Carlo (MCMC) simulation. All convergence criteria were satisfied with four MCMC chains, each 100,000 iterations long. The updated model performed as well or better than the previously published model. This approach allows both oxidative and conjugative metabolites to be clearly applied to multiple key characteristics. Only a few could not be assigned to any pathway. The majority of pathways were assigned to the 9th key characteristic representing the 10th key characteristic of human carcinogens. Since both are also known to be genotoxic from routine toxicological testing, the gene expression data support the conclusion that these classic carcinogens express most of the key characteristics. We propose to extend the current findings to additional chemical agents.
of developing and curating MIE knowledge, discovery toxicology using omics and model species, target-based in vitro assays, SAR model generation, understanding of the quantitative trigger conditions can be used to develop integrated testing strategies to quantitatively predict DART effects.

### 2507 Predicting Chemical Mechanisms-of-Action Using High-Throughput Transcriptional Data


The EPA ToxCast effort has screened thousands of chemicals across hundreds of high-throughput in vitro assays. Now, the project is leveraging high-throughput transcriptomic (HTTr) technologies to substantially expand its coverage of biological pathways by measuring the expression of 19,290 genes across multiple cell types. Our objective is to deploy HTTr as an initial screen to prioritize the existing suite of high-throughput assays for additional testing. To accomplish this goal, we must elucidate the potential mechanisms of action (MoA) for each chemical using the transcriptomic profiles. Thus far, HTTr data have been generated for 2,200 chemicals in concentration response format in MCF7 cells at 6 h time point. We have also developed a computational pipeline in Python/R to streamline data processing and analysis as follows: (a) translating raw RNA-Seq data to normalized transcriptional profiles, (b) identifying statistically significant transcriptional perturbations using DESeq2, (c) finding concentration-responsive transcripts using benchmark dose modeling (BMD), and (d) predicting chemical mechanism of action (MoA). We used “connectivity mapping” to infer the 35% of a chemical based on similarity with a database of HTTr profiles from the Connectivity Map (CMap) project (Lamb et al, 2006). The CMap transcriptomic database contains 3,334 Affymetrix transcriptomic profiles (13,029 genes) for 1,176 chemicals and 482 chemicals annotated with >90% from classes curated from KEGG and DrugBank. Analyzing HTTr profiles for genistein (10μM), sirolimus (0.1μM) and trichostatin A (1μM) from MCF7 cells using connectivity mapping correctly identified their known targets as estrogen receptor (ESR), mechanistic target of rapamycin (mTOR) and, histone deacytelase (HDAC), respectively. However, connectivity mapping also produced high-scoring database matches that were not consistent with the known MoA for these chemicals. We are exploring machine learning techniques to mine higher-order dependences between genes in order to make MoA predictions that are biologically meaningful, sensitive and specific. We will present MoA predictions for 2,200 chemicals using concentration-response HTTr data and show their utility as an initial screen for high-throughput toxicity testing. This abstract does not reflect US EPA policy.

### 2508 Confidence in Fitting and Hitting Concentration-Response Data: Tox21 10k Library Pipeline Comparison


The Tox21 program has generated high-throughput screening data on thousands of chemicals. While the data are publicly available through partner websites, PubChem, and publications, the analyses are different. We developed a pipeline consensus to identify higher-confidence chemical-assy calls and are developing a public web application. Tox21 chemical-assay pair activity calls (active, inactive, and, in some cases, inconclusive) were compared among the four hit-call methods: CurveP and 35% of a chemical based on similarity with a database of HTTr profiles from the Connectivity Map (CMap) project (Lamb et al, 2006). The CMap transcriptomic database contains 3,334 Affymetrix transcriptomic profiles (13,029 genes) for 1,176 chemicals and 482 chemicals annotated with >90% from classes curated from KEGG and DrugBank. Analyzing HTTr profiles for genistein (10μM), sirolimus (0.1μM) and trichostatin A (1μM) from MCF7 cells using connectivity mapping correctly identified their known targets as estrogen receptor (ESR), mechanistic target of rapamycin (mTOR) and, histone deacytelase (HDAC), respectively. However, connectivity mapping also produced high-scoring database matches that were not consistent with the known MoA for these chemicals. We are exploring machine learning techniques to mine higher-order dependences between genes in order to make MoA predictions that are biologically meaningful, sensitive and specific. We will present MoA predictions for 2,200 chemicals using concentration-response HTTr data and show their utility as an initial screen for high-throughput toxicity testing. This abstract does not reflect US EPA policy.

### 2509 Use of In Silico Models for Compound Property Prediction to Reduce the In Vitro Screening Burden


In order to maximize the return on investment for early ADMET screening assays, a way to prioritize testing of novel compounds was developed to remove compounds from the automated submission system when the outcome of the assay could be predicted with high confidence. To demonstrate feasibility, a pilot experiment was initiated to apply such an in silico model filter for the human protein binding (hPPB) assay. Human protein binding is an important piece of data in the assessment of safety margins when considering toxicological data. An in silico support vector machine model incorporating the conformal prediction framework was developed for hPPB and its utility in selecting compounds for which no measurement was required initially was evaluated. Compounds where the predictions had a low confidence (< 80%) were submitted to automated testing, for the remaining compounds predicted hPPB data were stored in the internal database accessible to scientists. During the first quarter of 2017 more than 35% of all project compounds to be removed from automated testing, thus, saving unnecessary testing and expense. Moving forward we have investigated how confidence predictions can be applied to ADMET assay panels rather than individual assays. Since there are cost advantages in running these assays as one panel the ability to predict all assays with high confidence will be essential but may be challenging to achieve.

### 2510 Integrated Analysis of Transcriptomics Data and the Adverse Outcome Pathway Framework for Risk Assessment of Chemicals: An Exploratory Case Study Using Piperonyl Butoxide and Liver Models


The integration of existing knowledge to support the risk assessment of chemicals is still a challenge for scientists, risk assessors, and managers. International initiatives like the Adverse Outcome Pathways (AOP) programme have a role in supporting the integration of information from various sources and building collaboratory platforms enabling the scientific community to address risk assessment issues. In this exploratory case study, gene expression data from HepaRG and HepG2 liver cell lines for piperonyl butoxide (PBO) housed in the Data Infrastructure for Chemical Safety (diXa) database were used. The differentially expressed genes were used in pathway enrichment analysis, chemical similarity associations (similar mode of action (MOA) as PBO), and disease associations using tools from the LINCS Consortium, and the Comparative Toxicogenomics Database (CTD). The resulting pathways, chemical analogs, and disease associations were combined with specific liver AOPs and key events from the AOP knowledge base to show evidence supporting a case for PBO as a liver toxicant. There was an 8% overlap in pathways between the two cell lines in response to treatment, and a 6.1% overlap of chemicals with similar MOA to PBO across both lines 146 and 133 known chemicals for HepaRG and HepG2, respectively. Overall, there were fewer associations for the HepaRG cells compared to the HepG2 line, highlighting the mechanistic differences that exist between the different liver models as illustrated by the low overlap in association results. The results also highlight the reference bias that may be introduced by the computational tools used in analysis and the reference data they depend on. This work shows that human in vitro transcriptomics data and modeling tools can identify potential toxicity
by highlighting the biological pathways, diseases, and related chemicals based on the biological signatures. When mapped to existing AOPs, this information can be used to identify relevant AOPs for a chemical, highlighting knowledge gaps where new AOPs could be defined, and assemble AOP networks relevant for the chemical. This approach could support the evidence-based risk assessment of individuals or groups of compounds by using the transcriptomic profiles to identify data gaps and eventually propose additional testing.

2510a Structure-Based QSAR Models to Predict Systemic Toxicity Points of Departure

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Human health risk assessment associated with environmental chemical exposure is limited by the tens of thousands of chemicals with little or no experimental in vivo toxicity data. Data gap-filling techniques, such as quantitative structure activity relationship (QSAR) models based on chemical structure information, are commonly used to predict hazard in the absence of experimental data. This study presents a set of QSAR models developed for chronic or subchronic in vivo points of departure (POD, the point on the dose response that marks the beginning of a low-dose extrapolation). The in vivo data are taken from the EPA’s ToxValDB, a compilation of information on ~3,000 chemicals from a variety of public data sources. Using PubChem fingerprints and Chemistry Development Kit (CDK) descriptors as physchem descriptors (with feature selection), and support vector machines, random forests, K-nearest neighbor, and gradient boosting regressor as machine learning algorithms (with hyper-parameter tuning), models were developed and evaluated using five-fold internal cross-validation and external test validation. Quantitative POD models were developed for mouse (538 chemicals) and rat (811 chemicals), using point estimates for the experimental POD values. The best mouse model had an external test root mean square error (RMSE) = 0.86 and R² = 0.36. The best rat model had an external test RMSE = 0.18. Since the training data for both mouse and rat models was skewed, they were reconstructed by creating bootstrap samples with 10% duplicate data (randomly selected from the long tail), and the models were rebuilt. Reconstructing the datasets to reduce the skewness in original data did not result in significantly improved models. A second set of models was built that accounted for both lab to lab variability in the POD values. To do this, a POD distribution was constructed for each chemical using mean = median experimental POD value and standard deviation = 0.5 log-units, based on the typical lab-to-lab variability. Bootstrap models were built with random sampling of values from the pre-generated POD distribution to derive point estimates of POD values and confidence intervals for each prediction. The best mouse model had an average external test RMSE = 0.96 and R² = 0.31. The best rat model had an average external test RMSE = 1.15 and R² = 0.02. All units are log10 mg/kg/day. These models will inform chemical screening and prioritization efforts. This abstract does not necessarily represent US EPA policy.

2511 Uncovering Drug-Drug Associations by Data Mining of Drug Adverse Event Database

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The FDA centers receive reports from consumers, healthcare professionals, manufacturers, and others regarding the safety of various regulated products, such as drugs, vaccines, artificial hearts, surgical lasers, and nutritional supplements. FDA Adverse Event Reporting System (FAERS) is a database that contains spontaneously reported adverse events submitted to the FDA. It is a challenge to extract the information in these reports for better assessment of product safety and rapid detection of adverse event signals. A total of 63,082 drug adverse event pairs were identified as the significant association between 936 drugs and 10,316 adverse events. Report ratio (RR) values and p-values of chi square test of drug adverse event pairs indicated that the identified signals were significant. New safety signals were identified when comparing with the currently available information in various sources. After applying random network algorithm to DDI network, 14 drug groups were obtained. Some groups were dominated by drugs with the same first-level Anatomical Therapeutic Chemical (ATC) classification system code, indicating the high purity of identified drug groups. It was distinguished that similar side effects could infer similar target of drugs. The outcome of this study is expected to enhance information input to the decision-making process for drug safety detection and postmarketing surveillance.

2512 Bioinformatics Resource Manager: Web Tool for Data Integration and Systems Toxicology

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The Bioinformatics Resource Manager (BRM) is a web-based application that provides computational tools for biologists to assist with analysis and integration of high-throughput (HTP) or high-content omics data sets. BRM allows users to cross-reference gene identifiers within data sets or to integrate across datasets, including integration of multi-omic platforms (e.g., proteomics and transcriptomics) or integration across different model organisms with simple to follow workflows. In this study, we identified the functional consequences of miRNA regulation in human airway epithelium organotypic 3D culture model after exposure to polycyclic aromatic hydrocarbons, benzo(a)pyrene (BAP) and dibenz[o(def)]chrysene (DBC), through integration of global miRNA and mRNA. The BRM miRNA workflow provides the ability to identity conserved miRNA gene targets from multiple public databases or identify potential regulatory miRNAs for genes of interest. BRM allows users to not only identify predicted targets of miRNAs from both computational and experimentally validated sources, but also to integrate predicted gene targets with experimental miRNA in a single step for human, mouse, and zebrafish data. Cells were exposed to 500 µg/ml BAP and 10 µg/ml DBC for 48 hrs and samples were collected for parallel analysis of miRNA and mRNA by RNA sequencing using Illumina HiSeq 3000. Differentially expressed targets for mRNA and miRNA were integrated using the BRM miRNA workflow. The output from BRM can easily be imported into other commercial or public data analysis software and visual analytic tools. Comparison of miRNA networks for BAP and DBC showed common regulation of genes predominantly affecting extracellular matrix remodeling, cell adhesion, oxidative stress and inflammation. BRM was developed using Java™ and other open-source technologies and is freely available to users (http://ccb.pnnl.gov/brm/). This study was supported by P42 ES016465.
Our aim is to apply mechanistic and clinical understanding to develop a next-generation risk assessment (NGRA) approach for skin allergy that doesn’t require new animal test data, addresses novel exposure scenarios, and better characterizes our uncertainty. Our NGRA approach for skin allergy involves a comprehensive multi-omics approach that integrates predictive chemistry expertise, historical in vivo data, and existing or new in vitro data using two model-based, defined approaches (DAs) to predict the probability of human skin sensitization occurring following a given product exposure, with explicit uncertainty. The first computational model is a probabilistic, weight-of-evidence (WoE) approach that predicts the outcome of a hypothetical in vivo (HRIPT and mouse local lymph node assay [LLNA]), in vitro (direct peptide reactivity assay [DPRA]), human cell line activation test (hCLAT), KeratinoSens, SENS-IS and U-SENS or in silico (DEREK-NEXUS) hazard information. The WoE model output can be expressed as the probability of observing one or more incidences of inducing skin allergy following exposure to a chemical under the conditions of exposure, with hazard information. The WoE clinical potency model is a high-dimensional probability distribution constructed using skin sensitization-relevant in vivo (HRIPT and mouse local lymph node assay [LLNA]), in vitro (direct peptide reactivity assay [DPRA]), human cell line activation test (hCLAT), KeratinoSens, SENS-IS and U-SENS or in silico (DEREK-NEXUS) hazard information. The WoE model output can be expressed as the probability of observing one or more incidences of inducing skin allergy following exposure to a chemical under the conditions of the HRIPT and can therefore be used for risk assessment decision-making. Depending on initial WoE model prediction, a second model-based approach can be used for risk extrapolation from a HRIPT exposure to a product exposure scenario by applying skin exposure and toxicokinetic models that use consumer exposure, skin penetration, and protein reactivity data. Our NGRA for skin allergy has been evaluated using six cases: (2,4-dinitrochlorobenzene DNCB), eugenol, lactic acid, propyl paraben, 1,4-toluenediamine (PTD), and resorcinol used at case study ingredients (2,4-dinitrochlorobenzene DNCB), eugenol, lactic acid, propyl paraben, 1,4-toluenediamine (PTD), and resorcinol used at 0.2% in two hypothetical product exposure scenarios (face cream and acid, propyl paraben, 1,4-toluenediamine (PTD), and resorcinol used at case study ingredients (2,4-dinitrochlorobenzene DNCB), eugenol, lactic acid, propyl paraben, 1,4-toluenediamine (PTD), and resorcinol used at 0.2% in two hypothetical product exposure scenarios (face cream and shampoo); 2% from this analysis will be described alongside conclusions and next steps.

Targeted gene panels have been used in clinical settings to specify breast tumor type and to predict breast cancer prognosis and response to treatment. Separately, panels have also been curated to predict systemic toxicity and xenobiotic activity as a part of chemical screening strategies. However, currently available panels do not specifically target the biological processes relevant to breast development and carcinogenesis. We have curated a gene panel called the Breast Cancer Screen (BC Screen) as a tool to identify potential mammary carcinogens and characterize mechanisms of toxicity. First, we used seminal papers and reviews to identify 14 key characteristics of carcinogenesis and breast cancer development. Next, we applied a hybrid data and knowledge-driven gene selection system to identify combinations of information from publicly available whole transcriptome data, reported chemical-gene interactions, and primary literature review to generate a panel of 500 genes representing these key characteristics. We found that BC Screen partially overlaps with existing gene lists used in breast cancer prognosis, and with lists currently used in chemical toxicity screening. For example, 47% of BC Screen genes are included in the Tox21 S1500+ gene list, although representation is not uniform within each characteristic. Overlap with S1500+ ranged from 16% for the growth hormone characteristic to 76% for xenobiotic metabolism. One third or fewer BC Screen genes selected to represent effects on epigenetics, genotoxicity or immunotoxicity were included in the S1500+ list. Enrichment analysis showed that BC Screen includes genes involved in cell cycle signaling pathways, responses to steroid hormones, and nuclear receptor activation, as well as genes associated with breast cancer and reproductive diseases. BC Screen has the potential to highlight the key molecular initiating events and pathways that are involved in chemically-induced rodent mammary carcinogenesis for a diverse set of chemicals, and to serve as a tool in high-throughput chemical screening to identify potential breast carcinogens. Furthermore, our biologically-based systematic approach to gene prioritization for breast carcinogenesis can be extended to develop gene panels for other endpoints in order to support discovery related to etiology and strengthen toxicological screening.
The U.S. Environmental Protection Agency (EPA) Computational Toxicology (CompTox) research effort helps prioritize chemicals for research based on potential human health risks by integrating advances in biology, chemistry, and computer science. As an outcome of these efforts, the National Center for Computational Toxicology (NCCT) has generated an enormous quantity and diversity of data for the environmental sciences including high-throughput in vitro screening data, in vivo and functional use data, exposure models and physicochemical databases. EPA CompTox research is publicly accessible through a series of software applications. The CompTox Chemistry Dashboard web application is the most recent of these tools and is based on a software architecture that provides a web-based data integration hub associated with ~760,000 chemicals. These data include experimental and predicted physicochemical property data, bioassay screening data associated with the ToxCast and Tox21 programs, product and functional use information and a myriad of related data of value to environmental scientists. This presentation will provide an overview of the CompTox Chemistry Dashboard, its capabilities for delivering data to the environmental toxicology community and how the architecture provides a foundation for the development of additional applications to support chemical risk assessment. This abstract does not reflect US EPA policy.

CompTox Chemistry Dashboard: A Web-Based Data Integration Hub for Environmental Chemistry Data

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Data Sharing and Linking Across the Entire Drug Development Continuum

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Prior knowledge about how a chemical interacts with genes or proteins is valuable in predictive computational toxicology. Many relationships between chemicals and proteins (or genes) have been catalogued in various databases. However, these databases are incomplete; some information can be found only in the literature. Here, we describe a text-mining approach that leverages information in databases to improve the quality of text mining with the goal of identifying relationships missing from those databases. Automated relation extraction from text is difficulty due to the many ambiguities in natural language. The current state of the art consists of selecting features such as words, word stems and syntactic information, and using them as inputs to a machine learning classifier. Here, we demonstrate that automatic identification of relationships between chemicals and proteins found in existing databases to the features used in machine learning. We integrate knowledge from many different databases using the KaBOB [1] knowledge base, to automatically identify a set of five possible relations ("upregulation," "downregulation," "agonist," "agonist," and "substrate of") between a chemical and a protein mentioned in PubMed abstracts. The knowledge base incorporates information about the chemicals and proteins (i.e., "participates in kinase activity," "has N aromatic rings," "it’s lipoxygenase activating," etc). We tested our approach on an extensive manually annotated set of relations from the ChemProt [2] database (including therapeutics), using this prior knowledge in conjunction with text-derived features. Feature selection algorithms and post-hoc analysis of the machine learning results identifies the aspects of the prior knowledge that were most helpful. Furthermore, these results can now be used to estimate the probability of each of these relationships between any chemical-protein pair, based on their attributes in the knowledge base.

Knowledge-Based Chemical Relation Extraction

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Applying Bioinformatics to Unravel Mechanism of Toxic Effects of E-Cigarette Aerosol Exposure

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Multi-billion dollar ECIG industry continues to grow as more people use ECIGs, out of curiosity, a desire to quit smoking, or a safer way to continue a nicotine addiction. However, there is still little evidence regarding the physiological effects of exposure to ECIG aerosol and the aim of this study was to investigate toxicities induced by electronic cigarette aerosol. We analyzed gene expression data of primary human bronchial epithelial cells grown at Air Liquid Interface exposed to four different ECIG aerosols. Two different flavors and presence or absence of nicotine in ECIGs were compared. From the whole genome analysis, 204 signature genes (p<0.05, FC>1.5) were detected as significantly deregulated after exposure to any ECIG aerosols. We created a computational model of biological pathways describing cellular processes in human bronchial epithelium by manually annotating and processing molecular information from the publicly available data (PubMed articles and FDA reports) and made the information computable. ECIG exposure induced genes involved in oxidative stress pathways and decreased expression of genes involved in cilia assembly and movement. These changes were more pronounced in ECIG products containing nicotine than those without nicotine. Furthermore, we generated a comprehensive database of known side effects. By applying bioinformatics analysis tools to this data, we have identified a number of adverse effects associated with cardiovascular, nervous and hepatic system, and generated the mechanistic hypothesis describing the involvement of ECIG products in development of those toxicities.

BioCelerate Toxicology Data Sharing Initiative: Development of a Centralized, Searchable, Preclinical Data Repository for the Biopharmaceutical Industry

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BioCelerate, a subsidiary of TransCelerate, was formed in 2015 as a preclinical industry consortium that could identify and implement initiatives to drive efficiency and productivity in early stage R&D. Toxicology Data Sharing (TDS) is the first initiative launched under BioCelerate and involves creation of a centralized, searchable toxicology data repository that will enable participants to make more informed decisions on compound progression based on increased understanding of on-target and off-target toxicity. The project has also been extended to include a repository of background control data. Moreover, the initiative has implemented a framework for successful and secure collaboration and data sharing within a biopharmaceutical industry consortium. Motivated in part by the FDA’s 2011 Strategic Plan for Regulatory Science and Innovation, the TDS Initiative partnered with a technology vendor to design, develop, host, and maintain a cloud-based data lake to facilitate sharing and analysis of deidentified unstructured (e.g. PDF) and structured (e.g. SEND) data sets. The platform was designed for flexibility and modularity, built in line with our future-state vision - to enable voluntary data sharing and linking across the entire drug development continuum, from preclinical discovery through late phase clinical studies. Presented here are the core capabilities, use cases and value story driving the TDS Initiative, the processes and system architecture for the core data sharing platform, and the principles guiding data sharing and collaboration amongst BioCelerate participants.

Development of a Computerized Workflow to Predict Nicotinic Acetylcholine Receptor-Mediated Acute Mammalian Toxicity

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While techniques such as sub-structure scaffolding and ligand-docking are well-established in the field of computational drug discovery, their utility in predicting the toxicity of xenobiotics via receptor-mediated interaction is unclear. To this end, we aimed to develop automated in silico workflows to predict the acute toxicity of compounds based on their interactions with known biological targets. As an initial proof-of-concept effort, we investigated the nicotinic acetylcholine receptor (nAChR) as a model target receptor. The nAChR is a cholinergic cys-loop receptor that functions as a ligand-gated cation channel that responds to its endogenous ligand (acetylcholine; ACh) as well as numerous xenobiotics (e.g. nicotine), representing an important therapeutic target and a potential off-target of toxicity. We curated a database of over 4,400 nAChR agents using a combination of automated and semi-automated data-mining approaches. Using a cluster-based chemical scaffolding algorithm coupled with machine-learning techniques, we identified 92 unique scaffolds that describe the chemical space of nearly all known nAChR agents with high sensitivity (96.4%) and specificity (82.4%) when iteratively tested against a challenge set comprising 10% of the known nAChR non-binders from a public ToxCast and in vitro database. Using high-throughput molecular docking, we evaluated the ability of compounds within various scaffolds to interact with the nAChR at its ACh binding site, peripheral allosteric binding site and receptor channel. Based on the docking results, we developed a set of regression models to predict the intravenous LD50 or in vitro EC50 values for several classes of nAChR agents with reason-
2523 Applying a High-Throughput PBTK Model for IVIVE

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The ability to link in vitro and in vivo toxicity enables the use of high-throughput in vitro assays as an alternative to resource intensive animal studies. Toxicokinetics (TK) should help describe this link, but prior work found weak correlation when using a TK model for in vitro–in vivo extrapolation (IVIVE) (Wetmore et al., 2013). In this work, we evaluated the assumptions in the use of a high-throughput, physiologically based (PBTK) model to relate in vitro and in vivo toxicity data. The generic, high-throughput PBTK model in this study used rat in vitro measured values of fraction unbound in plasma (fu) and intrinsic hepatic clearance for 92 chemicals. In vivo doses (endpoint-specific low effect levels for rat, EPA’s ToxRefDB) were transformed to concentrations (cPBTK) via the PBTK model, and compared with in vitro AC50 (EPA’s ToxCast program, 394 assays) relative to a randomized parameterized result (rand) and untransformed dose (xasso). For each pair of in vitro assay and in vivo response, simple regressions were performed of standardized AC50 vs the three separate predictors: PBTK, xasso, and fu. Different combinations of assumptions in the use of the PBTK model were then evaluated based on how frequently PBTK, xasso, fu, and xasso 8 predictor had the largest absolute slope. The best result for the PBTK model was achieved by using maximum plasma concentration and assuming metabolism independent of fu (PBTK = 82 %, xasso = 7 %, and fu = 11 %). Using in vitro free vs cell culture also improved results for cell-based assays (PBTK = 87 % vs 78 %). Results demonstrate that use of the PBTK model improves the correlation between the in vitro and in vivo toxicity data relative to the untransformed dose and the randomized result in the rat. This suggests that incorporating TK may enhance human IVIVE. This abstract may not reflect US EPA policy.

2524 Virtual Screening of Chemicals for Estrogen and Androgen Activities

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Toxicity assessments of chemical substances, only using their structures, can cause damage to the respiratory organs and organism as a whole. While regulatory bodies, e.g., European Union, require inhalation toxicity evaluation to be performed for high-volume production chemicals, the use of animals is not permitted in such evaluations. In place of animal testing, it is often recommended to use data, identification of structural alerts to judge toxicity, grouping of chemicals for read-across analysis, and the development and use of quantitative structure-activity models. However, the volume of experimental data and number of reliable QSAR models, concerning the acute inhalation toxicity is limited which hinders all in silico approaches. In this study, we developed a set of QSAR models built using publically available acute inhalation toxicity data in rat. The set consists of four categorical models, designed to predict one of the four GHS categories available acute inhalation toxicity data in rat. The set has assign the correct GHS category to 75% of the external validation compounds. Coverage was maintained in the range of 71-84%. Identified alerts are discussed along with the advantages and disadvantages of using the categorical versus the default model. Overall, this approach provides a reliable QSAR method for predicting acute inhalation toxicity of chemical substances, only using their structures.
Gaps in the database of points of departure (PODs) constitute a major hurdle in public health chemical risk assessment. Methods of computation and selection of high-throughput screening are often viewed as an adjunct to laboratory mammalian toxicology that can help filling the gaps. In the present work, statistical relationships between oral health guidance values (HGVs) for different exposure durations and associated PODs were examined. The endpoints specific to acute, intermediate, and chronic exposure durations were extracted from the ATSDR MRL and US EPA RFD databases; these sources represent a unique data collection concerning adverse health effects due to low-dose chemical exposures. The strength of association between the acute, intermediate, and chronic oral endpoints were investigated using the correlation and regression analyses. The correlation coefficients for acute, intermediate (AI), acute-chronic (AC), and intermediate-chronic (IC) log-HGV pairs in molar units were all similar at about 0.93. Both type I and II regression approaches were explored. Because the relationships were log-linear with slopes statistically equal to 1, simple linear regression with ordinary least squares was applied. The derived relationships suggested the possibility of cross-extrapolation between HGVs for different exposure durations within the limits of statistical uncertainty. The estimated extrapolation factors between HGVs for AI, AC, and IC durations were 5, 5, and 2, respectively. Because of remarkable strength of correlation, in the future, the statistical uncertainty is expected to decrease as the number of HGVs available for regression analyses increases. The proposed relationships are consistent with recent US EPA conclusions (Thomas R.S. et al, Toxicol Sci 134:180-194) concerning “temporal concordance between apical and transcriptional points of departure for chemical risk assessment.” Disclosure: The findings and conclusions in this presentation have not been formally disseminated by the CDC/ATSDR and should not be construed to represent any agency determination or policy.

The potential for neurotoxicity in adults and children following exposure to environmental chemicals remains a high public priority due to concerns that recent increases in the prevalence of neurological disorders may in part be due to chemical effects. Thus, the need for reliable and efficient screening tools to identify, prioritize, and evaluate chemicals for their potential to induce developmental neurotoxicity (DNT) is well recognized. To address this, the National Toxicology Program (NTP) created a library comprising a set of reference compounds (e.g., developmental neurotoxicants, flame retardants, PAH compounds, and so forth) that were not available for regression analyses. The findings and conclusions in this presentation have not been formally disseminated by the CDC/ATSDR and should not be construed to represent any agency determination or policy.

The scientific community.

OpenRiskNet is an EU funded infrastructure project with the main objective to develop an open e-infrastructure providing resources and services to a variety of industries requiring risk assessment, including chemicals, cosmetic ingredients, drugs and nanomaterials. The OpenRiskNet approach is to work on different case studies to test and evaluate requirements to overcome the fragmentation of data and tools and to provide solutions improving the harmonization of data, usability and interoperability of application programming interfaces (APIs) and the deployment into virtual infrastructure. The cases present real-world settings such as systems biology approaches for grouping compounds, read-across applications using chemical and biological similarity, and identifying areas of concern based only on alternative methods approaches. We discuss our progress on the OpenRiskNet goal of harmonizing data and metadata within APIs that can be added to already existing analysis and modeling services and data warehouses. We also demonstrate how these APIs can be easily be used towards the generation of full risk assessment workflows either using scripting languages or workflow managers. Finally, we show the first approaches to make these APIs semantically rich by annotating data with human- and computer-readable data schemata. OpenRiskNet has initiated the Associated Partners Programme strengthening the working ties between the OpenRiskNet members and other organizations within the scientific community.

Pharmacogenomics: A Genomics-Based Toxicity Screening Platform


Elucidating the in vivo molecular actions of drugs can provide insights into drug toxicity. However, systematic efforts in examining the molecular pathways influenced by individual chemicals in in vivo systems remain limited. Previous efforts, e.g., the Connectivity Map, have focused on genomic footprints of drug compounds based on in vitro cell lines mostly applicable to oncology studies, which may not cover a broad spectrum of toxicity. On the other hand, curated toxicity databases like the comparative toxicogenomics database (CTD), are largely based on previously published literatures and in vitro assays and may not provide a complete picture of toxicity in in vivo systems. Here, we present a framework to construct a comprehensive drug-toxicity knowledgebase, Pharmomics, using genomic footprints in a species- and tissue-specific manner, aggregating and meta-analyzing microarray and sequencing data relevant to drugs/substances from tissues and primary cell populations derived from human, mouse, and rat samples. Using multi-tissue/cell gene expression signatures as intermediate modulators, we can infer the perturbed molecular pathways and associate drugs with various potential toxicity endpoints and diseases that share similar gene expression signatures. We will first validate the platform using a training set of known drug toxicities and apply the platform to make novel predictions, followed by experimental validation in animal/cell models. We have established a prototype of the Pharmomics platform and constructed species- and tissue-specific gene signatures of select cardiovascular drugs. We envision this informatics-platform based on big data will offer species- and tissue-specific insights into the in vivo drug activities and can be broadly applicable to toxicity prediction.
2531 Deriving Pathways of Toxicity from Omics Data: Endocrine Disruption as a Case Study

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Systems toxicology has transformed toxicology from a reductionist approach to a more holistic viewpoint that takes advantage of the newer, high-content and high-throughput technologies. From a hazard assessment perspective, it offers an opportunity to move away from the limited mechanistic information provided by a traditional "blackbox" animal tests to a pathway based approach that can provide a detailed mechanistic understanding at a cellular level. Endocrine disruptors have proven very difficult to understand using animal models, as they have subtle effects over a long-term exposure and not necessarily with a monotonic dose response curve. Here, we show how using a network based approach to an in vitro microarray dataset of endocrine disruptors and a time course can derive a basic Pathway of Toxicity and better elucidate the complexity of cellular responses to estrogenic substances such as BPA, can identify thresholds, and elucidate target receptors with better accuracy than other approaches. Moreover, a network approach can reveal several important genes in humans that may not have analogs in other model systems and would also be likely be invisible in an annotation based approach to transcriptomic data.

2532 New Features in ToxRefDB to Improve Modeling Applications and Data Integration

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The Toxicity Reference Database (ToxRefDB) contains publicly available in vivo toxicity studies conducted largely to Environmental Protection Agency (EPA) guidelines, with information for >1,000 chemicals and >5,000 studies. ToxRefDB has been utilized for training and testing of alternative models yet a "positives"-only resource, i.e., only effects significantly different from controls were included. The lack of "true negatives" limited available balanced datasets for modeling. Other impediments to modeling may include a lack of quantitative dose-response information and controlled vocabulary for in vivo effects. The objective of this work was to address these challenges to improve ToxRefDB as a predictive modeling resource. First, endpoints were annotated as either required, triggered, recommended, or not required/mentioned based. The new annotations included "true negatives" to be lin- dignished from "not tested" endpoints. Next, quantitative data were extracted for subchronic and chronic studies, enabling statistical modeling on nearly 400 endpoints. In this process, units were standardized to enable comparisons, decreasing the total unit number from >800 to <400. Finally, the lack of controlled vocabulary for in vivo effects led to challenges in continued data extraction, QA/QC, and integration with other resources. As a solution, ToxRefDB vocabulary was standardized to reflect the language used in corresponding EPA 870 series guidelines and cross-referenced to the National Cancer Institute Thesaurus (NCIt). These cross-references are based on Clinical Data Interchange Standards Board (CDISC), an international effort to describe clinical and non-clinical data. NCIt cross-references enable mapping of in vivo pathological effects from ToxRefDB to PubMed (via MeSH terms) and subsequent genes (and other information) that may be relevant for toxicological research. The new annotations to ToxRefDB increase the potential for connections between ToxRefDB and other resources and enhance utility for toxicology modeling applications. This abstract does not necessarily reflect USEPA policy.

2533 In Silico Identification of Molecular Targets for Three Optical Brighteners


Optical brighteners (OB) are fluorescent white dyes that absorb ultraviolet light and produce fluorescence in the blue region, creating a false sense of whiteness and cleanliness. These chemicals have been massively used in laundry detergents, although few studies have been carried out on their toxic effects, implying a lack of knowledge regarding potential risks to environmental and human health. The goal of this study was to employ molecular docking to identify possible target proteins and signaling pathways modulated by three OBs (DAST, DASC-4 and FWA-1). Protein target identification was carried out using PharmMapper web server, selecting those human proteins contained in PharmTargetDB that generated the best 50 complexes with each OB. The proteins were subsequently downloaded from the Protein Data Bank database, prepared using Sybyl-X and then employed to dock the optimized OB structures. The best docking affinity values, lower than ~9.0 kcal/mol, were found in complexes formed between OBs and nuclear receptors, ERbeta, RAR gamma, PPAR, as well as for proteins involved in the metabolism of glutathione and phospholipids, apoptosis and glucose tolerance, among other processes. FWA-1 was the chemical that produced the greatest number of AOPs. Results suggested OBs may have the capacity to act as endocrine disruptors and alter mechanisms involved in oxidative stress and cell death. In short, OBs are potentially active chemicals that should be subjected to greater toxicological scrutiny to minimize adverse effects from current exposure. Unicartagena (2016-2017), Colciencias, 647-2014.

2534 ICE Tools for Aligning Assay Endpoints to Adverse Outcome Pathways

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A critical challenge to the implementation of non-animal approaches in chemical safety testing is linking endpoints measured in these approaches to adverse physiological responses in vivo. The adverse outcome pathway (AOP) framework allows these molecular, cellular, and tissue-level endpoints to be placed in a biologically relevant context. The National Toxicology Program's Integrated Chemical Environment (ICE) web resource houses curated data from in vivo, in vitro, and in silico assays and models. Many of these non-animal data sources measuring different key events in an AOP can be integrated to form defined approaches to testing and assessment for particular toxicology endpoints. The ICE ontology maps assay endpoints to key events within AOPs and enables use of ICE data with AOPxplorer, a Cytoscape plugin that allows visualization of data in AOP networks. The ability to map ICE data to AOPs can be used to identify data gaps and build confidence in the mechanistic plausibility and relevance of a proposed defined approach. This work is also relevant to understanding the respective role of active ingredients in mixtures, such as pesticide formulation products. ICE includes data on over 300 active ingredients in over 800 formulations, and provides the ability to highlight key events perturbed by the active ingredients, and compare that to formulation toxicity data. We demonstrate the utility of this workflow using an accepted AOP for skin sensitization and putative AOPs for acute toxicity pathways. This was funded with US federal funds from the NIEHS/NIH/NIH under Contract HHSN273201500010C.

2535 Use of ToxCast High-Throughput In Vitro Data to Develop a Computational Model to Identify Compounds That Interact with Neurological Receptors


The ability to predict the neurotoxicity of xenobiotics is a major goal of predictive toxicology. ToxCast data presents an important repository of structurally diverse compounds with high-throughput in vitro assessment of their activity against a number of toxicologically relevant targets. We sought to understand the utility of this resource by using it as the basis for developing two-dimensional structural schematic-based prediction models to screen compounds for their ability to interact with neurological receptors. We modeled the ToxCast database for compounds that interacted with the nicotinic and muscarinic acetylcholine receptors, acetylcholinesterase, the GABA receptors as well as the serotonin and glycine receptors. For each target, the active compounds were clustered and common structural motifs (scaffolds) identified using an automated in-house KNIME workflow. For targets with a sufficient number of active compounds, we were able to build accurate prediction models with sensitivities in the range of 80-96% and associated negative prediction values >93%. Using a similar workflow to analyze the 264 compounds that were active against any of the 21 neuronal targets in ToxCast, we identified 153 unique scaffolds and developed a prediction model that can identify the likelihood of a compound inter-
The PubChem database contains highly curated information "on the biological activity of small molecules". It can be used to get a high-level overview of available information about chemicals. Toxicologists seek to gain an understanding of the effects of chemicals by grouping them into classes based on their structure and toxicological effects. Researchers can discover chemicals which are novel or relatively unstudied in the same class as highly studied chemicals by seeing the density of public information on toxicologically and structurally related chemicals. By noting perturbed biological pathways or toxicological effects of highly researched chemicals, researchers can make informed decisions as to what evaluations they should run on novel compounds. 

PubChem-heatmap is written in Shiny, an R web framework. Users input compounds using chemical identifiers and view the data density (or word count) of sections of PubChem. The heatmap clusters chemicals using various similarity tests. By default, similarity is calculated by converting the "CACTVS Substructure Key Fingerprint" contained in the "Chemical and Physical Properties" section of PubChem into 880-bit descriptors which are used to calculate Tanimoto similarity scores. Individual chemicals and sections can be selected to see a word cloud or curated 'hit call matrix', identifying highly discussed organs, biological systems, cell types, and assays related to a chemical. Perfluorinated chemicals of interest to the NTP were surveyed using the tool. Out of 20 chemicals had little to no information in sections such as 'Toxicity' and 'Pharmacology and Biochemistry'. Perfluorohexanesulfonic acid, the compound with the most information in the 'Toxicity' section of those surveyed, has been linked to developmental neurotoxicity and endocrine signaling disruption. This information can be used to guide exploration of the additional chemicals in the class. Since the tool is written in R, it can be adapted to work with similar databases and extended to provide additional functionality. Future directions for the tool are to integrate it with other established software and services like SWIFT-Review to gain a broader view of the body of research related to a compound.

Digital PCR has been promoted as a method for obtaining absolute measures of the amount of nucleic acid in a sample and has gained widespread use since the first instruments were introduced approximately 10 years ago, but still lacks standardization in data reporting. In many cases, concentrations of target have been represented only on a per volume basis, making comparisons between data from different sets of assays and especially different platforms difficult. We considered that a more useful approach would be to normalize the measured copies to the amount of nucleic acid present in the assay. Since many factors can affect the actual concentration of nucleic acid in the final digital PCR assay, our goal was to develop a method which would account for as many of these factors as possible. Using droplet digital PCR as our platform and previously validated reference genes duplexed with the target genes, we have developed a method of normalization which can be used to estimate the amount of input nucleic acid in the individual assay, and have subsequently reported the number of copies of a target gene relative to this amount. Correcting for the actual amount of nucleic acid present demonstrated a much higher correlation between various dilutions of sample mRNA compared to that obtained by using the theoretical amount and allowed us to make more widespread comparisons of digital PCR results. Supported by the Intramural Research Program at NCI/NIHES.

Alternative splicing is a gene regulatory process that splices out introns (a segment of DNA that consist of noncoding material) and chooses specific exons (a segment of DNA that is intended to be included in the final mRNA product which can result in different protein products). Alternative splicing is important to the regulation of normal physiological functions and is dysregulated in pathologies. To analyze alternative splicing, researchers use software such as JuncBASE, MISO, and MapSplice. However, through reviewing RNA sequencing (RNA-Seq) of tumor samples, we found a previously uncharacterized and complex splicing event in CTNNB1, a gene known to be mutated in many cancers that software like JuncBASE cannot detect. The issue of not detecting these events could cause some genes with splicing changes to be unaccounted for in the analysis of RNA-Seq data. We present our new approach called MESA: Mutually Exclusive Splicing Analysis, that will be able to detect these complex splicing events in RNA sequencing data. The user will input junction coordinates (location where intron was spliced out) and as output will receive mutual exclusive junctions, and a calculated Percent Spliced In (PSI) of those mutually exclusive junctions. The program will allow researchers to identify all possible splicing events and identify new ways that genes can be alternatively spliced.

The EPA Adverse Outcome Pathway Database (AOP-DB) is a database resource that aggregates association relationships between AOPs, genes, chemicals, diseases, pathways, species, orthology information, and ontologies. The AOP-DB front end is a simple yet powerful AOP-DB user interface in the form of a web application. By replacing applications like MySQL Workbench and the MySQL command line interface (MySQL CLI), it allows users to interact with the SQL database conveniently within their preferred web browser on any operating system. This approach includes all information that the AOP-DB has to offer without the need of excess applications or knowledge of creating SQL queries. The front end itself is built using pure-JavaScript frameworks Node.js and AngularJS. These modern frameworks allow for increased modularity, responsiveness, and robust page features without impacting development time. Currently, the AOP-DB front end is to be utilized for exclusive in-house EPA use (intranet); though this is the case, the front end is being built with eventual public external (internet) use in mind by taking appropriate measures for administration and security. Alongside these considerations, the application will also serve as a framework for other databases housed at the EPA. One database integrating the front end framework is the EPA Nanomaterials Research Database (NaKnowBase), a database responsible for organizing physical and chemical parameters of nanomaterials and their potential actions on environmental and biological systems. The workflow described here includes a finished mockup for the AOP-DB, a prototype that includes essential features such as database navigation, and site administration. This abstract does not reflect US EPA Policy.

COSMOS Database, maintained through the COSMOS DataSharePoint, is part of a suite of tools for managing and storing toxicity and chemical data to assist in the early assessment of cosmetics-related substances under the paradigm of non-animal testing strategies. This public resource comprises a total of 102,285 substance records spanning 31 toxicity endpoints searchable within COSMOS DB. Also included in the database is the US EPA’s most extensive and openly accessible database compiled from 15 sources, including regulatory inventories such as Cosling, Korean Cosmetics Industry Institute, US Personal Care Products Council,
and Cosmetics Ingredient Review, as well as the European Food Safety Authority (EFSA). In order to assure data quality, the COSMOS project established a robust data quality evaluation process through application of the COSMOS MINIS criteria. These were developed from toxicity data, including the oRepeatToxDB and used to derive a quantitative data reliability metric, which in turn is essential for estimating uncertainties in information used for Read-Across. Assessment of the uncertainties based on multiple sources of data includes consistent comparisons of data quality, which depends on the reliability and relevance of the data. Reliability scores define five categories, ranging from “meeting all COSMOS MINIS criteria (score=5)” to “meeting none of the major criteria (score=1)”. In addition, COSMOS DB allows for convenient research reproducibility, significant challenges persist with data sharing in toxicity and environmental health sciences. This study provides an initial look into the state of data sharing in environmental health sciences with the goal of informing the additional resources and infrastructure that are needed to increase the discovery, accessibility, and reuse of NIH-funded data. A sample of NIH-funded journal articles that were published over a two-month period in 2015 (N = 357) were analyzed for evidence of data sharing. In total, 20% of NIH-funded supported publications exhibited some level of data sharing. Of these articles, more than half (11.4%) cited human subject data, while few articles with human subject data had evidence of sharing. Further, impacts of publisher data sharing requirements were examined. Journals were classified using a published rubric for strength of their data sharing policy, and publications in journals with strong data sharing policies exhibited a greater proportion of data sharing (30.3%) that those with a weak (15.5%) or unclear (15.5%) sharing policy. Overall, these findings provide a representation of the current landscape of data sharing in environmental health sciences, which are largely consistent with previous analyses of data sharing in journal articles funded across all NIH institutes, and suggest that additional efforts to increase data sharing are warranted.

2541 Digging for Data: Understanding Data Sharing in Environmental Health Sciences


Environmental health research is becoming increasingly data-centric. Data sharing is essential for maximizing the utility and impact of the rich and diverse datasets being generated by the environmental health research community. Despite many known benefits of sharing data, including enhanced research reproducibility, significant challenges persist with data sharing in toxicology and environmental health sciences. This study provides an initial look into the state of data sharing in environmental health sciences with the goal of informing the additional resources and infrastructure that are needed to increase the discovery, accessibility, and reuse of NIH-funded data. A sample of NIH-funded journal articles that were published over a two-month period in 2015 (N = 357) were analyzed for evidence of data sharing. In total, 20% of NIH-funded supported publications exhibited some level of data sharing. Of these articles, more than half (11.4%) cited human subject data, while few articles with human subject data had evidence of sharing. Further, impacts of publisher data sharing requirements were examined. Journals were classified using a published rubric for strength of their data sharing policy, and publications in journals with strong data sharing policies exhibited a greater proportion of data sharing (30.3%) that those with a weak (15.5%) or unclear (15.5%) sharing policy. Overall, these findings provide a representation of the current landscape of data sharing in environmental health sciences, which are largely consistent with previous analyses of data sharing in journal articles funded across all NIH institutes, and suggest that additional efforts to increase data sharing are warranted.

2542 Use of ToxPrint Chemotypes for Exploring Chemical Feature Enrichments across the ToxCast Chemical-Assay Landscape

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EPA’s ToxCast library spans diverse chemical uses, functionalities, structures and features potentially relevant to toxicity and environmental exposure. This diversity, along with assay noise and low average hit rates across the high-throughput screening (HTS) technologies used in sets, poses challenges to traditional, global structure-activity relationship (SAR) modeling approaches. A publicly available set of 729 ToxPrint chemotypes (CTs), representing a diverse set of chemical features spanning toxicity alerts, common scaffolds, and varied ring, bond, and atom types, is being used to explore the dimensionality of the ToxCast inventory and codify local chemistry domains to amplify structure-activity signals within those domains. An automated CT-enrichment workflow (CTEW) has been developed, with preliminary functionality released via the EPA Chemistry Dashboard (https://comptox.epa.gov/). ToxPrints were computed for the entire DSSTox database (>700K structures), and CT-enrichment results were generated for >800 ToxCast/Tox21 HTS assays. Over 460 CTs are statistically enriched (Odds Ratio >3, Fisher’s Exact p-value <0.05) in ≥ 1 assay, with 191 of these CTs enriched in ≥ 40 assays; 600 assays are enriched with ≥ 3 CTs, with 140 of these assays enriched in ≥ 40 or more CTs. These results offer a rich vein of chemical inferences to mine, such as CTs associated with promiscuous HTS activity. The CTEW has been applied to a variety of datasets, including ToxCast assays grouped by target type (e.g., nuclear receptors) and by common detection technologies (e.g., autofluorescence); analytical QC “failed” Tox21 chemical samples; microelectrode array (MEA) neurotoxicity assay results where enriched CTs support mechanistic linkages to ToxCast ion channel assays enriched with the same CTs; and various activity subsets within the ToxRef vivo dataset. The approach offers an intuitive, flexible complement to traditional SAR methods, with results that are easily interpreted, anchored to visualizable chemical features, and that can productively guide more targeted SAR investigations. Abstract does not reflect US EPA policy.

2543 Novel Approaches to Safer Chemical Identification for the US EPA’s Safer Chemical Ingredients List


Candidate chemicals for the US EPA’s Safer Chemical Ingredients List (SCIL) are routinely obtained via product formulation submissions to the Safer Choice program (SCP). Chemicals used in Safer Choice-certified products are published on the SCIL and represent a palette of commercial chemicals that meet specific functional use criteria. These criteria include comprehensive review analyses for human health, ecotoxicity, and environmental fate. Efforts to further expand EPA’s SCIL were conducted with the goal to provide stakeholders, such as product formulators and manufacturers, with a dynamic and informative tool that can be used to formulate new products and support the development of Safer Choice-certified products in new product sectors. This presentation will describe how the current universe of commercial chemicals was screened and prioritized to identify candidates for the SCIL. Two novel approaches were developed using publicly available datasets and tools. Multiple chemical inventories containing functional use information were employed in this study, including the EPA’s Chemical and Product categories (ChemCat) database, the ECHA’s Candidate List (CL) database, the Environmental Working Group (EWG’s) Skin Deep® database and the National Institute of Health (NIH)’s Household Products Database. In the first new approach, the functional use chemical inventories were combined and duplicate entries and known PBT and CMR chemicals were removed. Chemical structures were obtained for the chemicals in the combined data set, using SRC internal databases of chemical structure information. The structures for the chemicals in the combined dataset were clustered using EPA’s ChemACE software. Clusters containing substances that were structurally similar to those chemicals already meeting SCIL criteria were chosen for further SCP review as SCIL candidate chemicals. In the second approach, the REACH and the Danish QSAR toxicological data sets were queried to identify chemicals with reported experimental or estimated toxicity values that would likely pass SCP criteria. The chemicals with known toxicological uses were identified as candidate chemicals for further SCP review which included a comprehensive hazard screening assessment. Over 40 chemicals have been added to the SCIL as result of these screening and prioritization methodologies.

2544 Identifying Attributes That Influence In Vitro-to-In Vivo Concordance by Comparing Tox21 Bioactivity versus DrugMatrix Transcriptional Responses to 130 Chemicals


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The concordance between chemical-induced responses observed in vitro high-throughput screening (HTS) vs. in vivo models represents a critical research area, as the integration of HTS data in chemical toxicity assessments is increasing in prevalence. This investigation set out to (i) measure concordance between chemical-induced responses observed in vitro and in vivo across many diverse classes of compounds, including pharmaceuticals and environmental/industrial chemicals, were compared between in vitro data produced in the Tox21
suit of bioassays and transcriptomic data from the liver of rats exposed to a chemical for ≤5 days, available through the DrugMatrix database. A comparison of pathway-level responses showed both similar and different signaling profiles between datasets. A global comparative analysis (i.e., in vitro vs. in vivo) resulted in a Cohen's kappa concordance statistic of 0.02 and a percent agreement of 79%, with results ranging on a per-chemical basis between -0.25-0.76 and 41-100% for the Cohen's kappa statistic and percent agreement, respectively. Three attribute categories were then analyzed for their potential to influence response concordance. First, experimental design attributes were found to influence concordance, including cell type and target pathway. Second, in silico-derived drug similarity was shown to influence concordance by filtering DrugMatrix data to only include experimental doses administered to rats that were within a 10-fold factor of those related to likely bioactivity, derived previously using ToxCast/Tox21 data and high-throughput toxicokinetic modeling. Third, chemical-specific attributes were evaluated by modeling physicochemical properties and structure fingerprints, and the partition coefficient (logP) and a subset of structural attributes were predicted to influence response concordance. Together, findings suggest that experimental design, dose applicability, and chemical-specific attributes should be considered when using in vitro HTS data to predict or inform in vivo toxicological responses.

2545 Development, Validation, and Integration of QSAR Models to Identify Androgen-Active Chemicals within CoMPARA Project

S. Manganelli1, A. Manganaro1,2, A. Roncaglioni1, K. Mansouri3, E. Benfenati4, and P. Ruiz5. 1 of animals that potentially could be used for testing. This roundtable discussions will explore the opportunities and challenges in utilizing in vitro and other alternative toxicology testing methods for the assessment of e-cigarettes. The panel members, representing a wide range of viewpoints, including academia, industry, and government, will discuss: 1) how global regulations are impacting research; 2) what innovations are necessary to make the science ready for regulatory decision making; 3) how to foster collaboration to ensure standardization of approaches; 4) what lessons can be taken from other industries and agencies adopting alternative approaches; 5) what role industry and other stakeholders can play; and 6) how progress can be accelerated.

2547 Arsenic, a Gift and Malice: From Discovery to Detrimental Effects, a Historical Perspective

B. Mahadevan, Abbott Laboratories, Mumbai, India.

From natural/industrial toxin to chemical warfare, murder to crime fiction, healer to poison, arsenic remains a powerful force in modern life. Arsenic toxicity is a global health problem affecting millions of people. Apart from the limit on levels of arsenic (10 parts per billion (ppb)) in drinking water established by the US Environmental Protection Agency, the US Food and Drug Administration (FDA) has been measuring total arsenic concentration in foods through its Total Diet Study and has come up with limit levels. In order to better understand the toxicity of arsenic, one needs to consider its toxicokinetic and toxicodynamic interactions. This session will elaborate on our understanding of the interactions of arsenic (As III, As V, and total), as well as the role of arsenic metabolites (dimethylarsinic acid and monomethylarsonic acid). The US FDA found that inorganic arsenic exposure in infants and pregnant women can result in a child's decreased performance on certain developmental tests that measure learning based on epidemiological evidence about arsenic, including dietary exposure. More recently, the US FDA has proposed an action level of 10 ppb for inorganic arsenic in apple juice. The objectives of this symposium are to provide: 1) historical and scientific understanding of arsenic from toxicology and therapeutic perspective; 2) safety policies; 3) recent updates on mechanism of arsenic toxicity and carcinogenesis; and 4) the thought process on arsenic exposure through food.

2548 In It to Win It: How to Negotiate During the Interview Process

K. Sant, University of Massachusetts, Amherst, MA.

After years of professional training, early-career toxicologists are eager to start interviewing to finally secure their dream job. While nailing the interview is important, navigating the delicate process of negotiations is critical to successfully sealing the deal. However, negotiations are often kept private, giving trainees little knowledge of negotiation logistics and etiquette. Further, negotiating procedure and tactics can vary widely between academia, industry, and government. This session is designed to provide trainees with tips and strategies that will help them successfully navigate the negotiation process. Speakers, representing successful toxicologists from academia, industry, and government, will: 1) provide an overview of the negotiation process; 2) give advice on specific items that are included in recruitment packages; and 3) present practical examples of negotiating skills and techniques. The presentations will be interactive and will engage the audience through live polling technology, role playing, and mock negotiations. These discussions will be highly relevant to all student and postdoctoral attendees, as well as senior toxicologists considering a transition across the professional sectors. This career development session will stimulate an active discussion about how negotiations proceed and provide trainees with strategies, tips, and the confidence to navigate this daunting process and secure their dream job.

2546 Alternative Toxicology Approaches to Evaluate Next-Generation Nicotine Products

J. Fowle III. Science to Inform, LLC, Pittsboro, NC.

The development and uptake of novel nicotine products, including e-cigarettes, has grown rapidly around the world in the last decade, creating a need to evaluate the potential health risks associated with the use of these products. The US Food and Drug Administration Center for Tobacco Products and the European Union Tobacco Products Directive have made recommendations and issued guidance documents outlining the criteria for assessing the risks of these novel products. As part of these proposed frameworks, significant nonclinical testing is required. A wide variety of stakeholders are concerned about the large number of animals that potentially could be used for testing. This roundtable discussion will explore the opportunities and challenges in utilizing in vitro and other alternative toxicology testing methods for the assessment of e-cigarettes. The panel members, representing a wide range of viewpoints, including academia, industry, and government, will discuss: 1) how global regulations are impacting research; 2) what innovations are necessary to make the science ready for regulatory decision making; 3) how to foster collaboration to ensure standardization of approaches; 4) what lessons can be taken from other industries and agencies adopting alternative approaches; 5) what role industry and other stakeholders can play; and 6) how progress can be accelerated.

2549 Application of Data from New Approaches in Regulatory and Product Safety Decisions

R. Thomas, US EPA, Research Triangle Park, NC.

Following more than 10 years of evolution in applied toxicology from the release of the seminal National Research Council report, Toxicty Testing in the 21st Century: A Vision and a Strategy, and the progress to date highlighted in the 2017 report, Using 21st Century Science to Improve Risk-Related Evaluations, there is now broad recognition of the problem to be targeted with innovations in applied toxicology: Hazard and exposure assessments are needed for thousands of chemicals, and the data gaps present cannot be filled using solely traditional methods in toxicology and exposure science due to time and resources. High-throughput predictions for bioactivity and exposure are beginning to inform both
regulatory and product safety decisions, including prioritization, screening-level assessments for emerging contaminants, read-across, and product development and safety assessment decisions. Critical to the use of high-throughput and alternative methods for decisions informed by toxicology is definition of the qualitative and quantitative uncertainty of these methods to ensure conservative protection of human and ecological health. The purpose of this symposium is to provide details on successful first implementations using high-throughput toxicology tools in specific types of decisions and how the associated uncertainty with these tools was understood and accounted for within the decision. Importantly, the lessons learned from these early applications of high-throughput methods to regulatory and product safety decisions will provide the context for modification of high-throughput tools and data interpretation to meet the ongoing challenges of more rapid and efficient safety assessments.

2550 Application to Prioritization for Health Canada’s CMP
T. Barton-MacLaren. Health Canada, Toronto, ON, Canada. Sponsor: R. Thomas

Following advancements in toxicology and risk assessment, Health Canada has been working toward the development of strategic tools for the prioritization of existing chemicals in the Canadian marketplace under the Chemicals Management Plan (CMP). The next goal for the CMP is consideration of the remaining 1,550 priority chemicals out of the original 4,300 chemicals identified as priorities by 2020. To meet this goal, Health Canada’s current approaches for identifying priorities and conducting screening level assessments are being further developed to make use of new approach methodologies including QSAR, adverse outcome pathways, integrated approaches to testing and assessment, and other approaches to support regulatory decision-making, including the use of high-throughput toxicokinetic data to support incorporation of in vitro bioactivity with appropriate consideration of uncertainty.

2551 GenRA: From Research and Implementation to Practical Application

Read-across is a data gap-filling technique in chemical category and analogue approaches that has been used in various regulatory programs. There are a number of steps in the category/analog workflow, though the two critical steps in the process are analog identification and analog evaluation. Source analogs are typically identified on the basis of their structural similarity whereas analog evaluation relies upon mechanistic similarity. One way that mechanistic information can be packaged to justify a read-across is by using an approach known as generalized read-across (GenRA). The read-across prediction is a similarity weighted activity of nearest neighbors characterized by chemical and mechanistic similarity. Here we present several examples where GenRA has been practically applied to identify source analogs, evaluate their validity, and make read-across predictions for specific chemicals of interest. We draw specific examples from PFAS and Superfund chemicals and highlight the insights gained from being able to make reproducible predictions with quantitative measures of uncertainty. The views expressed are those of the authors and do not necessarily reflect the views or policies of the US EPA.

2552 Utilizing Novel Data Streams to Characterize Emerging Contaminants in the Superfund Program
A. Frame. US EPA, Arlington, VA.

The Comprehensive Environmental Response, Compensation, and Liability Act, as amended, (CERCLA; also known as Superfund), authorizes the President to respond to chemical releases or threatened releases of chemicals into the environment. A significant challenge facing the Superfund program is proactively identifying novel substances of concern, understanding their health effects, and providing robust, consistent evaluations of potential chemical properties and toxicity. To address this problem, US EPA’s Superfund Program has part-nered with the Office of Research and Development’s National Center for Computational Toxicology to develop a program specific dashboard to aggregate data on over 740,000 chemicals. These data include experimental and predicted physicochemical property data, bioassay screening data associated with the ToxCast and Tox21 efforts, product and functional use information, predictive toxicity models, analytical methods, and a myriad of related data of value to environmental scientists. Specific to the Superfund program, this dashboard incorporates “screening levels” (SLs) for chemicals using standard Superfund approaches. These screening levels are risk-based concentrations derived from standardized equations incorporating physicochemical properties, toxicity, and assumptions about human exposure to calculate medium specific concentrations which are considered by the agency to be protective for humans over a lifetime of exposure. This dashboard enables users to quickly access information on novel compounds and provides estimates of human health risks, along with SLs, to facilitate site-specific risk assessments. The data in the dashboard are intended to assist risk assessors, project managers, and the public to identify emerging contaminants and facilitating access to available in vitro, in vivo, and in silico data. The views expressed are those of the authors and do not necessarily represent the views or policies of the US EPA.

2553 Utilization of High-Throughput Data for Product Safety Assessment
R. J. Rasoulipour, Dow AgroSciences, Indianapolis, IN.

Technological developments for in silico and in vitro screening assays have created the opportunity to perform 21st century product safety assessment that does not necessarily rely on anchoring to animal-intensive in vivo studies to derive all of the endpoints to perform human health risk assessment. In this talk, the high-level challenges and opportunities posed by discovering and developing novel agricultural plant protection products will be discussed, followed by a deeper dive into many of the predictive in silico and in vitro assays that are currently being utilized to design molecules of the future with more favorable human health and environmental safety profiles.

2554 Application to Risk Assessment: Can Bioactivity Predictions Be Used as a Conservative Point-of-Departure?
K. Paul Friedman, and R. Thomas. US EPA, Research Triangle Park, NC.

Use of high-throughput, in vitro bioactivity data in setting a conservative point-of-departure (POD) has the potential to accelerate the pace of human health risk assessments. Advancement toward this goal requires greater confidence that in vitro bioactivity data, in concert with high-throughput toxicokinetic and reverse dosimetry, can be used to estimate administered dose equivalents at or below the PODs derived from traditional animal studies. The primary goal of this work is to elucidate whether a “region of safety,” i.e., a threshold below which no bioactivity or toxicity would be anticipated, can be identified for nearly 400 chemicals. Using human health evaluations as well as high-throughput predictions of bioactivity, reverse dosimetry, and exposure. PODs were compiled from in vivo guideline study data available to the US EPA, Health Canada, ECHA, and EFSA, and were compared to a distribution of administered dose equivalents calculated for ToxCast bioactivity and predicted exposure (ExpoCast). This work demonstrates more broadly the feasibility, and challenges, of using high-throughput predictions of bioactivity as a conservative POD in screening level assessments. The views expressed are those of the authors and do not necessarily represent the views or policies of the US EPA.

2555 Effectively Leveraging Cellular Functional Genomics Strategies for Elucidating Chemical Mechanisms of Action
A. Bone. US EPA, Research Triangle Park, NC.

High-throughput toxicity testing has become increasingly important in the field of toxicology, as evidenced by the advent of programs such as ToxCast and Tox21. However, these programs cover a fairly limited amount of biological space and are constrained by current knowledge of biological pathways of toxicity. In order to comprehensively assess the potential for chemical hazard without the bias of only investigating known pathways, more wide-ranging screening techniques are needed. In recent years, new technologies have been developed that broadly can be referred to as functional genomics (or proteomics), which are capable of globally determining genes/gene products that are criti-
cally involved in chemical interactions. Techniques include, but are not limited, to: 1) yeast knockout collection with haploinsufficiency profiling/homozygous deletion profiling (HIP/HOP) technology and the like, which uses chemical tolerance or intolerance to identify toxicity targets; 2) CRISPR-Cas9 knock-out screening in human cells, which functions similarly to the HIP/HOP screen; 3) proteomics methods that identify specific chemical-protein interactions in the context of the environment or milieu; and 4) transcriptomics methods which use the technologies of next-generation sequencing (NGS) to study the cellular state of healthy, chemically perturbed, and/or diseased tissue. These techniques cover more comprehensive biological space that may provide data leading to discovery of new biological targets and pathways of toxicity. In addition, since these techniques provide some measure of cell health as a result of gene manipulation linked to chemical insult, the role of each gene in either chemical tolerance or intolerance can be functionally ascertained. The purpose of this session is to provide an overview of the cell-based techniques that are currently in use or being developed to demonstrate powerful new ways of determining mechanisms of toxicity for environmental and other chemicals. One of the co-chairs will provide an introductory overview of functional genomics and describe how these technologies could be applied in 21st-century toxicology. The first speaker will describe the use of a large diversity of cell lines including cell lines, iPSC and primary cells, with critical gene targets modified using CRISPR-Cas9 technology to identify critical chemical targets. Validation of results using both CRISPRi (inactivation) and CRISPRa (activation) of targets identified also will be demonstrated and use of these approaches in understanding off-target effects of drugs. The second speaker will discuss the use of CRISPR technology to identify genes following arsenic exposure that promote the endoplasmic reticulum stress response and apoptosis in human cells. Experimental data presented will include novel validated gene hits, in particular, those of the classical repressive complex and microRNAs. The third speaker will continue the discussion of the use of CRISPR technology in human cells for functional genomics by presenting work done in human erythroleukemic K562 cells exposed to arsenic trioxide or acetaldehyde. The speaker will present data that demonstrates not only the utility of this approach for identifying novel toxicity pathways, as he will show in the case of arsenic trioxide, but also the capability to assign potential roles to uncharacterized proteins based on known toxicity pathways, as he will show in the case of acetaldehyde and DNA repair. The fourth speaker will compare use of gain of function and loss of function genomic screens using CRISPR-Cas9 to deliver an open reading frame library into human cells to characterize mechanisms of action of drugs in cancer cell lines and compare this approach to CRISPR-Cas9 methods. The final speaker will discuss an unbiased chemical proteomics platform that identifies chemical-protein interactions in intact cells by a variety of techniques including cell lines, iPSC and primary cells, with critical gene targets modified using CRISPR-Cas9 technology to identify critical chemical targets. Validation of results using both CRISPRi (inactivation) and CRISPRa (activation) of targets identified also will be demonstrated and use of these approaches in understanding off-target effects of drugs.

**2558 CRISPR Genetic Screens on Cellular Stress Response to Proteotoxins**

Q. Lu. Harvard T.H. Chan School of Public Health, Boston, MA. Sponsor: A. Bone

We report the identification and characterization of multiple cellular suppressors of ER stress response and the associated apoptosis that is induced by environmental proteotoxins such as arsenic. Using a genome-wide CRISPR library targeting both protein coding genes and microRNAs, we screen for genes whose inactivation further increased ER stress-induced upregulation of C/EBP homologous protein 10 (CHOP)-the transcription factor central to ER stress-associated apoptosis. Among the top validated hits are components of the polycomb repressive complex and microRNAs. Our study reveals previously unrecognized mechanisms by which cells suppress the ER stress response and its associated cell death, and may lead to the development of new therapeutic strategies aimed at restoring cellular homeostasis. This approach showcases how CRISPR-mediated inactivation of genes and microRNAs can identify novel mechanisms of toxicity that may be broadly shared by many proteotoxics.

**2559 Genome-Wide CRISPR-Cas9 Screens in Human Cell Lines Provide Novel Mechanistic Insights into Toxic Responses to Arsenic and Acetaldehyde**

A. Sobh. University of California Berkeley, Berkeley, CA.

Comprehensive identification of pathways involved in cellular response to a toxic substance has recently become more feasible due to the emergence of novel genomic approaches. The CRISPR (clustered regularly interspaced short palindromic repeats)-Cas9 (CRISPR associated protein 9) gene knockout system enables genome-wide functional screening, a powerful tool to study mechanisms affecting cellular sensitivity to a toxic substance. We utilized the CRISPR-Cas9 system to perform genome-scale loss-of-function screens in human K562 erythroleukemic cells. Using this approach, we investigated mechanisms affecting cellular response to acetaldehyde, the primary product of alcohol metabolism, and arsenic trioxide (ATO), a potent anti-leukemic agent. Our acetaldehyde screen revealed 18 candidate genes whose disruption increased resistance to acetaldehyde exposure and 21 candidate genes whose inactivation exacerbated acetaldehyde toxicity. Consistent with the reported role of aldehydes in DNA damage, multiple candidate gene products that we identified as components reducing acetaldehyde toxicity were DNA repair enzymes. The screen further revealed functionally uncharacterized genes whose role in acetaldehyde toxicity was validated by individual gene knockout. Our primary arsenic trioxide screen identified 74 genes whose inactivation decreased sensitivity and 26 genes whose disruption increased sensitivity to ATO. Using a secondary screening approach, we validated the relevance of the vast majority of the identified candidates to ATO toxicity. Pathway analysis on validated genes showed a predominant role of reactive oxygen species (ROS) in ATO toxicity and revealed a novel link between YO1/TO loss-of-function and nucleocytoplasmic biosynthesis/incorporation into selenoproteins. Our work further demonstrates the strength of functional screening using the CRISPR-Cas9 system in deciphering mechanisms of toxicity.

**2557 The Use of CRISPR-Cas9 Technology for Validation and De-Validation of Targets from Functional Genomics**

L. Mayr. AstraZeneca, Cambridge, United Kingdom. Sponsor: A. Bone

This presentation will cover recent advances in the field of Precise Genome Editing (PGE), CRISPR-Cas9, and other RNA/DNA-guided endonucleases, which enable identification and validation of drug targets and off-targets at unprecedented ease, speed and precision. It is believed that CRISPR-Cas9 has the potential to substitute the currently used technologies for target validation which are mostly based around RNA-interference (RNAi), such as siRNA, shRNA, and their variations. The presentation will give examples about target validation and de-validation in vitro and in vivo models, and will show the advances and limitations of this novel technology over existing technologies.
High-throughput genetic screening is a powerful tool for target discovery in cancer, it can be used to systematically identify genes that support cancer cell viability and regulate cancer drug. The latest development of CRISPR-Cas9 and ORF technologies enables us to knock out, activate, and introduce precise mutations in genes, and makes possible to perform loss-of-function and gain-of-function studies at genome scale to interrogate mechanisms of action of drugs or toxicants. Novel genetic screens offers improvements in speed and scale compared to arrayed-based screening. Negative selection proliferation screens can be used to detect genes that affect sensitivity of cancer cells to drug treatment. Synthetic lethal phenotypes can be detected using negative selection proliferation wide screens, where thousands of genes can be simultaneously disrupted and tested in presence of a small molecule to find genes that are synthetically lethal with the compound. However, the power of pooled genetic screens is particularly evident in the setting of positive selection, such as identifying genes that mediate resistance to cytotoxic or targeted chemotherapy agents. A subset of drug targets/ cancers, representing a diverse range of cancers chemotherapy and targeted therapies, were screened for causal drug resistance perturbations using genome-scale ORF and CRISPRa lentiviral libraries. Our screens identified classical genes involved in resistance mechanisms as well as novel and potentially drugable genes. The identification of resistance mechanisms to targeted and traditional cytotoxic therapies can be used to evaluate the most effective path forward for resistance studies to new therapies, and it helps the understanding of the biology of tumor responsiveness to inhibition of specific targets or cytotoxic drugs. Loss-of-function genome-wide screens are powerful sets of unbiased tools that allow the identification of critical genes involved in susceptibility or resistance to environmental toxicants and provide novel mechanistic insights in the field of toxicology.

Unbiased high-throughput methods are urgently needed to monitor the spectrum of protein-compound interactions since the toxic modes of actions remain unclear for most bioactive environmental chemicals. Yet while chemical-genomics methods have been developed to assess the impact of chemicals on biological pathways, most existing approaches do not pinpoint the actual proteins physically bound by bioactive compounds. Here we developed a hybrid platform by incorporating complementary chemical proteomics assays to identify physical protein targets of environmental chemicals. Specifically, target identification by chromatographic co-elution (TICC), target identification by ligand stabilization (TILS), activity based protein profiling (ABPP), and affinity pull-down system combined with untargeted chemical analysis (PUCA with ~6000 His-tagged protein library) methods will be incorporated into the platform, and the broad applicability of the platform was validated by identifying both known and unexpected protein targets of bioactive molecules. For example, enoyl-acyl carrier protein reductase (FabI) was identified to be the bacterial target of 6-OH-BDE-47 in Escherichia coli. The physical interaction was further confirmed by PUCA assay with recombinant protein. Overexpression of FabI rescued the growth inhibition of Escherichia coli by 6-OH-BDE-47, validating it as the primary in vivo antibacterial target. Our study demonstrates the strength of chemical proteomics platform in unbiased identification of protein targets of environmental chemicals. The work presented here will highlight the value of identifying the physical protein targets of environmental chemicals, which is a key part of understanding their pathways to adverse outcomes.

Air pollution research has traditionally focused on exploring the mechanisms linking acute and chronic exposure to lung injury/inflammation and, ultimately, the development and exacerbation of airway diseases. More recently, evidence has emerged linking air pollution to adverse cardiovascular health effects, neurological diseases, systemic inflammation, diabetes, obesity, steatohepatitis, and poor reproductive and developmental outcomes. A novel paradigm has been proposed involving the role of central nervous system activation to explain pulmonary and extra-pulmonary effects of inhaled pollutants. The new evidence in general brings forth a compelling common mechanism involving sympathetic-adrenomedullary and hypothalamos-pituitary-hypothalamic axis-mediated biological homeostatic processes that can explain the widespread multi-organ metabolic and immune effects of air pollution. The role of these neuroendocrine axes in mediating systemic effects of pollutants has been overlooked in the past. This mechanism emphasizes the importance of considering a systems biology approach for inhaled pollutants and other stressors and proposes a common mechanistic pathway for chemical and non-chemical stressors. The unifying hypothesis involving the neuroendocrine axes will be presented and supported by each speaker to specifically explore: 1) the basic understanding of how acute physical and psychological stresses (good stress) through neuroendocrine activation modulate the cell egress and extravasation and other homeostatic changes and, in the long term, contribute to chronic inflammatory diseases (bad stress); 2) epidemiological evidence linking environmental stressors, specifically air pollutants, to the neuroendocrine pathway leading to exacerbation of inflammatory conditions of asthma and chronic obstructive pulmonary disease (COPD), which will include looking at the interactive effects of psychological and environmental stressors during development and susceptibility to chronic respiratory diseases; 3) how irritant pollutants likely activate neuroendocrine stress response through nociception by stimulation of sensory nerves, including vagal C-fibers, and integrate sensory signals to the brain to stimulate stress-responsive centers including hypothalamus, which is involved in a flight-or-flight response upon physical stress encounter; 4) experimental studies examining how exposure to inhaled pollutants through neuroendocrine stress response pathways activate adrenergic and steroid mechanisms and alter systemic metabolic homeostasis to affect liver, muscle, and adipose tissue lipid and glucose metabolism and influence lung injury/inflammation; and 5) the development of a glucocorticoid receptor antagonist adverse outcome pathway to integrate the new mechanistic information on environmental and sensory irritant and metabolic and immune function through neuroendocrine stress axes activation and tissue-specific activation of stress hormone receptors. The goal of this session is to synthesize and discuss these emerging findings in order to build a consensus that neuroendocrine stress axes play a major role in air pollutant and environmental health effects and disease susceptibility. The topics covered also will highlight areas where redirection of research efforts might better address the most critical knowledge gaps in the understanding of air pollutant-induced modulation of neuroendocrine pathways and disease susceptibility and improve health risk assessment.
Widespread metabolic changes observed after air pollution exposure, how the release of stress hormones through the activation of sympathetic challenges an accepted mechanistic paradigm of how irritant air pollutants induce systemic metabolic impairment and lung injury.

New experimental evidence involving the role of neuroendocrine activation demonstrates that, in addition to the traditional effects of both chemical and non-chemical stressors on respiratory outcomes. Exposure to air pollutants, including ambient particulate matter has been associated with asthma development and exacerbation in children in sex-specific manner. The evolving research provide insights in to how these complex interactions may more accurately identify vulnerable human populations and to more fully characterize health risks due to common airborne pollutants. Adverse effects of stressors (psychological or environmental) during development are postulated to interactively increase the susceptibility of children to subsequent air pollution exposures. Exposure to environmental stressors can modify lung development, especially in susceptible sub-populations. Because fetal development occurs through a complex orchestration of developmental events, toxins that disrupt these processes can have variable impact depending on the nature of the pollutant as well as timing and/or dose of exposure. The air pollution epidemiology and clinical evidence linking neuroendocrine stress response to respiratory diseases such as COPD and asthma provides mechanistic insights for potential therapeutic interventions.

The airways are densely innervated by sensory nerves from the trigeminal and vagal ganglia. Airway sensory nerves are heterogeneous with respect to their receptor and neurotransmitter expression and anatomical distribution, and thus different subpopulations serve different functions. The two major functions of airway sensory nerves are the homeostatic regulation of breathing and the defense of the airways. The latter is regulated by sensory nerves that sense noxious stimuli, thus are termed 'nociceptors'. Airway nociceptors express the ion channels TRPA1 and TRPV1, which are activated by a host of inhaled pollutants including ozone, acrolein, particulate matter, aldehydes and disocyanates. Acute inhalation of these pollutants triggers airway nociceptive responses including cough, bronchospasm, mucus secretion, dyspnea and changes in blood pressure and heart rate via the recruitment of nociceptor-associated CNS and autonomic networks, including neuroendocrine stress axes. Furthermore, neuronal protein expression is not static, and plasticity in receptor or neurotransmitter expression can lead to altered sensory functionality in both respiratory and cardiovascular disease. For example, activation of airway nociceptors via inhalation of TRPA1 agonists evokes parasympathetic-mediated reflex bradycardia in normotensive rats, but in spontaneously hypertensive rats the same sensory stimulus evokes a complex combination of parasympathetic-mediated reflex bradycardia and sympathetic-mediated tachycardia. Inhibition of pollution-induced sensory nerve activation will likely decrease the impact of air pollution on respiratory and cardiovascular disease.

New experimental evidence involving the role of neuroendocrine activation challenges an accepted mechanistic paradigm of how irritant air pollutants induce systemic metabolic impairment and lung injury. We focus on recent air pollution studies highlighting how the release of stress hormones through the activation of sympathoadrenal-medullary and hypothalamic-pituitary-adrenal axes lead to downstream systemic metabolic imbalance and stimulation of an innate immune response, two fundamental survival mechanisms. Widespread metabolic changes observed after air pollution exposure, typical of a flight-or-fight response, are characterized by adipose lipolysis, muscle protein catabolism, inhibition of pancreatic insulin secretion and increased glucogenogenesis as determined using metabolic assessment in rodents and humans. There is also evidence of a major metabolic shift in the liver associated with transcriptional depression of lipid catabolism and increased mitochondrial stress. Furthermore, the oxygen-induced innate immune response involves extravasation of immune cells to the lung and is associated with changes in stress hormones. The roles of circulating epinephrine and cortisol/corticosterone are examined using interventional approaches involving pharmacological agents specific for adrenergic and glucocorticoid receptors with or without the surgical removal of the whole adrenal glands or adrenal medullas. These studies emphasize that the presence of circulating stress hormones is necessary in mediating irritant air pollutants-induced metabolic and lung inflammatory effects and demonstrate selective modulation based on the differences in the tissue distribution of specific adrenergic and glucocorticoid receptors. Changes in induced alterations in homeostatic mechanisms and dysregulated stress response pathways are postulated to contribute to diabetes, obesity and cardiovascular disease susceptibility. This abstract does not reflect US EPA policy.

The Adverse Outcome Pathway (AOP) framework represents an ideal tool for integrating diverse data relating chemical exposures to adverse outcomes. Glucocorticoid signaling is tightly controlled and numerous adverse health outcomes have been associated with either elevation or suppression of these glucocorticoids including cardiovascular health, diabetes, growth, gonadal function, osteoporosis, myopathy, and increased disease susceptibility. The requirement for tight regulation of hormone levels leaves this system vulnerable to chronic elevation or depression associated with exposure to environmental stressors. Recently, impacts of air pollutants on glucocorticoid signaling have been identified. A network of AOPs describing glucocorticoid signaling have been developed based on the wealth of mechanistic and clinical data resulting from the long-term use of glucocorticoids as therapeutic agents. This framework has been used to integrate data from chemical structure-based predictions, in vitro toxicity tests, animal toxicology, and human clinical data to evaluate the potential impact of environmental exposures on glucocorticoid dysregulation. The broad range of clinical applications for this class of compounds has resulted in clinical information in humans at a wide variety of doses. In addition, there are both acute and chronic indications for these compounds including diverse such as congenital adrenal hyperplasia, where lifelong hormone replacement is sometimes required. When considered in the context of the AOP framework, this information can be used to predict the potential impacts of environmental stressors from air, food, and water considering pre-existing disease and the treatment thereof. These studies highlight the value of the AOP framework for integrating information from chemical and non-chemical stressors to provide a holistic view of the etiology of complex diseases. This is an abstract of a proposed presentation and does not necessarily represent US EPA policy.

You have performed your experiment or analysis, generated or used toxicological big data, want to publish it, and need it to be transparent and reproducible. What do you do? This is one of the challenges faced when using or generating toxicological big data (high-throughput screening, high-content analysis, or omics technologies). Data analyses have become more complex, with increasing scrutiny being placed on the computational methods to ensure analyses are clearly communicated (transparency) and easily reproducible. Supplementary data files may be insufficient to adequately relay the necessary detail or metadata, nor meet the data quality needs and minimum information criteria required for risk assessors to utilize such data/analyses. This session seeks to address these challenges facing toxicologists highlighting platforms for data sharing, how to cite alternative data sources, and the minimum information required for reproducibility and transparency that will help lead toward regulatory acceptance of big data. Furthermore, in order for complex models to be broadly utilized and reproducible, and
clear methodologies, as well as developing fit-for-purpose models that address specific needs, are required. This session will encompass various viewpoints—from the research perspective to that of regulators and industry—providing suggestions and resources for establishing reliable and useful models, as well as promoting transparent communication regarding the use of big data and computational approaches in the toxicological sciences. An aim of the session is to promote awareness regarding the dissemination and use of big data in toxicology, and it will conclude with a panel discussion including all speakers.

2569 Challenges Focusing Publication and Transparency for Big Data in Toxicology

L. Burgoon, US Army Engineer Research and Development Center, Research Triangle Park, NC.

Toxicology is coming of age: The Big Data age. Since the birth of toxicogenomics, the amount of toxicological data has increased exponentially. Today, the wealth of data goes beyond ‘omics, as large databases of curated “classical toxicological data” come online, and more and more chemical entities are screened in high-throughput screening assays. As data size increases, so does analysis complexity. This is not the first time we faced these challenges. In the early days of transcriptomics, the Minimum Information About A Microarray Experiment (MIAME) standard and its associated tools sought to address many of these same challenges: How do we share data in a way that anyone can use it; how do we share analysis details; can we standardize information to facilitate interpretation; how do we make our research results reproducible? Although a MIAME for big data in toxicology may or may not be warranted, we can learn important lessons from that time period regarding data sharing and availability, clearly communicating protocols, providing sufficient meta data, sharing analysis code, and the ethics of citation when using alternative data sources in a new publication. This talk will briefly touch on our current challenges specifically faced by toxicologists with data sharing, data discovery, and ethical use of data. Furthermore, minimum information required for regulatory use/acceptance will be discussed, as criteria for meeting needs of the risk assessment workflow can present unique challenges for the use of big data in toxicology.

2570 Big Data in Toxicology: Remodeling the Publishing Landscape

G. Miller, Emory University, Atlanta, GA.

The classical model of scientific publishing was developed at a time when datasets were manageable and supplemental data were a rarity. The past decades have witnessed a transformation in toxicology research with a rapid expansion of data volume and complexity. Although there have been significant improvements with the ‘omic revolution, publication models still struggle to deal with the ever-growing data. Investigators and funders need tools and resources to make complex data accessible and useful. This talk will discuss some of these challenges and provide examples of how journals and publishers can assist with the handling of big data. Many journals, including Toxicological Sciences, are partnering with data repositories to make the data and code underlying the scientific findings available to the larger scientific community, but to date these resources have been underutilized. These efforts parallel those in the area of open access publishing that are designed to disseminate research findings to as large an audience as possible. Strategies to improve the use of these tools will be discussed with a goal of optimizing the storage, access, and use of complex toxicological data.

2571 Resources and Alternative Publication Streams for Big Data

A. Williams, US EPA, Research Triangle Park, NC.

The volume of data and associated myriad of data analyses conducted to support toxicological research studies has been expanding for years, and there is little indication that it will be slowing. Traditional publication methods offer, at best, a summary of the data underlying a research publication, and in general are not sufficient for meeting the needs of data sharing and transparency. Datasets can be too large to be supplied as supplementary files in peer reviewed publications, commonly cannot be distilled into a PDF file (the publishers preferred format for data distribution), and this sometimes necessitates release in the form of large file downloads of complex databases. Analyses are commonly conducted using custom-written computational codes, unique to the study, which are currently not commonly published alongside the results. There are many resources available to toxicologists that can be utilized to effectively share data and methodology in the toxicology community. Such approaches can deliver data in a more readily available format for public use when supplied in a computable format and using standards amenable to integration into other systems. This presentation will address some of the challenges associated with data sharing and highlight options and tools available to publish toxicological data and analysis methods to facilitate reproducibility and transparency. This abstract does not reflect US EPA policy.

2572 Utility of Big Data: Developing Reliable Fit-for-Purpose Data Models

M. Martin, Pfizer, Inc., Groton, CT.

Big, complex data and models are most frequently compiled for hypothesis generating and data exploration. Such bottom-up approaches to knowledge discovery present unique challenges; follow-up hypothesis testing experimentation is often applied, which can confirm whether data are good enough and of sufficient quality (reliable), results are generalizable (repeatable), and are of value (relatable). While knowledge discovery efforts should continue to be developed and explored bottom up, evaluation and communication of evolving big data approaches generally requires a top-down approach. Data models should be evaluated by their inherent accuracy in the context of how such models will be used, i.e., fit for purpose. This permits clearly defining what is good enough for a specified purpose, how repeatable it needs to be, and ensures the models are described in non-statistical terms from the perspective of the potential user. If deemed "good enough," a snapshot of the data and model can be extracted, further ensuring reproducibility. From an industry perspective, establishing a clear use case and evaluating big data and models through the lens of the user are critical to ensure adoption, and effectively communicate utility, of any alternative toxicological data and models.

2573 Establishing Confidence in Prediction Models


Increased availability of data has greatly influenced the development of predictive modeling technologies, as well as methods to increase predictivity, interpretation, and confidence in the results. Computational approaches such as using conformal prediction can be used on both small and large datasets, and are suitable for a variety of applications such as prioritizing compounds for experimental testing and chemical risk assessment. These methods are honest in the sense that they display model utility when the user is making predictions. We will look at cases from AstraZeneca such as prioritizing compounds for experimental testing, and chemical risk assessment, to highlight the benefits of these methods, including how data quality influences interpretation of results and decision-making, and quantification of the risk associated in various situations. Further examples from AstraZeneca on how results are shared will be presented. Ultimately, these examples will focus model development to address specific needs, requiring effective sharing of data and methods to effectively communicate results and maximize utility.

2574 Mitochondria: Critical Targets in Pharmaceutical and Environmental Toxicity

B. van de Water, Leiden University, Leiden, Netherlands.

Mitochondria are essential for cellular metabolism, and mitochondrial damage is a key event in adverse outcome pathways. Toxic insults causing mitochondrial dysfunction can lead to cellular necrosis due to loss of adenine triphosphate production, to apoptosis through cytochrome-C leakage, and to many other adverse events, including reactive oxygen species production. Speakers from academia and industry will discuss several aspects of mitochondrial toxicity in the context of pharmaceutical and environmental toxicity. The goal is to present current insights into the central role of mitochondria in distinct toxicity pathways and state-of-the-art approaches to investigating mitochondrial toxicity. The latest technologies, including transcriptomic, proteomic, and metabolomic, as well as fluorescent protein reporters in combination with high-resolution microscopy, provide time-resolved mechanistic insight into cause and consequences of chemical-induced mitochondrial injury. The session will start with cutting edge work that centers on the mechanisms by which mitochondrial quality is maintained and how damaged mitochondria are removed from the cell. This
will be followed by new findings obtained through metabolomic characterization of isolated human mitochondria and definition of the mitochondrial exposome. The next presentation will feature exciting work showing how off-target adverse drug effects on mitochondrial energetics cause muscle dysfunction. The following presenter will discuss innovative approaches to unraveling mitochondrial toxicity pathways as developed in the EU-ToxRisk project. The final presenter will address how changes in mitochondrial biogenesis serve as a cellular adaptive mechanism counteracting drug-induced liver toxicity. This session will be of general interest to scientists interested in cellular and tissue-level mechanisms of toxicity. In addition, attendees with an interest in state-of-the-art approaches to unraveling pathways of toxicity in a time- and concentration-resolved manner will benefit from the session. Finally, this session will be of interest to regulatory scientists incorporating information from in vitro assays in the decision-making process.

### W 2575 New Insights in Mitochondrial Quality Control and Mitophagy

**A. Gustafsson. University of California San Diego, San Diego, CA.**

**Sponsor: B. van de Water**

Damaged mitochondria pose a lethal threat to cells that necessitates their prompt removal. Recent findings from our lab have shed new insights into mitochondrial quality control and removal of damaged mitochondria. It was found that NF-kB on the one hand activates inflammation but on the other hand restricts inflammasome activation through elimination of damaged mitochondria. The latter involves parkin-mediated mitochondrial ubiquitylation and subsequent mitophagy via the autophagy receptor p62/SQSTM1. Moreover, an alternative pathway for mitochondrial elimination was discovered, in which mitochondria are sequestered into Rab5-positive early endosomes and subsequently delivered to lysosomes for degradation. Endo-lysosomal clearance of mitochondria and mitophagy are activated by the same stressors and share regulation by Beclin1, suggesting cross-talk between these two pathways. These findings reveal novel mechanisms for mitochondrial quality control and mitophagy. The work reveals mechanisms that are highly relevant to our understanding of mechanisms of mitochondrial toxicity. Recent progress of this work will be presented.

### W 2576 The Mitochondrial Exposome

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

Over the course of a lifetime, humans are subjected to a diverse chemical experience that varies spatially, temporally, and in magnitude. While it is well recognized that environmental factors have the potential to contribute to disease, relationships between exposures and human health are largely unknown. Thus, the cumulative measure of exposures and associated biological response, defined as the human exposome, is a critical component of understanding how environmental factors contribute to disease. To date, exposure assessment in human populations has largely focused on circulating biomarkers of exposure; in vivo mitochondrial exposure and accumulation in humans is unknown. In this presentation, research efforts at the Emory University HERCULES Exposome Research Center to delineate the mitochondrial exposome in human tissue will be presented. Mitochondrial phenotyping using powerful untargeted chemical profiling platforms and integrated ‘omic approaches provide new insight into the burden of environmental chemicals in humans. Application to mitochondria from human adrenal glands, which provide critical endocrine and hypothalamic-pituitary-adrenal (HPA) axis function, shows the presence of a diverse range of exposure-related chemicals. Identified compounds were consistent with NRF-1 and 2 mechanisms, and herbicides, endocrine disrupting chemicals were also detected, including Bisphenol A, di-n-hexylphthalate, styrene, and piperonyl butoxide. These results represent the first characterization of the human mitochondrial exposome. Efforts are ongoing to further define the presence of environmental chemicals accumulated in human mitochondria and identify blood-based biomarkers that can be used to assess the mitochondrial exposome. This work will be discussed.

### W 2577 Drug-Induced Myopathy through Adverse Effects on Mitochondrial Energetics

**F. Russel. RIMLS, Amsterdam, Netherlands.**

Mitochondrial dysfunction is a common outcome of drug-induced toxicity. Understanding the mechanisms by which drugs perturb mitochondrial function is of significant importance to identify patients at risk, and assist in the development of more effective and safer drug therapies. Many of these adverse mitochondrial effects are not predictable consequences of the known mechanism of a drug, but result from off-target pathways. The studies have applied a systems medicine approach to identify the long-sought mechanism of statin-induced muscle toxicity, by integrating molecular, cellular, chemical, and clinical data on the mitochondrial off-target effects of these drugs. *in vitro* determination of muscle cell viability and characterization of mitochondrial energetics (e.g., oxygen consumption, ATP production, and individual respiratory chain complex activities) were combined with *in silico* protein-binding pocket similarity predictions to select potential off-targets. A strong inhibition, up to 84%, of the third complex of the respiratory chain by statins in their lactone form, was found. The inhibitory effect was associated with muscle complaints, as confirmed in patients suffering from these clinically cumbersome adverse effects. This approach appeared also successful to reveal the mitochondrial ATP/ADP exchanger as off-target of the anti-obesity drug ibipinabant, which explained its previously observed muscle toxicity in a preclinical safety study in dogs. To conclude, the developed strategy will be discussed, demonstrates to be a powerful tool to explore mitochondrial off-targets, and has the potential to unravel the molecular mechanisms underlying drug-induced adverse effects on mitochondrial energetics.

### W 2578 EU-ToxRisk: Advances in High-Content Microscopy Analysis for Mitochondrial Toxicity

**E. Danen. Universiteit Leiden, Leiden, Netherlands.**

**Sponsor: B. van de Water**

An integrated network-based approach has been developed to help unravel adverse outcome pathways. Data generated for this approach include RNA interference screens, global transcriptomics, phospho-protein and metabolomics. Recently, a panel of generally encoded reporters has been generated for key signaling pathways to complement these approaches with real-time high-content microscopy analysis. Data will be presented of the EUToxRisk “mitochondrial toxicity” case study, where this integrated approach was used in the context of a set of compounds representing different mitochondrial toxicant classes, including uncouplers, complex I inhibitors, complex II inhibitors, and complex III inhibitors. For mitochondrial toxicity, the studies included tools to quantify changes in mitochondrial membrane potential, mitochondrial ROS formation, and mitochondrial morphology. The time and concentration-resolved high-content microscopy parameters in relation to changes in mitochondrial functionality and cell fate outcome, provides the information required to connect a series of events to points of departure for adversity. This approach is applied in a single-exposure and repeated-dose setting. The obtained information will serve as input for the establishment of quantitative adverse outcome pathways (qAOPs) specific for compound-induced mitochondrial toxicity and ultimately, these AOPs could contribute to the improvement of toxicity prediction, both for known and unknown chemical classes. This ongoing work will be presented.

### W 2579 Increased Mitochondrial Biogenesis as a Cellular Adaptive Mechanism Counteracting Drug-Induced Liver Toxicity

**P. Carmichael. Unilever, Sharnbrook, United Kingdom.**

Mitochondrial dysfunction has been implicated in acute, severe liver injury caused by overdose of acetaminophen. A recent collaboration between the Chinese Academy of Military Medical Sciences and Unilever points to an important role for altered mitochondrial biogenesis in this process. Low, non-cytotoxic concentrations of acetaminophen triggered increased mitochondrial biogenesis in liver cells in vitro. In parallel, expression of key transcriptional regulators of mitochondrial biogenesis, such as PGC-1α, NRF-1 and TFAM, as well as expression of antioxidants including glutathione, MnSOD, HO-1, NQO1, and Nrf2, was upregulated. In contrast, exposure to high, cytotoxic concentrations of acetaminophen did not trigger this response but, instead, led to inhibition of mitochondrial biogenesis and reduced expression of these regulatory proteins and antioxidants. A computational model was for-
Color plays a significant role in food choice by influencing consumer preference, taste perception, and acceptability. With an increasing number of consumers looking for foods with “clean labels,” the market demand for “natural” colors has recently been rising significantly. There have been a growing number of food manufacturers turning away from synthetic colors and towards extracts from plant sources, primarily because of the increasing public interest in “natural” products. Currently, global regulatory agencies do not have a harmonized viewpoint as to the definition of color additives from botanical sources and their use requirements. In the United States, any unapproved color additive from a natural source is subject to premarket safety review by the US Food and Drug Administration (USFDA) with the same safety standard as for synthetic color additives. For plant-based color additives, considerations include chemical identity and composition, manufacturing process, source plant material, heat, pH, light stability, and pesticide and toxic element contamination. In the European Union, a decision tree is used to help determine when an ingredient can be considered a coloring food versus a coloring additive, with the latter being subject to scientific safety assessment. Failure to be aware of the regulatory review requirements can lead to compliance and labeling challenges and possibly charges of adulteration and misbranding upon importation of finished food items. Dialogues and collaborations among stakeholders, including color manufacturers, industrial end users, researchers, and regulatory scientists, are needed. They should work together to harmonize and optimize the safety evaluation of plant-based color additives. This workshop brings together food color experts from these stakeholder groups to share their experiences and perspectives on this topic. Following an introduction that will summarize the use pattern of food colors, speakers will discuss wide-ranging topics related to the safety evaluation of plant-based color additives used in foods, including: (1) the differences between synthetic and natural color with regard to manufacturing and practical applications; (2) issues related to adulteration and contamination that impact the safety of these colors; (3) issues with product quality related to adulteration and contamination that compromise the safety; (4) United States Pharmacopeia (USP)’s experiences in establishing identity and specification standards for plant extracts; (5) the US FDA review processes and special considerations; and (6) highlights of the most recent developments by global regulatory authorities. Examples of data packages sent to the US FDA, European Food Safety Authority (EFSA), and the Joint Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) Expert Committee on Food Additives for support to safety will be presented.
safety evaluation of an article to determine whether or not it is qualified for admittance to the USP monograph development process. Using an example of a USP botanical monograph article, this talk will illustrate how the USP admission evaluation and quality monograph together can serve in ensuring the quality and safety of natural extracts. Similar principles could be applied in assessing the safety of natural colors.

2585 Assessing the Safety of Plant-Based Food Colors in a Regulatory Setting

Y. Zang. US FDA, College Park, MD.

Color additives are extensively used in food. In the United States, they are under the regulatory oversight of the US FDA. All new color additives, synthetic and naturally-sourced, are subject to premarket safety review by the US FDA, and there is no “generally recognized as safe” (GRAS) exemption applicable to color additives. Once approved, a color additive will be listed in the regulations as either subject to or exempt from US FDA batch certification, the latter historically includes those that are not synthetic (e.g., plant, animal, and mineral sources). Though the safety standard for synthetic or natural color additives is the same, the source materials inform the assessments. The toxicology data package required to establish safety for plant-based color additive is judged on a case-by-case basis. In addition, special consideration is given to the chemical identity and characterization, heat and light stability, pesticide and toxic element contamination, etc. The speaker will delineate US FDA’s review process of color additives used in foods, with examples of both synthetic and plant-based color additives. Typical safety testing submitted to US FDA in support of the safe use and common deficiencies will be discussed.

2586 Global Regulations for Plant-Based Food Colors

S. McAvoy. Sensient Colors LLC, St. Louis, MO. Sponsor: M. Bastaki

Consumers globally are demanding clean label and colorants which are plant-based. Consumer products companies (CPG) are responding to this demand with 75% of new product launches in the United States containing “ exempt from certification” colors in 2016. This shift will likely continue in the subsequent years, and will expand to other regions. With this shift away from the traditional synthetic dyes, there are new challenges for the color suppliers and the CPG. International regulations, with non-synthetic color additives are not harmonized. This talk focusing on plant-based color additives, provides comparison on the current regulations in the United States and the EU, criteria that differentiate food colors and coloring foods under the regulation of the EU, and safety assessment monographs from different regulatory bodies including EFSA and JECFA. Failure to be aware of these differences can lead to compliance and labeling challenges, and charges of adulteration and misbranding upon importation of finished food items into countries outside of the manufacturing country.

2587 Communicating Science: Unconventional Oil and Gas Operations as a Case Study

E. Bruce. Baylor University, Waco, TX.

Ten years ago, there was a rapid increase in oil and natural gas development activities (OGD) in various unconventional resource basins around the country. The increase was the result of an innovative use of existing technologies (horizontal drilling and hydraulic fracturing). The transformation also led to a rapid increase in the build out of the infrastructure required to deliver the product to the markets (i.e., pipelines, compressor stations) and facilities for processing the product (i.e., natural gas process facilities, liquefaction facilities). While the increase was a boon to the economy, it also generated scrutiny from policymakers, researchers, and advocates who commonly cited health concerns. As the decade mark of the nationwide ramp up in industry development activities, Texas has a rich history of OGD going back to the early 20th century and is home to some of the largest shale basins. The publication of the group’s work in 2015 (PLoS One. 2015 Jul 15;10(7):e0131093. doi: 10.1371/journal.pone.0131093. eCollection 2015) led to extensive multimedia interest (the study has been downloaded more than 22,000 times). This level of interest is likely a reflection of a deficit of health information related to UGOD. The presentation will discuss the experience of CEET in disseminating the findings to interested parties that have included testimony to state legislators, the Marcellus Shale Documentary Project, through international, national and local news media, presentation at national conferences, and discussion with community organizations. The talk will address the need to provide accurate and understandable risk assessment to audiences when gaps in hazard identification and hazard characterization exist and how to communicate this uncertainty. The presentation will address how this lack of knowledge leads to gaps in health information and how these gaps frame the risk discussion to audiences that have a preconceived risk perception of UGOD.
2590 Perspectives from the Texas Commission on Environmental Quality (TCEQ) Related to Risk Communication and Unconventional Oil and Gas Operations in Texas

T. Bredfelt, Texas Commission on Environmental Quality, Austin, TX.

The TCEQ utilizes data to evaluate potential risks associated with the development and production of shale gas via hydraulic fracturing. The data includes air monitoring data, the use of infrared cameras either by hand or helicopter mounted to assess potential sources of emissions, and hand held Jerome units to detect reduced sulfur compounds. This data are made publicly available, and the use of these devices and the subsequent data output are often demonstrated at public open house meetings wherein the public can directly see these tools used and the types of data that they produce. The Agency has worked with the public and with the media to communicate observed risk sources and potential management strategies. In addition, the TCEQ has worked collaboratively with industrial entities to identify potential sources of air emissions or groundwater contaminants, and produced an inventory of risk sources. This effort has led to the genesis of programs that enable industry to fix problems before they pose a risk through the “Find it and Fix it” Program. This experience has enabled the public to understand how transparent communication of information with the public has provided us with policies, rules, and guidelines that mitigate risks associated with unconventional shale gas facilities. These programs offer a microcosm of greater programs that can be implemented for risk communication.

2591 Perspectives from a Risk Communication Expert: Focus on Unconventional Oil and Gas Operations


While it is essential to convey facts clearly, that alone is often insufficient to quell public concerns. Research shows that public concerns typically are based 95 percent on perceptions, and only five percent on facts. People’s behavior usually is predicated on perceptions - often misperceptions - that differ from reality. Concerns and question raised by stakeholders typically fall into two groups: 1) Information concerns and questions typically related to concerns and questions focused on the following six questions: who, what, where, when, why, and how; and; 2) challenge concerns and questions that relate to trust, credibility and consistency of the message. This presentation discusses how utilizing proven science-based collaborative techniques, key issues can be identified. Also discussed is how tailored risk communication tools can be adapted to build trust and foster informed support among stakeholders. A panel discussion to encourage audience interaction will follow this presentation.

2592 Communication of Scientific Findings and Risk Perception Associated with Unconventional Oil and Natural Gas Development

E. Craft. Environmental Defense Fund, Austin, TX.

Public concern around the development of unconventional natural gas resources continues to be a challenge in communicating health risks associated with development of the sector. Many studies have relied on exposure estimates based on proximity to drilling activity rather than actual pollution measurements, and the increasing influence of citizen science reports has complicated explanation of peer reviewed findings. Nonetheless, evidence of associations between drilling activity and adverse health outcomes continues to grow, especially with regard to birth outcomes and respiratory disease. How the findings in the literature are presented is critically important in communicating health risks to the public. In addition, special consideration of the risks to communities that may be burdened by both chemical and non-chemical stressors (e.g., neighborhoods of recognized low socio-demographic status located close to drilling activity) is a difficult task because of the multiple facets of risk present and challenges in measuring and controlling for them. New efforts in evaluating cumulative risks of chemical and non-chemical stressors are underway to assess exposure risks posed to vulnerable communities. This presentation will focus on a few of these initiatives as part of the effort to secure vital environmental and health protections for communities that may experience an increased burden as a result of pollution from unconventional oil and natural gas development.

2593 Radiation Toxicity: Historical Perspective on Epidemiological and Experimental Evidence Informing Standards

R. McClellan. Toxicology and Risk Analysis, Albuquerque, NM.

The literature on the health effects of external radiation exposures and internally-deposited radionuclides, most of which was developed post-World War II, is extraordinarily voluminous and robust. This literature includes information on the sources of radiation and radionuclides, environmental and occupational exposures, substantial epidemiological findings, and complementary findings from controlled exposures of molecules, cells, tissues, and populations of multiple species of laboratory animals. The substantial scientific literature has been used to inform the settings of radiation exposure standards for workers and the general population. The radiation protection scheme takes account of radiation quality and dose projection. The presentations in this session will briefly review the history of key discoveries in the radiation field and the development of radiation protection standards, as well as the historical development of knowledge on radiation effects discovered using both epidemiological and experimental approaches. Mutations and cancer, which have traditionally been primary endpoints in radiation protection standards, will be highlighted. Various models describing radiation dose-response relationships, including the use of the linear no-threshold model, will be covered. Uncertainties and controversies in the knowledge base will be discussed, including the extent to which the setting of radiation protection standards has subsequently influenced the standard setting for chemical exposures. Further, in light of the thousands of nuclear weapons tests conducted since World War II, this session will also put into perspective the human and environmental consequences of ionizing radiation that could ensue from their potential use.

2594 Skeletal Toxicity Resulting from Exposure of Growing Male Rats to Coplanar PCB126 Is Associated with Disruption of Calcium Homeostasis and the GH-IGF-1 Axis

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Polychlorinated biphenyls (PCBs) are ubiquitous persistent environmental pollutants. Several studies have described skeletal toxicity following exposure to PCB mixtures, and coplanar PCBs. However, molecular mechanisms remain poorly understood. We exposed groups of N = 6 male Sprague-Dawley rats age 4-5 weeks to PCB126, a pentachloro-substituted, dioxin-like PCB congener by a single i.p. injection of 5 μmol/kg in soy oil vehicle or vehicle alone. After 4 weeks rats were sacrificed and serum, liver, kidney, bone (tibia and vertebrae) were collected. PCB exposure resulted in hypocalcemia (P<0.05) and significant increases in serum PTH without changes in serum phosphorous. Hyperparathyroidism was accompanied by increased expression of mRNAs encoding vitamin D3-metabolizing cytochrome P450 enzymes CYP27B1 and CYP24 in the kidney (P<0.05). PCB exposure also reduced serum IGF-1 and hepatic expression of mRNAs encoding the male-specific GH-pattern-regulated cytochrome P450 enzymes CYP2C11 and CYP3A2 relative to controls (P<0.05). PCB exposure also reduced serum CYP3A2 relative to controls (P<0.05). In addition, microCT analysis revealed that PCB exposure resulted in smaller long bones with reduced diameter and surface area but increased trabecular thickness and volume (P<0.05). This was accompanied by a reduction in serum osteocalcin, a marker of bone formation (P<0.05) but no effect on the bone resorption marker, RatLaps. These data suggest that skeletal toxicity after exposure to PCB126 is a result of disruption of calcium homeostasis and the GH-IGF-1 axis and may also involve direct effects on bone formation. Funded in part by the Iowa NIEHS Superfund Center P42 ES013661 (L.R.) and R37 AA18282 (M.J.R.).

2595 Environmental PCBs in the Serum and Breast Milk of Lactating North Carolina Women

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PCBs, are ubiquitous, persistent organic compounds that form a family of 209 structurally-related congeners. PCBs are lipophilic compounds that bioconcentrate in the food chain and are present in detectable amounts in human populations. There is a paucity of data about recent human exposures through breast milk to the PCBs, especially in US studies and even fewer studies with repeated measures over time from the same individual. The goal of the US EPA Methods Advancement
for Milk Analysis (MAMA). Study was to develop or adapt methods to measure PCBs, and other environmental chemicals in milk and serum twice during lactation (at 2-7 weeks and 3-4 months postpartum) in 34 North Carolina women. Milk and serum were quantified for 35 PCB congeners. A majority of the milk congeners were measured at concentrations above LOD, including PCBs 132, 137, 138, 140, 158, 170, 209, 178, 189, 194, 196, 197, 199, 206, 207, 208, 213, 218, and 226. In descending order of concentration with mean concentration ranges of 19.6 to 0.8 ng/g lipid. Similar trends were measured in serum (mean concentrations ranged from 13.3 to 0.86 ng/g lipid). There was no evidence of depuration between visits. These data suggest that breastfeeding North Carolina mothers are exposed to these environmental PCBs and that the PCBs can partition to breast milk. This abstract is the opinion of the authors and does not represent US EPA or NIEHS policy.

2596 Polyaromatic Hydrocarbons and Their Oxygenated Metabolites Directly Alter Ion Channels and Alter Eye Development in Zebrafish Embryos

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Polyaromatic hydrocarbons (PAH) present in crude oil may alter the movement of ions across the membrane of cardiomyocytes contributing to impaired excitation-contraction coupling, possibly resulting in diminished cardiac function and changes in morphogenesis. Alterations are proposed to occur through the ether go go related K+ channels (ERG), L-type Voltage-Gated Calcium Channels (CaV), ryanodine receptors (RyR) or sarco/endoplasmic reticulum calcium ATPase (SERCA) but direct binding of PAHs or oxygenated metabolites with these channels has not been demonstrated. We utilized the ryanodine receptor ligand binding assay (RRLBA) to assess whether PAH parent compounds, and several oxygenated metabolites, interact with these ion channels in zebrafish. We also evaluated the potential of PAHs and their metabolites that cause ion channel disruption to contribute to other forms of developmental toxicity; namely eye development. Zebrafish embryos were treated with 0.5, 5 and 10 µM of PAHs from 2 hours post fertilization (hpf) to 72hpf. In vitro assays demonstrated that, of the 14 compounds assessed, anthraquione-1 and 1-hydroxyquione caused greater than a 200% overactivation and 2-hydroxyfluorene caused an 80% reduction in the activity of the RyR in zebrafish skeletal muscle homogenates. Channel disruption was concentration dependent and altered activity occurred 10 µm or below. In zebrafish embryos exposed to 0.5 µM of phenanthrene-dione, pyrene and phenanthrene showed significantly decreased eye size, and 5 µM of phenanthrene and anthraquinone caused a greater than 30% reduction in eye area than control groups. These results support the direct interaction of PAH or PAH metabolites with ion channels with important implications on various forms of developmental toxicity.

2597 Exposure to BDE-99 Leads to Vascular Toxicity and Disruption of Gene Expression Related to Angiogenesis and Barrier Function in Endothelial Cells

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Perfluorinated compounds (PFCs) are widely used in industry as surfactants and in the production of plastic, electronics, textile, and clothing. Due to their stability, persistence in the environment, and their propensity to bioaccumulate, PFCs are of significant toxicological concern. PFCs have been detected ubiquitously in bodies of water, human serum, cord blood, and human breastmilk, worldwide. These chemicals have also been associated with developmental, hepatic and carcinogenic effects in animal studies. Furthermore, there are many PFCs that have yet to be characterized for potential health effects. The objective of this study was to assess the cell viability of the HepaRG cell line, a dual lineage, hepatocyte/cholangiocyte human cell line, when exposed to a set of 22 PFCs. This cell line was chosen because it is known to have metabolic capability, and the liver has been reported as a sensitive tissue to the effects of legacy PFCs in previous work. Assessment of cytotoxicity was performed using a CellTiter-Glo luminescence assay and a JC-10 mitochondrial membrane potential assay. HepaRG cells were dosed with these PFCs at half-log concentrations, in a range of about 1 µM to 100 µM. Three modes of impaired cell viability were evident and some of the PFCs demonstrated EC50 within human exposure range; no effect, moderate and dramatic impairment were evident with increasing con-
centrations of PFCs. Interestingly, the JC-10 assay revealed either mildly decreased, increased at high doses (between 1-100 μM), or no effect on mitochondrial activity. These data suggest that structural information on the varied PFCs tested may help identify events leading to a decline in cell viability. Further analysis of mitochondrial events, such as oxygen consumption rate, extracellular acidification rate, and mtDNA quantification may more accurately determine the magnitude and direction of mitochondrial effects occurring as a result of PFC exposure.

2600 Paradoxical Protective Effect of PFOA and PFOS against High-Fat Diet-Induced Hepatic Steatosis in Mice

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Perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) are persistent organic pollutants (POP) exhibiting worldwide bioaccumulation because of prolonged half-life. In liver, PFOA and PFOS toxicity presents as fat accumulation (steatosis) and hepatomegaly. It has been suspected that PFOA and PFOS may exacerbate existing liver diseases. The objective of this study was to determine effect of PFOA and PFOS on high fat diet induced hepatic steatosis and metabolic disruption. Eight week old male C57BL/6J mice were fed either normal Chow diet (ND) or a high fat diet (HFD) ad libitum with or without 0.0001% w/w (1 mg/kg) of either PFOA or PFOS for 6 weeks. Interestingly, PFOA/S induced hepatomegaly in ND fed mice but failed to exacerbate hepatomegaly induced by HFD feeding. Immunohistochemical analysis of PCNA positive cells indicated no proliferation across all treatment groups. Oil Red O staining revealed increased hepatic steatosis in ND fed mice treated with PFOA/S. However, PFOA and PFOS treatment protected HFD fed mice from hepatic steatosis. Quantitative analysis of hepatic lipid content confirmed this result. None of the treatment groups exhibited changes in serum triglycerides or cholesterol. Serum glucose levels were not affected in ND fed mice after PFOA treatment, but were elevated in the PFOA treated group and were not affected by PFOA/S plus HFD feeding. We further explored the mechanisms of apparent protection offered by PFOA/S against HFD feeding by qPCR analysis of metabolism related genes. Expression of triglyceride synthesis genes and that of CD36, involved in hepatocyte lipid uptake were not affected by PFOA/S treatment in ND fed mice but was decreased by PFOA/S treatment in HFD fed mice. Fatty acid oxidation genes did not change or were decreased in ND fed mice treated with PFOA/S. PFOA and PFOS treatment inhibited fatty acid oxidation genes in HFD conditions. Together, our data indicate that PFOA/S induce hepatomegaly by altering hepatic lipid storage rather than inducing hepatocyte proliferation. Gene expression analysis suggested that PFOA/S inhibit triglyceride synthesis and fatty acid oxidation in HFD fed conditions. These studies have revealed a potential potometric effects of PFOA and PFOS on hepatic steatosis. Further studies to determine underlying molecular mechanisms and effects on metabolically important organs such as adipose tissue and muscle are warranted.

2601 Temporal Trends in Perfluoroalkyl Substances in Bottlenose Dolphins (Tursiops truncatus) of Indian River Lagoon, Florida, and Charleston, South Carolina

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Perfluoroalkyl substances (PFASs), are fluorinated chemicals that are persistent and globally distributed. We performed a temporal trends analysis of serum concentrations of PFASs in bottlenose dolphins (Tursiops truncatus) inhabiting the Indian River Lagoon, FL (IRL) and the waters surrounding Charleston, SC (CHS). Samples were collected during capture-release sessions from free ranging bottlenose dolphins, from June-July of years 2003-2006, 2010-2012, and 2015 for IRL dolphins (n=146), and August of years 2003-2005, and 2013 for CHS dolphins (n=101). We analyzed the data using parametric quantile regression assuming log-normal distribution with robust standard errors to estimate the ratios of median PFAS concentrations per increasing year. We analyzed the nine PFASs that were detected in enough dolphins (<30% below level of detection), and had an adequate range of sample years from both locations. For PFASs with values reported below the level of detection (LOD), we replaced the values with LOD/√2. We performed analysis by sex, and of adult dolphins separately (≥10 years for males, ≥ 6 years for females). The strata were too small to analyze the trends in juvenile dolphins. Median concentrations for all PFASs were higher in CHS dolphins than IRL dolphins. For both CHS and IRL dolphins, the median perfluoro-octane sulfonate (PFOS) concentrations, collapsing over time, were the highest compared to other PFASs, with medians (25th percentile, 75th percentile) of 690.0 (569.7, 1554.3) for CHS dolphins and 389.8 (246.5, 966.4) for IRL dolphins. The ratios suggested similar trends between the two locations for most PFASs. Among CHS and IRL dolphins, perfluorodecane sulfonic acid (PFDS) seemed to have the greatest decrease over time, with a median ratio (95% CI) of 0.79 (0.74, 0.85) for CHS dolphins and 0.84 (0.78, 0.89) for IRL dolphins, considering all ages and both male and female dolphins. In general, the PFASs had median ratios below or close to 1, suggesting decreasing or mostly stable levels over time. Among CHS dolphins, perfluorohexanoic acid (PFHxPA) had a median ratio of 1.12 (1.01, 1.25), which could indicate a slight increasing trend.

2602 Perfecting Your “Elevator” Speech

J. Cichocki. Alnylam Pharmaceuticals, Cambridge, MA.

Effectively communicating science to the general public and to experts in the field is a skill that must be mastered in order to build your career. For most individuals, and especially most trainees, orally presenting a seminar or formalized science speech is much easier than giving a brief, two-to-three minute talk about one’s science and its impact on the general public. This “elevator speech,” however, is often the most important talk one will ever give, as you often have to communicate your science in a brief, succinct, and clear fashion in order to convey your message to lay individuals and experts alike. Further, during job interviews, you will not have one hour to talk to every interviewer individually, but, rather, will have to quickly summarize your work in a couple of minutes. This session will provide tips and tricks to deliver your “elevator speech,” which will be useful for your future presentations, in SOT Annual Meeting and ToxExpo, but also in your daily professional (and maybe personal) life. Following a brief introduction, a panel of experts and early-career scientists will provide examples and advice on how to quickly summarize a scientific project into a brief two-to-three minute speech. Following the short panel discussion, a 35-minute “hands-on” session will follow in which the session chairs will facilitate attendees as they perform their two- to three-minute “elevator speeches” in small groups. Five minutes will be allocated to wrap-up the session following the hands-on activity.

2603 Cardiovascular Adverse Effects Are Still Causing Late Attrition of Novel Therapeutics: Developing Solutions to Detect and Avoid Cardiovascular Toxicity in the Clinic

J. Kremer. Covance, Madison, WI.

Cardiovascular (CV) liabilities continue to be a leading cause of drug attrition in late-stage clinical trials and post-market approval. Aspects of the current paradigm have adequately characterized (e.g., benefit vs. risk) compounds for hERG blockade and QT interval prolongation, but a method to detect arrhythmogenesis directly, rather than using surrogates, still needs to be defined. Even so, the rate of attrition due to CV liability has remained intrinsically high, primarily driven by non-QTc-related liabilities (e.g., blood pressure, contractility). This session will start by highlighting several high-profile drug withdrawals due to unexpected CV liability to establish the problem statement. Specific examples will include Terfenidine (QT prolongation, Torsades de Pointes); Vioxx/COX-2 inhibitors (myocardial infarction, stroke); and Torcetrapib (increased cardiac events, hypertension). The individual speakers will address this problem by describing new scientific and strategic approaches to detect and characterize potential liabilities, culminating in a panel discussion with the audience. Specifically, the first presentation will describe the current state of CV liabilities and the most common mechanisms responsible for drug-induced arrhythmia or structural cardiotoxicity. The second and third presentations will focus on the use of rodent and large animal models, respectively, in safety profile safety and potential mechanisms for drug-induced arrhythmia or structural cardiotoxicity. The second and third presentations will focus on the use of rodent and large animal models, respectively, in safety profile safety and potential mechanisms for drug-induced arrhythmia or structural cardiotoxicity.
how researchers across multiple disciplines and organizations can share approaches and data to improve the testing paradigm. The goal of the session is to provide a data-driven, comprehensive discussion on how to revise CV safety assessments to improve patient safety and reduce the rate of late-stage compound failures.

### 2604 Use of hiPSC-Derived Cardiomyocytes for Cardiac Safety Evaluation

#### L. Guo, Frederick National Laboratory for Cancer Research, Frederick, MD.

Mechanism-based in vitro safety profiling of compounds early in the drug discovery pipeline holds a promise to mitigate safety-related attrition in the late-stage development. This presentation will discuss the potential role of human-induced pluripotent stem cell (hiPSC)-derived cardiomyocytes in shaping the current testing paradigm toward a more comprehensive and translational assessment of both functional and structural cardiotoxicity. Data obtained from studies on cardiomyocyte characterization and model validation as part of CIPA initiative and beyond by siRNA knockdown and small molecule inhibitors are presented. hiPSC cardiomyocytes continue to develop a more mature and adult-like phenotype with increased time in culture, and remarkable changes in responses to selective modulators of major cardiac ion channels, were observed in older cells (cultured for 6 months) compared to those with a short (two weeks) culture, therefore, maturation status of hiPSC-cardiomyocytes may need to be taken into account for study design and data extrapolation. To explore apoptosis as a cellular mechanism responsible for drug-induced cardiotoxicity, expression of apoptosis-related proteins were analyzed at gene and protein level, their functions in maintaining cell viability were characterized by siRNA knockdown and small molecule inhibitors. The results demonstrate that thorough characterization and “fit-for-purpose” validation, hiPSC-cardiomyocytes may be utilized as a valuable tool to improve safety evaluation of both drug-induced arrhythmia and on-target cardiotoxicity of anti-cancer drugs such as ErbB2 and Mcl-1 inhibitors.

### 2605 Incorporation of Cardiovascular Assessment in Early Efficacy or Safety Studies in Rodents

#### R. Macia, Covance Labs, Madison, WI. Sponsor: J. Kremer

This presentation will focus on the use of rodents in early screening and efficacy studies to improve outcomes or assist in the development of early strategies for drug candidate selection and development. Latin-square cross-over and parallel design studies with or without clinical pathology, toxicokinetics, and pathology can be used to assess short term effects on the cardiovascular system and to provide an outlook for possible complications in the development of test articles. Rodent models including blood pressure, left ventricular pressure, body temperature and activity can be used for assessment. Specific data from these models will accompany this discussion and demonstrate the ability to routinely screen candidates for development. Study design considerations and data evaluation from these studies is critical to the success of potential candidates assist in the initial evaluation of potential CV issues, which still remain a major hurdle in bringing compounds to market. Examples comparing CV changes associated with various CV-active agents (aminorin and atenolol (contractility and lusitropy), phenotlamine (hypotensive/increased HR), methoxamine (hypertensive) and amphetamine (increased activity)) will be discussed in comparison to the standard large animal models that are discussed in later presentations. Also described will be the use of rodents to evaluate secondary CV effects as complications from human disease states (e.g. diet-induced obese mice, diabetic and spontaneous hypertensive rats).

### 2606 Clinically-Relevant Large Animal Models of Heart Failure: Model Selection, Limitations, and Optimization

#### K. Hoagland, Amgen, Thousand Oaks, CA. Sponsor: J. Kremer

Large animal disease models have been developed to investigate heart failure (HF) mechanisms and the goal of providing insight that translates to the clinical situation from a pathophysiological perspective. This presentation will focus on the use of large animal heart failure models to study efficacy of new drug candidates, and will include a discussion of model selection considerations (tachypacing, versus ischemic-induction; species selection; anesthetized versus conscious state), and best practices for validation and benchmarking. Additionally, a discussion of what clinically relevant parameters to monitor in HF with the use of telemetry and echocardiography will be included. Both dog and pig models can be valuable to evaluate efficacy of cardioactive agents and be relevant for evaluating safety endpoints, especially proarrhythmia in the HF setting. Our investigations show that ejection fraction (EF) decreases dramatically in tachypaced beagle dogs after 4 weeks of pacing at 240 bpm. Decreases in EF were modest in the ischemia - reperfusion Yucatan minipig model of HF, with individual animal EF values correlating with the ischemic scar area produced by 90-minute occlusion of the left anterior descending artery. Blood pressure and ECG intervals were notably not affected by induction of heart failure, although pigs tended to exhibit more instances of arrhythmia after heart failure induction than dogs. In the tachypaced beagle and ischemia-reperfusion Yucatan minipig models, cardiac functional reserve was blunted, as demonstrated by reduced heart rate and contractility responses to dobutamine (relative to the normal healthy state), consistent with what is observed in clinical heart failure. Collectively, these observations demonstrate the importance of designing and using appropriate model validation protocols with the goal of achieving clinically relevant data sets with test compounds from high-confidence preclinical animal models.

### 2607 Translation from Nonclinical to Clinical Cardiovascular Safety: What Constitutes a Safety Risk?

#### M. Holbrook, VASTA Pharma Solutions, Harrogate, United Kingdom. Sponsor: J. Kremer

In a healthy individual, routine daily activities such as sleeping, walking, eating, and exercise result in marked changes in cardiovascular (CV) parameters such as QT interval, heart rate and blood pressure without any detriment or concern. Whereas in the disease situation or through drug intervention, even modest increases in these parameters are associated with an increased risk of serious adverse CV events such as torsades de pointes, stroke, and ischaemic heart disease (Sager et al., 2013). Hence, it is important to consider the situation and the degree of change in a CV parameter when assessing drug induced cardiac toxicity. In this clinical situation, the challenge is to identify a potential hazard and assess the safety risk preclinically before progressing into clinical studies and eventually the intended patient population. Drug attrition data indicates that, for some CV outcomes our ability to manage this risk has been suboptimal (Redfern et al., 2017). This has brought focus onto translation including the relevant of preclinical models and their statistical and predictive powers (Valentin et al., 2009, Bhatt et al., 2016). This presentation will examine our ability to manage CV safety using nonclinical studies to predict outcome in healthy volunteers and patients. Testing paradigms employing in vitro, ex vivo, in silico, and in vivo techniques will be described along with a discussion of their sensitivity, specificity, and predictive power to the clinical situation. Specifically, the ongoing development of models using human stem cell-derived cardiomyocytes and biophysical in silico models will be described. Importantly, these model systems support the study of structure and function to investigate the content screening to the dissection of pathways and determination of the mechanism of action. There are reports of good translational value with high sensitivity and specificity (e.g. 74% and 74%, Pointon et al 2015) offering promise of improved risk identification. Questions such as What is a “positive” CV finding and how does this affect the novel clinical safety pharmacology evaluations of heart rate and blood pressure pose a risk of falls and injury. Evaluation of drug-induced effects on heart rate and blood pressure is included in the “core battery” of in vivo nonclinical safety pharmacology evaluations specified in the ICH S7A guidance document, Safety Pharmacology Studies for Human Pharmaceuticals. However, the sensitivity of the nonclinical safety pharmacology evaluations of heart rate and blood pressure that are reported to regulatory agencies is poorly understood. This presentation will report a retrospective analysis of the statistical power of the nonclinical heart rate and blood pressure data reported in recent IND submissions to the US FDA’s Center for Drug Evaluation and Research. Results will be reported for “stand-alone” in vivo cardiovascular safety pharmacology studies, for studies that evaluated heart rate and blood pressure during repeat-dose toxicity
studies instead of during stand-alone safety pharmacology studies, and for studies that evaluated heart rate and blood pressure during repeat-dose toxicity studies in addition to stand-alone safety pharmacology studies. As a complement to other presentations during this session, this presentation will also discuss the use of alternative in vivo models for cardiovascular safety pharmacology studies.

W 2609 Environmental Chemical-Microbiome Interactions in Disease Susceptibility

M. Nagarkatti, University of South Carolina, Columbia, SC.

The human body harbors trillions of microbes, and there exists a symbiotic association between humans and microbes. Such an interaction plays a critical role in maintaining homeostasis. Because the microbial ecosystem is found throughout the body, it is constantly exposed to environmental chemicals. Thus, there is a constant crosstalk between the environmental chemicals and microbiota, leading to altered bioavailability of chemicals and/or microbial dysbiosis that could trigger disease processes. It is critical to better understand the mechanisms of these complex interactions between environmental chemicals and microbiota. Such studies may help in understanding why certain individuals are more susceptible to certain diseases when compared to others. This session will explore recent findings that provide conclusive answers, demonstrating that certain environmental insults cause microbial dysbiosis and consequently trigger various diseases. This session will start with introduction to the field, followed by a presentation of data on how bacterially-generated and xenogenous chemicals alter the bacterial composition and metabolism within the gut, leading to altered host homeostasis by activating the Ah receptor (AhR). The session will provide evidence that inhalation of Staphylococcal enterotoxin-B (SEB) triggers acute lung injury (ALI) in mice that can be effectively blocked by resveratrol, an AhR ligand. Interestingly, SEB triggered dysbiosis in the lung and gut microbiota, thereby suggesting gut-lung axis. Moreover, resveratrol treatment reversed the dysbiosis, thereby suggesting that AhR activation may prevent SEB-induced inflammation through alterations in the microbiome. Presenters will provide evidence that specific microbial genotoxic activities originating from various microbial strains, such as Escherichia coli, Atoxoplasma parvulum, and Campylobacter jejuni, promote development of colorectal cancer (CRC). Also, it will be demonstrated that CRC development could be modulated using genetic and pharmacological intervention aimed at the microbiota. These studies represent the first step toward validating the microbiota as a potential therapeutic target for prevention/treatment of CRC. Presenters will discuss obesogenic chemicals and describe how they interact with gut microbiota and promote adiposity in animals. Talks will focus on obesogenic activity of tributyltin. Together, the presentations will be organized in such a way that they will transition from introducing the topic on how crosstalk between environmental chemicals and microbiota plays a critical role in health and disease to addressing, in the second talk, more specific questions, such as the gut-lung axis in regulating the inflammatory disease. The third and fourth presenters will focus on specific disorders, such as cancer and obesity, with the third talk also highlighting how altering the microbiota would serve as a therapeutic tool to prevent/treat colon cancer. Together, this workshop will provide an exciting opportunity for all participants to gain new knowledge of the complexity of the interactions between the environmental chemicals to which people are constantly exposed and the microbiota, as well as novel insights into how they regulate the pathogenesis of a variety of clinical disorders.

W 2610 Modulation of the AhR within the Gastrointestinal Tract Mediates Both Protective and Adverse Outcomes

G. Perdue, and A. D. Patterson, Pennsylvania State University, University Park, PA.

The gastrointestinal tract is exposed to dietary, bacterially generated, and environmental chemicals capable of activating the Ah receptor (AhR). Previous studies have suggested that the level of AhR activation and the class of ligands dictate whether the effects observed are beneficial or lead to toxicity. To explore this further, we have developed two distinct avenues of investigation. First, we investigate the beneficial effects of low dose dietary anthocyanins exposure through the broccoli consuming animal model. In order to assess whether AhR activation is a key chemoprotective effect of broccoli consumption, we administered isocaloric diets, with or without supplementation of whole broccoli (15% w/w), to mice expressing the high-affinity AhRb/b or low-affinity AhRd/d alleles, for 24 days. We then examined the effects of AhR activation on intestinal microecological structure, inflammatory status, and response to DSS-induced colitis. Cecal microbial community structure and metabolic potential were segregated according to host dietary and AhR status. Dietary broccoli that led to heightened intestinal AhR activity resulted in decreased microbial abundance of the family Erysipelotrichaceae, attenuation of colitis and inflammatory signaling. In our second approach, we examined the impact of low dose 2,3,7,8-tetrachlorodibenzofuran (TCDF), to alter intestinal homeostasis in mice. Dietary TCDF exposure (24 μg/kg for 5 days) induced significant changes in the microbiome community structure and metabolism in an AhR-dependent manner. TCDF exposure resulted in an elevation of hepatic lipid, triglyceride, and unsaturated fatty acids in conventionally-raised mice, which was associated with an up-regulation of hepatic SREBP-1C protein and related triglyceride biosynthesis and lipogenic genes. However, no significant changes in the level of lipid and triglyceride-related mRNA or protein expression were found in the livers of germ free (GF) mice or AhR-null mice after TCDF exposure. TCDF-treated GF-mice exhibited more severe inflammation and oxidative stress in the liver. These findings demonstrate that the intestinal microbiota significantly impact the host response to dietary TCDF with a significant role for AhR activation via a diverse set of ligands can lead to changes in the overall microbiome composition and metabolism within the gut, leading to altered host homeostasis.

W 2611 Staphylococcus Enterotoxin B (SEB) Triggers Acute Lung Injury (ALI) through Dysbiosis in Gut-Lung Axis and AhR Ligands Protect the Lungs through Reversal of Microbiome

M. Nagarkatti, University of South Carolina, Columbia, SC.

Staphylococcus Enterotoxin B (SEB), produced by Staphylococcus aureus, is a superantigen that triggers cytokine storm, and inhalation of SEB leads to toxic shock syndrome, respiratory failure, and often death. The gut microbiome plays an important role in the development and functions of immune system in different parts of the body, including respiratory and digestive systems. Also, crosstalk between microbiota in mucosal tissue of gut and lung through Gut-Lung Axis (GLA) can influence immune and respiratory functions of the lungs. The presentation will focus on intranasal exposure of SEB to induce acute lung injury (ALI) in mice and address the role of microbiota. For this purpose, 16S rRNA metagenomics sequencing was performed to investigate the nature of alterations in gut and lung microbiome after induction of ALI by SEB when compared to controls. Analysis of microbiome revealed that SEB inhalation caused a decrease in some species such as Ruminococcus gravis in the lung and gut while increasing Akkermansia muciniphila, which is responsible for degrading mucin barrier of the colon. This correlated with increased permeability and decrease in tight junction proteins in epithelial cell barrier in the lungs and intestines. There was also a decrease at the genus level in the beneficial microbiota in SEB group when compared to control group. Interestingly AhR agonists that suppressed ALI also caused reversal of microbial dysbiosis. Collectively, these data suggested that exposure to SEB that triggers ALI, is associated with similar alterations in the composition of microbiome in the lungs and gut, which promoted inflammation thereby regulating the pathogenesis in the lungs.

W 2612 Tributyltin Exposure Alters Post-Embryonic Growth, Adiposity, and Intestinal Microbiota Assembly in Zebrafish

J. Rawls, S. Gomez De La Torre Canny, and O. Mueller, Duke University School of Medicine, Durham, NC. Sponsor: M. Nagarkatti

Obesity is a ubiquitous public health problem that warrants the study of environmental factors that, by regulating energy balance, promote the storage of excess energy in adipose tissue. Two important environmental factors that promote adipose tissue accumulation are microbial communities residing in the intestine, and environmental chemicals that may act as obesogens. We developed an in vivo model to test the obesogenic effect of chronic tributyltin (TBT) exposure in post-embryonic zebrafish and to study the effect that this chemical has on intestinal microbiota. We have shown that zebrafish develop multiple adipose tissue depots, which consist of white adipocytes that display morphological and molecular homologies with those of mammals. Using fluorescent lipophilic dyes to measure adipose tissues together with nutritional factors that promote adipose tissue accumulation in zebrafish as it does in mice. Also, we show that 21-day exposure to TBT at a nominal dose of 1 μg/L was sufficient to increase the growth of adipose tissue in zebrafish compared to control animals. Unexpectedly, TBT exposure also inhibited somatic growth as early as 14 days post-exposure and at a dose as low as 0.1 μg/L. High-throughput sequencing of 16S rRNA genes revealed specific effects of TBT on intestinal bacterial community assembly including significant increases in Chitinibacter spp. abundance. Together, our
results indicate that TBT exposure promotes adipose tissue deposition even in the absence of a high fat diet, and affects other distinct aspects of post-embryonic growth and intestinal microbiota assembly. This work provides the first comprehensive analysis of the effect of a chemical onzebrafish somatic growth, adipose tissue accumulation, and intestinal microbiota composition, providing a critical frame of reference for future studies to test for mechanistic interactions between environmental toxicins, microbiota, and animal hosts.

2613 Cancer: Mining the Microbiota for Answers

C. Jobin, University of Florida, Gainesville, FL; Sponsor: M. Nagarkaraki

Gene-environment interaction plays a key role in disease susceptibility including colorectal cancer. Microbiota, particularly the intestinal microbiota, plays a central role in host physiology and the composition and activity of this consortium of microorganisms is directly influenced by known cancer risk factors such as lifestyle, diet, and inflammation. Accurately quantifying microbial contributions to a wide range of conditions is important. The mechanism by which microbiota impact cancer development is still unclear, but cancer risk factors such as inflammation and diet are known to influence environmental components that modulate microbiota. Specifically, inflammation represents a powerful condition by which microbial composition and biological activities are altered. Using gnotobiotic technology, microbial genetic manipulation, and genetically engineered mice (Apomucin+/-; IL10-/-), we investigated the role of specific bacterial consortium of bacteria in the development of colitis-associated colorectal cancer. Based on our data, the presentation will provide evidence that specific microbial toxic activities originating from various strains such as Escherichia coli, Atpobium parvulum, and Campylobacter jejuni promote development of CRC. For example, presence of DNA damaging toxins such as colicin from adherent invasive Escherichia coli or cytotoxic distending toxins (cdt) from Campylobacter jejuni are critical for development of colorectal cancer. In addition, we recently observed a correlation between presence of hydrogen sulfide (H2S)-producing bacteria (HSPB) and severity of new onset Crohn’s Disease (CD) in a pediatric population, with strain Atpobium parvulum able to promote development of colorectal cancer in Apomucin+/-; IL10-/- mice. Although single organism promotes development of colitis-associated colorectal cancer (CRC) in preclinical models, an ecologically complex microbial interaction is implicated in human pathology, including inflammatory bowel disease (IBD). This talk will provide evidence that consortia of bacteria obtained from human CRC tissues promote colorectal cancer in gnotobiotic Apomucin+/-; IL10-/- mice. These studies represent the first step in understanding mechanisms by which microbiota influence development of colorectal cancer, and identify new potential therapeutic target for prevention/treatment of cancer.

2614 Matching Analytical Methods to Markets: Balancing Regulatory Expectations and Technical Challenges

B. Henry, W.L. Gore & Associates Inc., Elkton, MD.

Today, regulators, supply chain partners, and customers want to know what is in the products they buy that might harm them. From a risk assessment perspective, the absence of clear consensus and regulatory guidance for chemical characterization of medical devices, drug-device combination products, and their components delays commercialization of life-saving technology, leads to increased animal testing as a clearly-defined path to market, and is expensive. This session will seek to open the dialogue on the challenges and potential paths forward for manufacturers of medical devices, drug-device combination products, and their components. An emerging area of focus is the use of new technologies and the roles they may play in streamlining product development, including new approaches to qualitatively, semi-quantitatively, and quantitatively measure chemical substances in biological matrices. The first presenter will briefly compare several extractables and leachables (E&L) approaches, including ISO 10993 and those by the United States Pharmacopeia, Product Quality Research Institute, the US FDA Container Closure Guidance, the US Pharmacopeia, and the Bio-Process Systems Alliance. (TTC) for a permanently-implanted medical device and a drug-device combination product. Now, that the opening presentations have shared a collaborative plan of attack for E&L, does it matter which lab does the analysis? Inter-laboratory variability related to compound identification and quantitation, including equipment sensitivity and the available chemical library, will be discussed by the next presenter. Diversity in the number of reported compounds among four test laboratories for the same test articles, despite the same extraction solvents, conditions, and analytical techniques, will be shared. Considerable quantitative and qualitative differences potentially impacting the risk assessment will be discussed. The final presentation will provide a high-level complex and complicated tunnel by sharing US FDA expectations of E&L studies for medical devices and combination products. The presentation will include LODs, LOQs, sensitivity, extraction methods, and the interpretation of qualitative, semi-quantitative, and quantitative data, as well as issues frequently encountered. To close out the session, the panel will answer audience questions regarding lab selection, unknowns, use of the TTC, Cramer Classes, and in silico approaches. This session will feature information and provocative discussions that will be transferable to other E&L applications and should be of interest to contract labs and manufacturers of parts intended for use in medical devices, pharmaceutical processing, or single-use systems, as well as regulators.

2615 Which Guidance to Follow for Which Market?

B. J. Henry, W.L. Gore & Associates Inc., Elkton, MD.

Framework: Lacking specific extractable and leachable (E&L) expectations from regulatory authorities, E&L testing is complicated and confuses for manufacturers of medical devices, combination products, and their components. A very brief comparison of several E&L approaches will be presented, including the US Pharmacopeia, ISO 10993-1, Product Quality Research Institute (PQRI), the US FDA Container Closure Guidance, the Extractables Work Group of the BioPhorum Operations Group (BPOG), and the Bio-Process Systems Alliance (BPSA).

2616 Extractables and Leachables Testing for Medical Devices: What About ISO 10993 Series?

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Regulatory authorities require the consideration of chemical characterization of Extractables and Leachables (E&L) with the corresponding toxicological risk assessment to ensure safety and successful submissions. For medical devices and combination products, the major standards, ISO 10993-1, -17 and -18 parts, acknowledge the importance of chemical characterization. The information provided in the current standards is not sufficiently detailed to plan extraction, analysis and interpretation for risk assessment. The preparation of sample extracts is a challenge as the conditions and extractions defined in ISO 10993-12, may not be relevant for the identification of E&L. This presentation will address the revisions currently in progress for these three standards. For ISO 10993-1 changes have been discussed to the flow-chart (describing the systematic approach to the biological evaluation of medical devices) to strongly focus on chemical characterization and the importance of in vitro and in vivo testing. Another change considered is to require chemical characterization while all other toxicological endpoints will be evaluated within a toxicological risk assessment. A major revision will be made on ISO 10993-18 to incorporate the technical and scientific experience developed during the last several years since its publication, including addition of several new annexes, and the description of experimental requirements for the investigation of E&L and a revision of the stepwise chemical characterization process, including the setting of the analytical evaluation thresholds (AETs) in alignment with the Threshold of Toxicological Concern (TTC) concept, already established and guidance on toxic pharmaceutical imputities. A major revision of ISO 10993-17 on allowable limits for leachable substances is in progress, though still early in the process. At the present in discussion are risk assessment approaches to use the concept of TTC, and, if it can be shown that an impurity is below the TTC, then the chemical substance is assumed to be of no significant risk. More work is necessary to
Variation in Chemical Characterization Results from Four Test Laboratories

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Chemical characterization is commonly conducted in the biological evaluation of medical devices. These test results may be impacted by laboratory-specific variables, including equipment sensitivity and the available chemical library. An investigation was conducted to evaluate inter-laboratory variations related to compound identification and quantitation. The test articles, two materials (polyurethane and polisoprene) and a medical device, are each known to generate many extractable chemicals. Test articles were extracted in water and isopropyl alcohol under exaggerated extraction conditions (50°C, 72 hours) and analyzed at four test laboratories in a battery of analytical tests per ISO 10993-18. Disparity in the number of reported compounds was observed among the four test laboratories. For example, the reported number of elements in the polyurethane water extract ranged between 0 and 12 for the four labs. For the polisoprene isopropyl alcohol extract, the reported number of gas chromatography-mass spectrometry compounds ranged between 12 and 156 for the four labs. For the polyurethane isopropyl alcohol extract, the reported number of liquid chromatography-mass spectrometry compounds ranged between 3 and 143 for the four labs. In addition, as much as a 10-fold difference in the amount of non-volatile residue was reported. The similarities between the reported compounds varied greatly depending on the test extract. Differences can be partially attributed to the granularity of compound identification and grouping of derivative compounds could improve similarities between test laboratories. Confidence in compound identification also varied, depending on the test laboratory. Comparison of results and the conduct of a risk assessment is further complicated by the reporting of unknown and non-descriptive chemical compounds. This study into inter-laboratory comparisons revealed considerable quantitative and qualitative differences among chemical characterization results, which can potentially impact the outcome of a risk assessment. These results highlight the importance of consistency in test laboratory selection in the identification of design- or process-related changes for individual materials or devices, as well as in the evaluation of potentially equivalent materials.
Corneal Injury Progression

Sulfur Mustard Exposure: A New Model of Corneal Injury Progression

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The eye is the most sensitive organ to sulfur mustard (SM) injury and greater than 90% of SM casualties present with ocular morbidities. While mild and moderate ocular exposures typically resolve without long-term complications, severe exposures involve a prolonged rehabilitation period during which victims are visually disabled. Persistent corneal manifestations subsequently develop in 35-90% of survivors, ranging from chronic ocular discomfort, to advanced corneal disease characterized by progressive corneal degeneration and loss vision. These persistent manifestations are collectively referred to as mustard gas keratopathy (MGK). While mild forms of MGK can be treated symptomatically, severe forms of MGK are progressive and difficult to clinically manage. Despite a century of research, the etiogenesis of corneal SM pathologies is poorly understood, and thus treatment strategies are limited to palliative measures. SM reactivity within biological tissues is rapid and irreversible, and consequently current treatment strategies are focused on mitigating pathological host responses and promoting healing. However, treatments to preserve the epithelium and reduce inflammation have been ineffective in preventing MGK. Hypothesizing that SM toxicity to the non-regenerative corneal endothelium may present a novel injury modality that could explicate ocular SM injury progression, our studies characterized corneal endothelial injury using a well-described vapor SM injury model in rabbits. Convergent functional and ultrastructural methods demonstrated that ocular SM exposure causes acute endothelial toxicity and disruption of endothelial barrier function. MGK eyes exhibit long-term endothelial pathologies, including endothelial cell cytotoxicity and deposition of a retrocorneal fibrous membrane, consistent with an endothelial-to-mesenchymal transition that is associated with endothelial failure. These features were absent in SM-exposed eyes that healed from the acute lesion. The association of endothelial pathologies with MGK suggests that SM damage to corneal endothelial cells provides a novel mechanistic basis for ocular injury progression. Based on these findings, studies hypothesize that (a) the severity of acute SM injury is determined by the degree of endothelial damage, and (b) the efficiency of endothelial repair influences whether corneas resolve or develop MGK.
**2624 Reaching Hazard Conclusions for Endocrine-Disrupting Chemicals: Adapting Systematic Review Methods**

J. Rochester, The Endocrine Disruption Exchange (TEDX), Grand Junction, CO.

Reviews of environmental chemicals, such as endocrine-disrupting chemicals (EDCs), are essential for identifying hazards to the health of humans and wildlife, yet they often lead to inconclusive results that can stall the regulatory process. Attempts to review this literature to arrive at hazard conclusions are often unstandardized, including weight-of-evidence reports or narrative reviews that lack transparency and reproducibility. To address the systematic review methodology has been developed to evaluate environmental health questions. These methods include the Office of Health and Translation (OHAT) Approach for Systematic Review, the University of California San Francisco (UCSF) Navigation Guide, and the systematic review and integrated assessment (SYRNA) of the National Academy of Sciences (NAS). SYRNA increases the objectivity and transparency in an evaluation by using a predefined, multistep process to identify, critically assess, and synthesize evidence. They also contain specific methods to address study quality, among other factors. When appropriate, systematic reviews employ meta-analytical techniques that allow in the interpretation of conflicting results and provide a clearer picture summarizing the overall body of evidence. These methodologies are adapted from the medical field, which has developed, used, and empirically tested systematic review methods to evaluate medical research and reach evidence-based clinical decisions about patient care and interventions. Recognition of the value of systematic review methods has increased among the environmental health community over the last five years. In fact, the US National Academy of Sciences (NAS) recently convened a panel to evaluate how systematic review procedures can be used as part of an overall strategy to evaluate potential low-dose “endocrine-mediated effects for environmental chemicals. The NAS panel, consisting of experts from academia, non-government organizations, government, and industry, developed systematic reviews of two EDCs to illustrate the utility of systematic reviews in decision-making. The resulting report will use the systematic review methodology. In this study, we conducted a systematic review of the literature on the neurological effects of polybrominated diphenyl ethers (PBDEs), as an example of non-reproductive health effects associated with EDCs. The last two speakers are members of the NAS committee and will provide an overview of the timely, cutting-edge evidence and insight from the NAS panel’s report of systematic review use in low-dose environmental toxicology, including its role in regulatory toxicity practices. The NAS strategy and recommendations for evaluating low-dose adverse effects that act through endocrine-mediated pathway will be outlined. The NAS report developed case studies that examined low-dose adverse effects of two EDCs: PBDEs and phthalates. These case studies will be used to illustrate how systematic review methods can be used to evaluate human and animal evidence of adverse effects of EDCs, considerations for health effects evidence and dose-response concordance in integrating the human and animal evidence, and how a published systematic review can be critically evaluated and used when the focus is appropriate for the research question under consideration. They also illustrate an evidence integration process to consider the observed adverse effect and the endocrine-disrupting activity. Now, as we begin to use this tool, we have identified the strengths and weaknesses of systematic review methodologies in the identification and assessment of EDCs. The recent NAS panel report will also be discussed, and their approach considered in the context of SYRNA.

**2626 PDBEs and Neurodevelopment: A Systematic Review Using the Navigation Guide**

J. Lam, University of California San Francisco, San Francisco, CA, Sponsor: J. Rochester

The presentation will provide a brief overview of the Navigation Guide framework as an example of an environmental health systematic review methodology. In this study, we conducted a systematic review exploring developmental exposure to PBDEs and intelligence or Attention Deficit/Hyperactivity Disorder (ADHD) and attention-related behavioral conditions in humans. The Navigation Guide systematic review approach to search articles published up to September 26, 2016 was applied, and included original studies that quantified exposures to PBDEs incurred any time in pregnancy or during in utero, perinatal, or childhood time periods. Fifteen studies met the inclusion criteria; 10 for intelligence and 9 for ADHD-related problems. The studies were reviewed individually with low to probably low-risk of bias. The meta-analysis of four studies estimated a 10-fold increase in PBDE exposure associated with a decrement of 3.70 Intelligence Quotient (IQ) points (95% CI: -6.56, -0.83). It was concluded the body of evidence was of "moderate" quality for ADHD with "limited" evidence for an association with PBDEs, based on the heterogeneity of association estimates reported by a small number of studies and the fact that chance, bias, and confounding could not be ruled out with reasonable confidence.

The first systematic review on this topic and the largest meta-analysis to date, covering over 3,000 mother-child pairs. However, a recurring limitation for systematic reviews remains the inability to combine more relevant studies in a meta-analysis due to the lack of or heterogeneity in reporting study details and measures of association in study publications. To advance the capacity to conduct robust systematic reviews in environmental health, more attention to reporting details and standardized effect estimates should be undertaken. Several high-impact journals have adopted checklists for the reporting of elements necessary to describe studies comprehensively and transparently, which can help to ensure that these details are available for incorporation into future reviews.

**2627 Developmental Neurotoxicity of PBDEs: Integrating Evidence and Consideration of Published Reviews in the NAS Case Study Systematic Review**

A. A. Rooney, NIEHS, Research Triangle Park, NC.

The National Academy of Sciences committee on low-dose toxicity outlined a strategy for evaluating and discussing the evidence for low-dose exposure to environmental chemicals that act through an endocrine-mediated pathway in a July 2017 report. The report includes two systematic review case studies developed to illustrate how these methods can be used to assess the evidence of low-dose effects of EDCs for human and experimental animal literature of polybrominated diphenyl ethers (PBDEs) on neurodevelopment, and (2) evidence that phthalates affect male reproductive-tract development. They also illustrate an evidence integration process to consider use of the health effects and dose-response data from the human and animal evidence streams in reaching hazard conclusions on low-dose effects of EDCs. The approach to evaluating the evidence that PBDEs effect neurodevelopment will be presented. A recent systematic review of the human PBDE epidemiological data was available. The case study developed a new systematic review of the experimental animal data and outlined a procedure to critically assess the published reviews of the human studies. The was moderate level of evidence for PBDEs and effects on learning in rodents based on studies of BDE-47, -99, and -209, with low or inadequate level of evidence to be able to evaluate effects on memory or attention. The Lam et al. systematic review concluded there was a decrease in 3.70 IQ points in children per 10-fold increase in PBDEs, and NAS review considered the human studies provided a moderate level of evidence that exposure to PBDEs is associated...
with a decrease in IQ. During evidence integration, the animal data on learning and memory were considered with the human IQ data to reach the conclusion that developmental exposure to PBDEs is presumed to pose a hazard to intelligence in humans, and that it was not possible to reach a conclusion on potential hazards for attention-related effects based on the available evidence. The lower human exposure levels, both intake and plasma concentrations, associated with effects suggests that animal toxicity testing may detect hazard, but might not be accurately predicting doses at which effects occur in humans. The PBDE case study also illustrates how a published systematic review can be critically evaluated and used for a research question under consideration.

**2628 Phthalate Male Reproductive Toxicity: Comparison of Hazard Conclusions and Dose Responses from the NAS Systematic Review and Traditional Toxicity Testing Studies**

K. J. Johnson, The Dow Chemical Company, Midland, MI.

This presentation will continue addressing the National Academy of Sciences (NAS) committee report on using systematic review to examine human hazard potential following low-dose exposure. One of the case studies developed in the NAS report was an evaluation of the evidence that phthalate exposure effects male reproductive tract development. The data for multiple phthalates were examined, with diethylhexyl phthalate (DEHP) composing a more comprehensive dataset. Upstreaming DEHP case study, systematic reviews of the human and animal data will be outlined, including the steps used to integrate the human and animal datasets, the utility and impact of available mechanistic data at the evidence integration stage, and the dose-response concordance between human and animal data. The DEHP systematic review concluded that in utero DEHP exposure was presumed to be a reproductive hazard in humans. This hazard conclusion will be compared to that obtained from traditional (i.e., guideline) reproductive toxicity studies with DEHP. In addition, the DEHP dose-response concordance between the NAS systematic review and traditional toxicity testing studies will be evaluated in order to highlight the NAS report conclusion that traditional toxicity testing methods “might not accurately predict exposures at which humans are affected.” Finally, key learnings from the NAS report gleaned from both the PBDE and phthalate systematic reviews will be presented, as well as the strengths and weaknesses of systematic review and traditional toxicity testing practices for evaluating human toxicity potential.

**2629 Reducing the Uncertainty of Read-Across Predictions by New Approach Methodologies: Application in Regulatory Human Risk Assessments**

I. Rusyn, Texas A&M University, Texas, TX.

A paradigm shift is ongoing in human risk assessment, away from the traditional in vivo animal studies toward new approach methodologies (NAM). NAM include in vitro, ex vivo, or ’omic technologies, as well as in silico and toxicokinetic modeling. Currently, hazard assessment and derivation of point-of-departure values are based on the apical toxicity findings in animal studies. These apical endpoints seldom provide detailed mechanistic information to inform extrapolation of these findings to humans. NAM have the potential to provide a deeper understanding of key and intermediate steps leading to a certain apical finding, a concept known as adverse outcome pathways (AOP). However, the integration of NAM data into risk assessments is challenging, in particular, for complex endpoints, such as repeated dose or reproductive toxicity. This session will provide an in-depth overview of the use of NAM in regulatory and investigative toxicology, starting with a regulatory perspective, followed by industry examples, and then broadening the scope to cover the most up-to-date developments from the EU-ToxRisk and US academic and government research programs. A focus will be on read-across case studies, by which the use of NAM and mechanistic data are demonstrated. Learnings from the proof-of-concept read-across approaches and case examples will help to develop new mechanism-based chemical safety testing strategies. This session will include a discussion on the limitations and advances of such approaches and the path forward to substantiate and support a paradigm shift in regulatory risk assessment practices.

**2630 Use of Read-Across under the REACH Regulation**

M. Rasenberg, European Chemicals Agency (ECHA), Helsinki, Finland. Sponsor: I. Rusyn

REACH is a regulation of the European Union, adopted to improve the protection of human health and the environment from the risks that can be posed by chemicals. Under this regulation, industry is obliged to assess and document the hazard, exposure, and consequent risk of the chemicals they manufacture or import. Minimum information requirements are defined for the hazard and risk assessment, including for classification and labeling of the substance. Under REACH, animal testing is the last resort, and the use of alternative ways of assessing the hazard of chemicals is highly encouraged. Considerable efforts in developing new approaches and methodologies (NAM) for investigating properties of substances have been made over the past years. Grouping of substances and read-across is one of the most common using alternative approaches for filling data gaps in registrations under REACH. If grouping and read-across are applied correctly, experimental testing can be reduced. The European Chemicals Agency (ECHA), which evaluates dossiers submitted under REACH, developed and published the Read-Across Assessment Framework (RAAF). In registration dossiers, ECHA has evidence for read-across assessment that can be based on theoretical considerations or expert systems, to results from in vivo or in vitro studies. (Q)SARs, alert-based mechanistic profiles, or in vitro assays (e.g., metabolism investigations in cells or cell homogenates) are submitted by registrants. Quantitative toxicokinetics information is unfortunately not so frequently available. Results obtained with ’omics techniques or high-throughput screening are also yet to be used in registration dossiers. This presentation will set out the context of read-across under REACH, describe the RAAF, and will discuss the learnings and paths forward for increased use of NAM-derived data in REACH submissions in the future. The viewers will be presented with a comparison of the hazard conclusions and paths forward for increased use of NAM-derived data in REACH submissions in the future. The viewers will be presented with a comparison of the hazard conclusions and paths forward for increased use of NAM-derived data in REACH submissions in the future. The viewers will be presented with a comparison of the hazard conclusions and paths forward for increased use of NAM-derived data in REACH registrations.
the concept of biological read-across. Each case study has specific scientific questions as well as technical and modeling challenges. Differences in toxicokinetics from in vivo and in vitro investigations are one key aspect of the integrated approach for testing and assessment of EUToxRisk. PBPK models informed by in vitro methods and reverse dosimetry are also used to determine toxicologically relevant test conditions. Overall, the case-study approach is aimed at developing demonstration blueprints on how NAM data may be used in regulatory submissions.

2633 Categorization of UVCBs Using Chemical-Biological Read-Across

I. Rusyn, Texas A&M University, College Station, TX.

Chemicals of unknown or variable composition, complex reaction products, and biological materials (UVCBs) present a major challenge for registrations under the REACH and US High Production Volume regulatory programs. In addition to frequent variations in their chemical composition, many gaps in available toxicity data preclude confident groupings of these substances for read-across applications. Here, we present a comprehensive experimental and computational approach to categorize UVCBs according to global similarities in (1) their chemical composition using ion mobility mass spectrometry (IMMS), and (2) their bioactivity in a suite of in vitro models. For chemical read-across, we analysed petroleum substances from distinct product groups by IMMS to determine substance-specific quantitative parameters including m/z distribution, drift time, carbon numbers, and double bond equivalents. For biological read-across, we exposed various human-induced pluripotent stem cell-derived organotypic models to DMSO-soluble extracts of petroleum substances comprising several product groups. We show how dose-response profiles for cell physiological, cytotoxicity, and targeted transcriptomics (Temp0-seq)-enabled product group-specific grouping. Data integration in ToxPi software and subsequent correlation analysis revealed group-specific clustering and a high degree of correlation between biological and chemical datasets. Altogether, we demonstrate how novel analytical chemistry and in vitro screening approaches can be effectively utilized to categorize UVCBs, thereby indicating their potential applicability in regulatory submissions.

2634 Current and Future Opportunities for US Regulatory Application of Read-Across

N. Kleinstreuer, NIEHS, Durham, NC.

Toxicology in the 21st century has ushered in a scientific revolution focusing on new approach methodologies to understanding chemical hazard and safety and performing human health risk assessment. A key tool in this evolving paradigm is the chemical read-across, by which similarity to chemicals with known toxic effects is used to inform decisions on chemicals with unknown effects. Evolving work on characterizing chemical similarity moves beyond exclusively structure-based methods to hybrid approaches incorporating physico-chemical properties and in vitro bioactivity profiles providing valuable mechanistic insight. Read-across, based on both structural and biological similarity, is currently a useful strategy to increase understanding of chemical hazard without de novo testing. However, expertise, application, and acceptance of the results of a particular read-across vary within and among organizations and regions. The NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) works with sixteen US federal agencies with an interest in non-animal testing, via the Interagency Coordinating Committee on Validation of Alternative Methods (ICCVAM), and focuses on efforts driven by agency priorities both regulatory and scientific in nature, including read-across applications. The risk assessment division of the US EPA Office of Pollution Prevention and Toxics routinely uses read-across in their existing pipeline, and other offices and regulatory agencies are investigating its utility as the supporting evidence grows. ICCVAM has established a cross-federal read-across working group focused on (1) identifying and evaluating the available approaches, (2) developing guidance and best practices, and (3) addressing both scientific and non-scientific issues, including regulatory challenges. This talk will cover existing regulatory use of read-across by US federal agencies as well as opportunities for data generation and future application.

2635 Conventional Fibrin Clot Formation Is Not Required for Fibrinogen-Directed Repair of the Acetaminophen-Injured Liver

A. K. Kopce1, H. Cline-Fedewa2, M. J. Flick2, and J. P. Luyendy1.1Michigan State University, East Lansing, MI; and 2Cincinnati Children’s Hospital, Cincinnati, OH.

Activation of the blood coagulation cascade and deposition of fibrinogen in the liver are hallmark features of acetaminophen (APAP) overdose. We recently demonstrated that fibrinogen deposits in the APAP-injured liver are critical stimuli of leukocyte-directed liver repair. The canonical blood coagulation cascade is defined by thrombin-catalyzed conversion of soluble fibrinogen monomers to insoluble stabilized fibrin polymers at sites of injury. However, a lack of tools capable of distinguishing the in vivo functions of fibrinogen monomer and fibrin polymer has left the exact nature of the fibrinogen deposits in the APAP-injured liver unknown. Here, we used novel mice (termed FibΔEK mice) expressing soluble fibrinogen completely insensitive to thrombin-mediated polymer formation (i.e., locked in the monomeric form). To induce liver injury, FibΔEK mice and wild-type (WT) mice were given a single hepatotoxic dose of APAP (300 mg/kg, i.p.) or vehicle (saline) followed by analysis after 24 hours. Unexpectedly, the levels of hepatic fibrinogen deposits were remarkably similar in APAP-challenged FibΔEK mice relative to WT mice, as indicated by immunohistochemistry and capillary-based western blotting. APAP-induced liver injury, as indicated by elevated serum alanine aminotransferase activity and centrilobular necrosis, was also similar in FibΔEK mice and WT mice. Whereas complete fibrinogen deficiency dramatically reduced hepatocyte proliferation, indicative of failed liver repair, the number of proliferating hepatocytes in APAP-challenged FibΔEK mice was identical to that of WT mice. Collectively, we demonstrate for the first time that fibrinogen accumulation in the injured liver is independent of thrombin-mediated fibrin polymer formation. Moreover, the results suggest that a novel coagulation-independent form of fibrinogen deposit drives repair of the injured liver.

2636 Chronic Trichloroethylene and Arsenic-Exposed MDR-Null Mice Develop Renal Damage but No Tumors

A. L. Perry, and D. W. Threadgill, Texas A&M University, College Station, TX.

Trichloroethylene (TCE) and inorganic arsenic (iAs) are environmental contaminants and carcinogens that target the kidney. Chronic exposure to TCE is associated with increased incidence of renal cell carcinoma. While co-exposure to TCE and iAs is likely in certain populations, such as those near Superfund sites, a review of the literature did not reveal any studies on co-exposure or co-carcinogenesis of these toxicants. Our objective was to determine whether multidrug resistance gene-null mice chronically exposed to TCE and iAs could be used to model the development of renal cell carcinomas in similarly exposed human populations. An F3 mouse population derived from the FVB/N-Apc11Em1blo, Abcb11Em1blo and CAST/EU strains was exposed to trichloroethylene and arsenic for one year and kidneys from these mice were processed for H&E staining before being analyzed for neoplastic and pre-neoplastic changes. Despite use of TCE and iAs in combination and at environmentally relevant concentrations, primary renal cell tumors failed to develop. A significant increase in histologic evidence of renal disease was observed overall with any level of iAs exposure, but not with TCE exposure. We expected that inclusion of multiple toxicants in environmentally relevant concentrations, primary renal cell tumors failed to develop. A significant increase in histologic evidence of renal disease was observed overall with any level of iAs exposure, but not with TCE exposure. We expected that inclusion of multiple toxicants in environmentally relevant concentrations, primary renal cell tumors failed to develop. A significant increase in histologic evidence of renal disease was observed overall with any level of iAs exposure, but not with TCE exposure. We expected that inclusion of multiple toxicants in environmentally relevant concentrations, primary renal cell tumors failed to develop. A significant increase in histologic evidence of renal disease was observed overall with any level of iAs exposure, but not with TCE exposure. We expected that inclusion of multiple toxicants in environmentally relevant concentrations, primary renal cell tumors failed to develop.
Frontloading Nonclinical Kidney Safety Evaluation in Staphylococcus aureus Minipig Surgical Infection Model Using Novel Biomarkers


Poorly translatable pharmacology models tend to lack significant failure of Staphylococcus aureus vaccines in clinical trials. To increase the success rate of candidate vaccines, we developed a minipig surgical wound infection model which has an immune system that is similar to humans. Several studies have described surgical infection models in rodents that were characterized by infection-induced glomerulo- and tubulo-interstitial nephritis. We developed a minipig model of surgical wound infection that is anticipated to closely resemble human disease. Various plasma, serum, and urine-based biomarkers of infection, inflammation, and nephrotoxicity were assessed. Infection in the deep thigh muscles of minipigs correlated with increased signal intensities detected using PET imaging. Elevations in circulating fibrinogen and haptoglobin were detected as biomarkers of systemic inflammation. Elevations in urinary neutrophil gelatinase associated lipocalin (NGAL) was detected as a biomarker of kidney tubular injury in the absence of changes in serum/plasma NGAL, sCr or BUN. This new, more relevant animal model will enable frontloading kidney safety evaluation in preclinical studies.

Use of Human/Mouse Alanine Aminotransferase (ALT) ELISA to Evaluate Hepatotoxicity in the Humanized-Liver Mouse Model


Predicting adverse effects in humans based on experimental animal model findings can be difficult because of interspecies differences in drug metabolism and disposition. We have developed experimental animal models that have reconstituted livers with human hepatocytes (Hu-liver) to overcome these interspecies issues. Hu-liver mice are a valuable in vivo model for predicting hepatotoxicity in humans. However, the enzymatic activity of alanine aminotransferase (ALT), a conventional liver toxicity marker, cannot be used to evaluate human-specific hepatotoxicity in Hu-liver mice because of the lack of species-specific enzymatic activity. Therefore, we aimed to establish a hepatotoxicity detection method applicable in Hu-liver mice to replace ALT activity. In this study, we used human and mouse ALT ELISA kits (Elabscience Biotechnology Inc.) to evaluate D-galactosamine-induced hepatotoxicity in Hu-liver mice. We administered a single intraperitoneal dose of D-galactosamine (1,000 mg/kg) to Hu-liver and control TK-NOG mice and analyzed routine parameters (ALT activity) and ALT protein level in mice plasma. In all cases, ALT activities were higher in the Hu-liver mouse groups than in the control TK-NOG mouse groups. ALT activity was significantly higher in both Hu-liver (4.0-fold) and TK-NOG (4.8-fold) mouse groups than in the vehicle control groups at 1 d after D-galactosamine treatment. We had confirmed the species specificity of each ELISA kit in advance by conducting cross-validation studies. Human ALT protein plasma level was significantly higher (1.8-fold) in only the Hu-liver mouse group with D-galactosamine treatment. Conversely, mouse ALT protein plasma level was significantly higher in both Hu-liver (3.3-fold) and control TK-NOG (1.7-fold) mouse groups than in the vehicle control groups. These results suggest that D-galactosamine is more toxic to mouse hepatocytes than to human hepatocytes in Hu-liver mice. Thus, a combination of human and mouse ALT ELISA can be used to quantitatively evaluate human liver-specific toxicities in Hu-liver mice model.

The Effect of Antibiotics on Gut Microbiome: A Metagenomics Analysis of Microbial Shift and Antibiotic Resistance in Antibiotic-Treated Mice

L. Xu, A. Chockalingam, S. Stewart, Z. Li, and R. Rouse. US FDA, Silver Spring, MD.

There are long standing concerns about the use of oral antibiotics and their impact on the normal gut microbiota/microbiome and the emergence of antibiotic resistance. More recently, drug-microbiota/microbiome interactions have been identified that have impacted drug efficacy and/or safety through altered drug metabolism. This project characterizes the effect of different antibiotic treatment on the composition of the gut microbiota/microbiome and the prevalence of antibacterial resistance in the gut in the context of UTI treatment. This knowledge provides a foundation for stratifying the risk potential of different antibiotics and antibiotic combination therapies to negatively impact health through a microbiota/microbiome. Standard NGS procedures were used with bio-informatics to conduct metagenome analysis on fecal samples from mice experimentally infected by inoculation of E.coli into the urinary bladder. Three antibiotics (ampicillin, ciprofloxacin, fosfomycin) commonly used for UTI were administered and fecal samples collected to compare and contrast treatment influence on the gut microbiota and microbiome composition.
with carbon tetrachloride (CCl₄) twice weekly for 4 weeks (1 ml/kg, ip).

Female wild-type mice and homozygous Fib AEK mice

plasma levels of mutant fibrinogen insensitive to thrombin-mediated

that fibrin clots promote liver fibrosis. Therefore, the goal of this study

anticoagulant drugs that inhibit activation of the coagulation pro-

fibrin in hepatic fibrosis is indirect and based largely on studies using

chronic liver diseases that produce hepatic fibrosis. Intravascular fibrin

larvae, measuring gene expression changes at doses of equivalent tox-

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of zebrafish with null mutations in the gene encoding Nrf2a. Using these

droperoxide, cumene hydroperoxide, and R-(-)-carvone) is due to those

are complex and interdependent in verte -

The biochemical pathways that respond to oxidative stress, and protect

oxidative damage, are complex and interdependent in verte-

Individual exogenous chemicals can activate multiple pathways, and

response genes can be induced via multiple pathways.

High throughput projects like ToxCast and Tox21 aim to measure large

numbers of outputs for large numbers of chemicals, but these expo-

sures are done on cultured cells. Metabolism and other processes in verte-

ane animals can result in divergent responses to chemical ex-

early differentiation in cell culture. For a set of seven model industrial

compounds, we examine the comparative potency of each to induce

antioxidant and oxidative stress gene expression responses in zebrafish

of mut null mutations in the gene encoding Nrf2a. Using these

fish, we measure changes in both constitutive and induced expression of

antioxidant genes compared to their wild-type siblings, particularly in the most responsive genes in our panel (gsp and prdx1).

Finally, we measure the extent to which loss of the Nrf2a oxidant response pathway affects the toxicity of each chemical, and we find that measuring the change in gene expression induced by a chemical in a wild-type animal is a surprisingly imperfect indicator of the extent to which the chemical’s mechanism of toxicity involves oxidative stress of the sort that activates Nrf2a.

The biochemical pathways that respond to oxidative stress, and protect

against oxidative damage, are complex and interdependent in verte-

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99 autoimmune-related genes by more than 1.5-fold (p>0.05) in the 1, 5, 9, and 13 wk cohorts, respectively. Categories of upregulated genes included interferon-driven proteins, chemokines, cytokines, complement, and macrophage/lymphocyte activation markers. Diets containing low and high DHA concentrations blocked expression of 80-90 % and 100 %, respectively, of the autoimmune genes triggered by cSiO2. These results suggest that DHA supplementation at physiologically relevant doses might be useful in preventing cSiO2 triggering of lupus and other human AD. Supported by NIH grant ES027353, Lupus Foundation grant 362470, and the Dr. Robert and Carol Diebel Family Endowment.

**2647** Dysregulated WNT Signaling in PTSD Patients Correlates with Altered Epigenetic Marks and Elevated Inflammation

M. Bam, X. Yang, P. S. Nagarkatti, and M. Nagarkatti. University of South Carolina School of Medicine, Columbia, SC.

It is now known that PTSD patients exhibit chronic systemic inflammation characterized by upregulated expression of pro-inflammatory cytokines such as IFNγ and IL-12. However, the mechanism of regulation of these genes and the cells producing these cytokines is inadequately understood. In this context, WNT/β-catenin signaling pathway is critical for the proliferation, differentiation, polarization, and survival of mature T cells. We have reported that T cells (Th1 and Th17) are increased in number in the PBMCs of PTSD patients which also correlated with the increased expression of IFNγ. However, it is not yet reported which regulatory molecules trigger a pro-inflammatory cytokine response in monocytes associated with ROS production, providing insights into potential systemic and pulmonary toxicity, and tissue damage in e-cigarette users. Supported by the 1R01HL135613, 1R01HL085613 and 1R01HL085613-S1, FDA-CTP 1R01DA042470, and T32 Toxicology training grant #T32ES007026.

**2646** Immuno-Toxicalogical Response in Monocytes to E-Cigarette Flavor Chemicals and E-Liquids


E-cigarettes are used as an alternative to smoking conventional cigarettes. These are available in over 7,000 unique flavors. Flavorings used in e-cigarettes are often generally recognized as safe (GRAS) for ingestion. However, the inhalation exposure to e-cigarette containing flavoring chemicals are not known. **Rationale:** We focused our study on the immuno-toxicalogical effects by these flavoring chemicals and flavored e-liquids on monocytes. We hypothesized that the flavoring agents and the flavored e-liquids induce reactive oxygen species (ROS) production associated with a systemic pro-inflammatory response. Monocytic cells (Mono-mac6 and U937) were exposed to flavoring chemicals, diacetyl, cinnamaldehyde, 2, 3-pentanedione, o-vanillin, maltol, coumarin, and aceton at different doses of microM ranges (10 µM to 1000 µM). Cell viability and the concentrations of the secreted inflammatory chemokine IL-8 were measured in conditioned media after treatment with selected e-cigarette flavors (café latte, melon mania, and cinnamon roll) at various concentrations. Cell-free ROS levels produced by commonly used flavoring chemicals and selected flavors of e-liquids were measured using 2′, 7′-dichlorodihydrofluorescein diacetate as a probe, and the results were expressed as hydrogen peroxide equivalents. Treatment of the cells with flavor chemicals and e-liquids caused varying degrees of cellular toxicity. Cinnamaldehyde, o-vanillin, diacetyl, café latte and melon mania produced significantly increased levels of IL-8 in a dose-dependent manner compared to their unexposed counterparts. Similarly, e-liquids and the flavoring chemicals produced significantly increased ROS concentrations in a dose-dependent manner compared to controls. Pentanedione, o-vanillin and café latte produced the highest hydrogen peroxide equivalents. Our data suggest that the e-flavor liquid constituents trigger a pro-inflammatory cytokine response in monocytes associated with ROS production, providing insights into potential systemic and pulmonary toxicity, and tissue damage in e-cigarette users. Supported by the 1R01HL135613, 1R01HL085613 and 1R01HL085613-S1, FDA-CTP 1R01DA042470, and T32 Toxicology training grant #T32ES007026.

**2648** Voltage-Gated Potassium Channel Kv1.3 Contributes to Sustained Microglial Activation and Neuroinflammation in Neurotoxicity Models of Parkinson’s Disease

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Protein aggregation is one of the major pathological hallmarks of Parkinson’s disease (PD). Recently, our lab demonstrated that metal toxicity leads to enhanced protein aggregation. The aggregation of misfolded α-Synuclein has been shown to activate microglia, thereby initiating chronic neuroinflammation in PD models, but the molecular targets that drive sustained inflammation is equivocal. Identifying the cellular targets contributing to microglial activation could provide potential therapeutic targets to slow down chronic neurodegeneration. In this study, we observed a significant upregulation of Kv1.3 voltage-gated potassium channel, is specifically upregulated in aggregated αSyn fibril (aSynfib)-stimulated primary microglia. Patch-clamp electrophysiological studies confirmed that the observed Kv1.3 upregulation translates to increased Kv1.3 channel activity. Additionally, we found Kv1.3 channel expression to be in three different mouse models of PD, including MPTP, AAV αSyn and MitoPark transgenic mice. Importantly, Kv1.3 channel was also upregulated in human PD postmortem samples. Furthermore, Kv1.3 knockout microglia treated with aSynfib had lower production of pro-inflammatory cytokines, demonstrating the proinflammatory functional relevance of this channel. Moreover, the Kv1.3 G protein-coupled receptor (NOD) ligands. The SMI microtissues are cultured using human monocytic cells producing these cytokines is inadequately understood. In this context, WNT/β-catenin signaling pathway is critical for the proliferation, differentiation, polarization, and survival of mature T cells. We have reported that T cells (Th1 and Th17) are increased in number in the PBMCs of PTSD patients which also correlates with the increased expression of IFNγ. However, it is not yet reported which regulatory molecules trigger a pro-inflammatory cytokine response in monocytes associated with ROS production, providing insights into potential systemic and pulmonary toxicity, and tissue damage in e-cigarette users. Supported by the 1R01HL135613, 1R01HL085613 and 1R01HL085613-S1, FDA-CTP 1R01DA042470, and T32 Toxicology training grant #T32ES007026.

**2649** In Vitro 3D-Human Small Intestinal Tissue to Study Ligand-Induced Acute and Chronic Inflammation in the Gastrointestinal Tract

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The intestinal epithelium is known to be involved in innate immune responses by recognizing pathogenic entities such as pathogen-associated molecular patterns (PAMPs) that drive innate immune receptors (PRRs). Abnormal innate immune responses have been implicated in the pathogenesis of inflammatory bowel diseases (IBD). To investigate PRR responses on the intestinal mucosa, we exposed an in vitro 3D human small intestinal (SMI) microtissue (EpiStem, MatTek) model to various TLR ligands (TLRs) and a broad-spectrum receptor (NOD) ligands. The SMI microtissues are cultured using human intestinal fibroblasts and enterocytes and their 3-dimensional polarity and morphology mimics that of native in vivo tissues. Characterization of the microtissues included evaluation of structural features, barrier properties, and expression of drug transporters and drug metabolizing enzymes. Ligands to TLR4 (LPS) and NOD2 (Muramyl dipeptide: MDP) induced gene expression of proinflammatory cytokines such as IL-1β, IL-6, and RANTES in a synergistic manner. Prolonged exposure of intestinal microtissue to IL-1β resulted in reduced tissue membrane integrity, which may be a precursor for IBD-like disease, and led to further induction of pro-inflammatory cytokines and chemokine gene expression (IL-6 and CCL20), which are known to stimulate acquired immune cell responses including release of TNF-α and IFN-γ. To simulate the effect of dysregulation of the WNT/β-catenin signaling pathway in PTSD which might be the reason for the elevated inflammation. The study provides further evidence that inflammation in PTSD is epigenetically regulated and this pathway could be useful for PTSD diagnosis and combating the inflammation. This work was supported in part by National Institutes of Health grants R01AT003961, R01AT006888, R01AI123947, R01AI129788, R01MH004375, and P20GM103641.
of immune cell responses on the intestinal epithelium, we exposed the intestinal microtissue to TNFα and IFNγ, which resulted in the reduction of membrane integrity and the release of proinflammatory cytokines. The effect of TNFα and IFNγ on the intestinal epithelium was further exacerbated if antigen-presenting cells such as dendritic cells were incorporated into the 3D intestinal tissue model. In summary, the results suggest that the intestinal microtissues in ZIKV infected brains are capable of producing various innate immune responses and that it will likely prove to be a useful tool to study the complex interactions of human intestinal epithelium with microbiome in the induction of IBD-like disease.

Zika virus (ZIKV) is an emerging virus from the family Flaviviridae. ZIKV is transmitted to humans primarily by mosquito vectors and has been responsible for epidemics throughout tropical and subtropical areas. ZIKV infection has spread rapidly in the tropical Americas since its identification in Brazil in 2014 and infections have been associated with microcephaly in newborns. However, the molecular mechanisms underlying ZIKV pathogenicity and tissue tropism are still unclear. The viral Envelope (E) protein is critical for viral attachment and entry to the host cells by binding to cellular receptors and by intra-endosomal acid catalyzed fusion. By using Mass spectrometry analysis and communoprecipitation assays, we show that the ZIKV-E protein is ubiquitinated by TRIM7, a member of the E3-ubiquitin ligase Tripartite Motif (TRIM) family of proteins. Using recombinant infectious ZIKV bearing Lysine to Arginine mutations that lack ubiquitination on E, we show that ubiquitiniation of sE is important for virus replication in specific cell types in tissue culture and for pathogenicity in vivo. Our data suggest that ZIKV hijacks the host ubiquitin system to enhance its own replication, and identifies a new host factor that could represent a potential target for development of antiviral drugs against ZIKV infections.
Flavivirus infections are a cause of great concern throughout the world due to recurrent epidemics and high mortality rates, being a public health problem. The relevance of the Zika virus (ZIKV) infection study was highlighted by the large number of infants born with microcephaly, especially in the Brazilian Northeast, in addition, some adults exhibited cases of Guillain-Barré syndrome (GBS) cause by ZIKV. In May 2016, the relationship between ZIKV infection during pregnancy and microcephaly cases was proved by our group. It is known that ZIKV, like other flaviviruses, has the ability to modulate innate and adaptive immune response of the host. Thus, in this work we aim to evaluate the role of CD8+ T cells in controlling viral replication and disease progression in murine infection by ZIKV. ZIKV Brazilian strain was used to infect C57BL/6 and CD8 deficient mice, all analysis were performed at 1, 3, 5 and 7 days post infection by flow cytometry or PCR. Wild-typeC57BL/6 animals controlled infection, unlike animals deficient in CD8 T cells, in which on the seventh day after infection it is still possible to detect viral particles in the spleen. In fact, ZIKV infection induces an increase in the frequency of CD4+ and CD8+, accompanied by CD8+ Foxp3+ and CD4+ Foxp3+ cells. The absence of viral load in spleen of control mice may be due to increase of Ifn-β expression different on the spleen of CD8 T cells deficient animals. CD8 T cells could regulate Ifn-β expression which is one of the most important cytokines in controlling viral replication and T suppressors cells could be evolved in suppressing immune response against zika virus infection.

### 2654 The Role of CD8 Cells in Experimental Zika Virus Infection

N. Ghabdan Zanluqui, C. Manganeli Polonio, L. Gomes de Oliveira, C. Rossato, C. Longo de Freitas, and J. S. Schatzmann Peron. São Paulo University, São Paulo, Brazil.

Viral infections always have been serious illnesses causes that have repercussions on morbidity and mortality increasing rates worldwide. Recently, a flavivirus transmitted by the vector Aedes aegypti, called Zika virus (ZIKV) was introduced in South America, causing a major public health problem. On February 1, 2016, the World Health Organization (WHO) declared a state of global concern after the alarming increase in the number of babies born with microcephaly and adults with Guillain-Barre syndrome in our country, both related to the infection. Genetic differences, mainly related to Interferons (IFN), may greatly influence the susceptibility to infection. The IFN are classified in general in two types: Type I IFN is a major cytokine involved in antiviral response and includes IFN-α and IFN-β, which can be secreted by all the nucleated cells during virus-triggered response, participating in apoptosis cell death induction of infected cells. On the other hand, Type II IFN (also known as IFN-y) is produced only by T cells and NK cells. It's known that during DENV infection, another flavivirus, IFN-γ-dependent mechanisms were associated with DENV resistance. Thus, in this project, we evaluated the role of IFN-y in experimental ZIKV infection. We infected the C57BL/6 WT and IFN-γ-/- mice with 1x10^5 PFU intravenously and the analysis was done 1 and 3 days post infection on spleen. Our results demonstrated an important role for IFN-γ in combating ZIKV infection, since IFN-γ-/- mice showed highest number of viral copies at spleen 1 and 3 days post infection compared to WT mice. In addition, we observed an increase in type I IFN cytokines production on the first day of infection in the WT mice, followed by a decrease, unlike the deficient animals, which production peaked on the third day. Consequently, the decrease was observed in the IRF7 and IRF9 transcription factors induction and in the interferon-induced antiviral genes (ISGs) expression, such MX1, ISG15 and IFITM3. Taken together, these data demonstrate the importance of the IFN-γ cytokine in the assembly of the antiviral response against ZIKV, once the expression of factors responsible for this response are produced later.

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### 2655 Evaluation of the Role of Interferon-y in Zika Virus Infection

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Zika virus (ZIKV) emerged as a global health problem and demands efforts of the scientific community to understand the underlying mechanisms involved in the host cell infection. ZIKV is part of the flavivirus genus, such as dengue virus, whose cellular invasion mechanism has been elucidated in the literature. It is clear the involvement of Axl receptor Tyro3 and Mer (TAM Family) during infection of dengue virus, a phosphatidylserine-dependent endocytosis mechanism. Studies conducted by our group showed a possible link between increased expression of TAM receptors and the infection of ZIKV, as well as the susceptibility of SJL mouse strain to ZIKV infection. This study aims to evaluate the role of TAM receptors in the Zika virus infection in SJL and C57BL/6 mice lineages. Mice were infected with 10^3 pfu of ZIKV intravenously and the organs were collected after 1 and 3 dpi. SJL strain expressed high amounts of Zikv virus RNA in the spleen compared to C57BL/6 mice. The presence of Zika virus infected Axl expression in SJL mouse at day 1, and when BGB324 (Axl blocker) was used the viral load decreased. From these data we can conclude that the treatment with Axl blocker decreases the viral load showing that Axl is one of the possible receptors that allows ZIKV infection. Furthermore, there was a difference between spleen viral load of SJL and C57BL/6 mice and also the expression of Axl receptor.

### 2656 The Role of Axl During Infection by Zika Virus in SJL and C57BL/6 Mice

L. G. Oliveira, N. G. Zanluqui, C. M. Polonio, C. L. Freitas, and J. S. Peron. University of São Paulo, São Paulo, Brazil.

Zika virus (ZIKV) is a flavivirus that causes a global health problem. The virus is transmitted by the mosquito Aedes aegypti and can cause severe diseases such as dengue fever, Zika fever, and microcephaly. Axl is a receptor that has been shown to play a role in the infection of dengue virus, which is part of the flavivirus genus. A recent study investigated the role of Axl during Zika virus infection in SJL and C57BL/6 mice. Mice were infected with Zika virus intravenously and the organs were collected after 1 and 3 days post-infection. SJL mice expressed high amounts of Zika virus RNA in the spleen compared to C57BL/6 mice. The presence of Zika virus infected Axl expression in SJL mice at day 1, and when BGB324 (Axl blocker) was used the viral load decreased. From these data we can conclude that the treatment with Axl blocker decreases the viral load showing that Axl is one of the possible receptors that allows ZIKV infection. Furthermore, there was a difference between spleen viral load of SJL and C57BL/6 mice and also the expression of Axl receptor.

Financial Supported by FAPESP (2011/18703-2 and 2017/11828-0).

### 2657 Regulatory Aspects of the Nonclinical Safety Evaluation of Zika Vaccines

C. Wrzesinski, and M. Green. US FDA, Silver Spring, MD.

FDA’s primary object in reviewing First in Human studies is to assure the safety of enrolled patients or healthy volunteers and stringent non-clinical evaluation of a new vaccine before testing in humans is a critical factor in the evaluation of safety. However, in the case of a potential pandemic outbreak a fast and efficient approach is needed to expedite the development of a safe and effective vaccine, particularly in areas of medical need without countermeasures. In the case of developing a vaccine for the Zika virus, a flexible approach was adopted that focused on assessing relevant new and available toxicologic information. Several approaches were adopted and included new toxicology studies, consideration of prior non-clinical and clinical experience with highly similar products, as well as focused toxicology studies. Additionally, toxicology studies including those evaluating potential reproductive developmental toxicities of the vaccine were considered as part of the future product development. For such vaccines developed for maternal immunization, reproductive toxicology studies need to be performed prior to starting clinical trials in pregnant women.

### 2658 Oral Exposure to 2-[1-Methyl-2-(4-phenoxyphenoxy)ethoxy]pyridine (MPEP) for 28 Days Alters Antibody Responses in Harlan Sprague-Dawley Rats

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2-[1-Methyl-2-(4-phenoxyphenoxy)ethoxy]pyridine (pyriproxyfen; MPEP) is a registered insecticide that exerts effects as a juvenile hormone analog and inhibits larval growth and development thereby preventing emergence of adult insects. MPEP is marketed for control of mosquito populations that are major vectors of infectious diseases including Zika virus. Due to the limited data available, this study was performed to determine the impact of MPEP exposure on the immune response in adult Harlan Sprague-Dawley (HSD) rats. Innate, humoral, and cell-mediated immune responses were assessed following 28 days of oral MPEP exposure at doses of 0, 62.5, 125, 250, and 500 mg/kg. Parameters evaluated included measurement of antibody production and cell-mediated immune responses. Antibody responses were measured in rats treated with ≥ 250 mg/kg MPEP, and a significant decrease in the anti-KLH IgM response was observed within the same rats following MPEP exposure. Minimal changes were observed in other immune endpoints. Neutralizing antibody responses influence the susceptibility of MPEP-exposed HSD rats to influenza virus infection and viral clearance. This work was supported by NIH contract HHSN272201400017C.
Synergistic Role of Nanoceria on the Ability of Tobacco Smoke to Induce Carcinogenic Hallmarks in Lung Epithelial Cells

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Ceferium oxide nanoparticles (nanoceria) have been proposed as a new promising agent in the treatment of oxidant diseases, including cancer, due to its known antioxidant properties. However, several biosafety concerns need to be solved before this nanomaterial can be applied in biomedical applications with security, as, i.e., there are no available data on the associated toxicological effects under realistic long-term exposure and co-exposure scenarios. This work aim to evaluate the transforming effects of long-term exposure to nanoceria in lung epithelial BEAS-2B cells, along with the effects associated to a common plausible tobacco co-exposure. Thus, BEAS-2B cells continuously exposed to 2.5 µg/mL of nanoceria alone or in combination with 1 and 5 µg/mL of tobacco smoke condensate (CSC) for up to 6 weeks were monitored for intrinsic and extrinsic changes associated with the acquisition of an oncogenic phenotype. Alterations in cellular morphology, growth and differentiation status were measured through the exposure, matrix metalloproteinase (MMP) activities were measured by zymography, colony formation and promotion were measured by soft agar assay, and cellular migration capacity was evaluated by wound healing assay. Results evidence no transforming ability of nanoceria in exposed BEAS-2B cells. However, results support a synergistic role of nanoceria on CSC transforming ability, as cells co-exposed to nanoceria plus CSC, when compared to cells exposed to CSC alone, showed a more noticeable spindle-like phenotype, an increased proliferation rate, higher degree of differentiation status dysregulation, higher migration capacity, increased anchorage-independent cell growth and a secretome with higher levels of MMP 9 and cell growth promoting capability. When mRNA expression of FRA-1 was evaluated as a mechanism of tobacco-induced transformation, nanoceria co-exposure was again found to exacerbate the observed expression changes. Our results point out the need for studying nanomaterials mixed exposure scenarios likely to be found in the environment and/or at working place.

Prothrombotic Risk of Silver Nanoparticles Mediated by Procoagulant Activation of Red Blood Cells

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Silver nanoparticles (AgNPs) have been estimated to present possible toxicity during transport, uptake and degradation as drug carriers in drug delivery system and cancer therapy. High concentration of AgNPs are exposed to circulating blood cells if taken intravenously. As the most abundant blood cells in human body, the potential risk of AgNPs on red blood cells (RBCs) could not be ignored. As well as platelet, RBCs are also included in the process of procoagulant activity and thrombosis. Here, we examined AgNPs (50-100 µg/mL) significantly exaggerated procoagulant activity via phosphatidylserine (PS) exposure, which is externalized from inner to outer membrane by key enzymes regulating membrane phospholipid asymmetry. Increase of intracellular calcium and generation of reactive oxygen species (ROS) due to AgNPs-induced oxidative stress were detected and further confirmed by chelation of calcium and application of antioxidants, respectively. Additionally, intravenous injection of AgNPs (10-50 mg/kg) significantly increased thrombosis via PS-exposing RBCs. As AgNPs are applied for cancer therapy, we evaluated the risk of thrombotic events through freshly RBCs isolated from cancer patients and found the prothrombotic effects induced by AgNPs were increased. Therefore, the intravenous AgNPs cancer therapy requires to be carefully concerned.

Functionnalization of Multiwalled Carbon Nanotubes Alters the Pro-inflammatory and Fibrogenic Response in Human Pulmonary Cells In Vitro and in Mice following Pulmonary Exposure


Multiwalled carbon nanotube (MWCNT) inhalation exposure is a potential risk to human health due to their escalating production and use in consumer products. In order to guarantee their safety, the toxicity properties and mechanisms of MWCNTs must be identified. Functionalization of MWCNTs has been shown to either decrease or increase pulmonary injury depending on the type of functionalization. The International Collaboration on Nanotube Safety (ICONS) is a USA/Europe research program funded to study the potential risks of a panel of functionalized nanotubes manufactured by the company Nanocyl. The panel of MWCNTs included the original manufactured MWCNTs as well as a grinded version and ones that have been chemically or thermally purified. The purified MWCNTs were then functionalized with carboxyl or amine groups. In order to test their inflammatory and fibrogenic potential in vitro, we measured mRNA expression in a panel of pro-inflammatory and fibrogenic mediators in human macrophages (THP-1), bronchial epithelial cells (BEAS-2B) and fibroblasts (MRC-5) 24h after exposure to 8 varieties of Nanocyl MWCNTs. To test this in vivo, male C57BL6 mice were dosed via oropharyngeal aspiration with either 1.6 or 4 mg/kg of 4 varieties of Nanocyl MWCNTs with Mitsui-7 MWCNTs used as a positive control. Necropsy was performed 3 days post-exposure and bronchoalveolar lavage fluid (BALF) and lungs were collected for analysis of inflammation and fibrosis. After MWCNT exposure to all cell types, the two carboxylated MWCNTs were determined to be the most fibrogenic. The greatest inflammatory were the ground up MWCNTs and the chemically purified, aminated MWCNTs. Exposure to mice resulted in increased numbers of neutrophils and eosinophils in the BALF. We found the aminated MWCNTs having the strongest effect. The aminated MWCNTs also resulted in increased levels of pro-fibrogenic mediators in the BALF. Based on our findings, the MWCNTs with the least fibrogenic potential are the unaltered MWCNTs and the purified MWCNTs with no functionalization. The least inflammatory MWCNT is the chemically purified MWCNTs with no functionalization. Based on the results in mice, the chemically purified/aminated MWCNTs are the most inflammatory and have the highest fibrogenic potential. Funded by: NSF 15-022.

Functionalization of Multiwalled Carbon Nanotubes Alters the Pro-inflammatory and Fibrogenic Response in Human Pulmonary Cells In Vitro and in Mice following Pulmonary Exposure


Because of the increased use of engineered nano-materials (ENM), it is important to develop a better understanding of ENM bioactivity and their potential for causing inflammation and disease. Some ENM have been reported to be bioactive and to trigger a pro-inflammatory response when inhaled. Alveolar macrophages have been demonstrated to be responsible for this inflammatory response due to their release of the cytokine, IL-1β. A key step preceding and linked to IL-1β release is phagolysosomal protein permeability (LMP). Some ENM have been implicated in inducing LMP in alveolar macrophages, which in turn results in release of cathepsin B from the phagolysosome. While, LMP has been implicated in this inflammatory pathway, the mechanisms of ENM-induced LMP remain unclear. It has been reported that ENM can have interactions with lipid membranes, therefore it is hypothesized that LMP is generated by a disruption of lipid packing in the membrane, caused by the ENM interacting with membrane lipids. The cell models used in this work were bone marrow derived macrophages (BMDM) and human red blood cells (RBC). BMDM from C57BL/6 mice were selected as a model for seamed macrophages. These cells were treated with 25-50µg/mL of crystalline silica (SiO2), a material reported to cause inflammation, or titanium dioxide (TiO2) for 16 hours and cytokines were measured, as a measure of LMP. Both SiO2 and TiO2 produced significantly increased cathepsin activity with SiO2 greater than TiO2. RBC were used as a simplified surrogate for internal cellular membranes, such as the phagolysosome. RBCs were exposed to SiO2 or TiO2 ENM for four hours at doses ranging from 25-200µg/mL. Subsequently, the RBC membranes were assayed for permeability and changes to lipid packing by a hemolysis assay and fluorescence lifetime imaging microscopy (FLIM), respectively. A significant increase
in hemolysis in response to SiO2 and TiO2. FLM results indicated increases in lipid packing around the fluorescence probe Di-4ANNEDPHQ, as a result of SiO2 and TiO2 exposure. These results suggest SiO2 and TiO2 generate LMP by inhibiting natural lipid mobility that can potentially induce membrane permeability.

**2663 Cell Cycle Alterations after In Vitro Exposure to Pegylated Gold Particles in Cancerous vs. Non-Cancerous Lung Cells**

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In the past few years engineered metal and polymer based nanoparticles have seen an increase in use in the pharmaceutical industry as drug delivery carriers (DDC) used for cancer therapy. The increased vasculature, altered morphology, and metabolism of cancer cells allow nanoparticles to be specifically engineered to preferentially penetrate these tissues over non-cancerous cells. PEGylated gold nanoparticles are highly utilized as an excitant (i.e. DDC, the non-active ingredient) due to their increased stability over polymer based DDCs while remaining bio-inert (i.e. does not react within the body). However, as the rate of administration of therapeutics containing gold nanoparticles increases, there is a need to characterize the interactions of the excitant with different biological test systems. To identify how the gold nanoparticles interact within the lungs, a pair of cancerous and non-cancerous cell lines from the bronchus (AS49 & BEAS-2B) and pleural space (Calu-3 & Met-5A) were examined for uptake of 20nm PEGylated gold nanoparticles and nanoparticle-induced cell cycle changes. Baseline gene and protein expression of Cyclin dependent kinases (CDKs) and CDK inhibitor p21, along with normal cell morphology were examined prior to exposure. Dose-responsive changes to the cell cycle were shown regarding changes in cell viability, proliferation, morphology, DNA integrity, and perturbed expression of CDKs and CDK inhibitors. While all cell lines exhibited changes in gene/protein expression, the two cancerous cell lines and pleural space cells were more susceptible to alterations in their normal cell cycle.

**2664 Screening In Vitro Toxicity Endpoints of Carbon Nanotubes and Nanofibers from United States Facilities**


nanotubes and nanofibers (CNT/F) to advance material science applications. Unfortunately, those same characteristics that provide significant potential for application may also confer adverse health effects. CNT/F represent a broad class of materials and we hypothesized that not all CNT/F produced or utilized in US facilities confer similar toxicities. Extensive characterization was done for seven different multiwalled CNT and two CNF that were selected based on primary particle diameter ranging from 10 to 150 nm. The specific surface areas ranged from 18 to 238 m2/g and decreased with increasing primary particle diameter. Key molecular-initiating events (MIE) and functional responses were screened over a wide dose range (0.03-60 µg/mL) in a human monocytic cell line (THP-1), both wild-type and NLRP3 inflammasome deficient cells, and in primary human lung fibroblast cells (PHF). Membrane damage and cell proliferation in THP-1 challenged with CNT/F for 24 h segregated by diameter with materials greater than or equal to 50 nm in diameter inducing greater toxicity. There was ~150 fold change in IL-1β secreted in THP-1 WT vs NLRP3 deficient cells and for all the CNT/F tested, apart from having a dose-dependent increase, the level of secretions was enhanced with increasing primary particle diameter. Similarly, CNT/F exposure (0.3 µg/cm2) to PHFs elicited increased cell proliferation and collagen I production that was greater with increased primary particle diameter. Structure activity correlations to date indicate that CNT/F could be broadly classified into two groups, materials below 50 nm in primary particle diameter were less bioactive/toxic than tubules greater than 50 nm. Ongoing research and modeling will further elucidate relationships between physicochemical characteristics and toxicity profile of various CNT/F.

**2665 Cytotoxicity of Applied Nanoclusters for Cellular Imaging**

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Nanoclusters (NCs) are recognized for their unique optical and biodispersion properties. NCs possess possible applications in tissue imaging and treatment strategies. However, the toxicity and optical properties of these compounds have yet to be evaluated in human cells. Recently a human microglial cell line was employed to investigate if noncancerous human neuronal cells can synthesize NCs and if pre-synthesized gold (Au) and iron (Fe) NCs result in cell stress and death. Cells were either treated with chloroauric acid (HAuCl4) or Au and Fe NCs for up to 72 hrs. Exposure to HAUCL4 or Fe NCs resulted in a dose-dependent loss of cell viability within the first 8hrs of exposure, with a continuing loss through 72 hrs. High HAUCL4 exposure lead to significant increased reactive oxygen species (ROS) within 24 hrs following exposure, which continued through the exposure study. Similarly, Fe NCs resulted in ROS within two hours after exposure, signal was found to rise through the exposure time. Interestingly, Au NCs did not produce a notable increase in ROS during the first 5 hours, however; once induced, ROS signal plateaued after another 24hrs. Fluorescent imaging revealed increased cell fluorescence following exposure to all compounds, with fluorescence seen in the cytoplasm during HAUCL4 and Au NC exposures; and dispersed throughout the cell during Fe NC exposure. Transmission electron microscopy confirmed microsome uptake of all compounds, and suggest localization within cellular vesicles. Inductively coupled plasma-mass spectrometry also indicated increased uptake of materials in a temporal and concentration-dependent manner. Taken together these results highlight i) the capacity for human neuronal cells to self-synthesize NCs following HAUCL4 treatment, ii) the bioavailability and cytotoxicity of pre-synthesized nanocluster, iii) the ability for human cells to uptake pre-synthesized NCs, and iv) the resulting increased intracellular fluorescence from all these material treatments.

**2666 Differentiation State of Airway Epithelial Organotypic Culture Models Is a Potential Mediator of Silver Nanoparticle-Induced Toxicity**


Engineered nanomaterials, including silver nanoparticles (AgNP), are one of the largest groups of emerging toxicants. They are used in hundreds of consumer products due to their antimicrobial properties, and have a potential to aerosolize through the manufacturing and usage of these products. AgNP are respiratory toxicants with potential to cause allergic airway inflammation; however, the genetic, environmental, and temporal factors that may mediate their toxicity have yet to be fully elucidated. This study has developed airway epithelium organotypic culture models (AE-OCM) as a platform to identify these potential mediators and use them to inform Adverse Outcome Pathways (AOP) for allergic airway diseases, such as asthma. We are quantifying gene × environment × time interactions (G×E×T) using AE-OCM derived from two inbred founder strains of Collaborative Cross mice (AJ and C57BL/6J), differentiated under two conditions (~ IL-13 and ~ IL-13; 25 ng/mL), and treated with AgNP (12.5, 25, and 50 µg/mL) at two time points—a four-hour repeated exposure over five days (5×4 hours), and 24 hours. Endpoints of interest include: changes in epithelial barrier function, cytotoxicity, and enrichment of gene ontologies for pathways associated with allergic airway inflammation. In AE-OCM + IL-13, we found significant reductions in epithelial barrier function, as measured by transepithelial electrical resistance (TEER; ohm×cm2), after AgNP treatment (25 and 50 µg/mL) at 5×4 and 24 hours compared to untreated control at day in vitro 24. We also found significant increases in cytotoxicity, as measured by LDH Release (%), after AgNP treatment (12.5, 25, and 50 µg/mL) at 5×4 and 24 hours compared to untreated control. We found few significant differences in cytotoxicity across strains, with A/J showing increased susceptibility compared to C57BL/6J. Compared to preliminary data for AE-OCM, AE-OCM + IL-13 shows reduced epithelial barrier function at baseline, and increased cytotoxicity after AgNP treatment, suggesting that differentiation state may mediate AgNP-induced toxicity.
Iron oxide nanoparticles (IONPs) are emerging as unique components of drug delivery systems, imaging techniques, environmental catalysts, components of thermoplastics, and more. Workers in IONP manufacturing facilities are known to be exposed to low doses of these particles over long periods of time. However, few studies have assessed potential adverse outcomes following this type of occupationally relevant exposure. Our previous research suggests that IONPs may induce a neoplastic-like cellular transformation, likely due to particle dissolution, release of free iron ions, and disruption of iron homeostasis. Other studies suggest that an amorphous silica coating may reduce particle dissolution, thereby reducing subsequent adverse outcomes.

We hypothesized that an amorphous silica coating (SiO2-nFe2O3) would prevent subsequent neoplastic-like cellular transformation and oxidative stress. To test this hypothesis, we used an occupationally relevant low dose of 0.02 mg/mL, while the LDH assay showed that significant cell death did not occur until the fifth day of exposure. The MTT assay indicated an IC50 of 0.02 mg/mL, while the LDH assay showed a significant (p<0.0001) reduction of cells in cultures treated with 400 μg/ml Er2O3 NPs when compared to controls. Caspase 3 and 8 levels were increased significantly (p<0.005) in cell cultures treated with 400 μg/ml Er2O3 NPs when compared to controls. Caspase 8 and 9 levels were determined to indicate a possible pathway for initiation of apoptosis. In the present study the toxicity of Er2O3 NPs was assessed under conditions.

The increased number of applications of engineered nanomaterials (ENMs) greatly increases the potential of direct human exposure to ENMs. However, the number of new ENMs presents a challenge in risk assessment. Grouping and ranking according to their potential hazard seems a promising approach. Here we tested a panel of nineteen ENMs procured from the Joint Research Centre (JRC) and commercial sources focusing on cytotoxicity and cytokine responses in the human macrophage-differentiated cell line THP-1. Macrophages are key players of the innate immune system. Physicochemical characterization of ENMs was performed using dynamic light scattering; moreover, all ENMs samples were shown to be endotoxin-free prior to testing. Following cytotoxicity screening in THP-1 cells using the Alamar Blue cell viability assay and ranking on the basis of IC50 values, the multi-walled carbon nanotubes (MWNTs), ZnO, Ag, and SiO2 NMs were found to be the most cytotoxic single-walled carbon nanotubes (SWCNTs), TiO2, BaSO4, and CeO2 NMs, as well as the nanocellulose materials, were non-cytotoxic (at doses up to 100 μg/mL). Profiling of cytokine and chemokine secretion using a multi-plex assay indicated that the TiO2, SiO2, BaSO4, CeO2, and nanocellulose materials induced potent inflammatory responses in THP-1 cells. Hierarchical clustering of cytokine responses coupled with gene expression analysis demonstrated that the panel of ENMs could be segregated into two distinct groups characterized by activation and deactivation, respectively, of PPAR (peroxisome proliferator-activated receptor) and LXR (liver X receptor/retinoid X receptor) nuclear receptor pathways. Both nuclear receptors are well-known for modulating inflammatory responses. Furthermore, using a PPAR-γ antagonist, we could show that PPAR-γ played an important role in the activation of inflammatory responses in THP-1 cells exposed to TiO2 and SiO2 NMs. These studies have shown that ENMs of different chemical composition can be grouped according to their inflammatory potential when tested under in vitro conditions.
Molybdenum trioxide nanoparticles (MoO₃ NPs) are used as: an additive in paint, an ingredient in glass and ceramics, a coating in nanowires and plastics, and in the production of metal alloys. Nanoparticles may be produced as a bi-product of copper and tungsten mining and also as a consequence of industrial applications. MoO₃ NPs use in industrial settings raises the concern for possible adverse health effects as a result of occupational exposure. The purpose of this study was to investigate the toxicity of MoO₃ NPs in rat pleural mesothelial cells (RPMCs; CCL-16; ATCC). RPMCs were cultured in Ham’s F-12 Medium supplemented with 10% fetal bovine serum, L-glutamine, and penicillin-streptomycin and grown in 5% carbon dioxide at 37°C. Cultures were divided into two groups: a control group, exposed to vehicle alone, and an experimental group that was exposed to 400 µg/mL MoO₃ NPs for 24 hours. TUNEL positive cells were evaluated at X400 and Giemsa staining. Toxicity was assessed by the MTT assay, TUNEL assay, and ROS fluorescence. Caspase 3 and 9 levels showed an increasing trend in control and treated cultures. Cultures treated with increasing concentrations of MoO₃ NPs had a decrease in cell numbers from 13% to 100 µg/mL exposures to 73% for cultures exposed to 800 µg/mL as compared to controls. A concentration of 400 µg/mL was selected for further testing as this concentration gave approximately 66% survival rate. Treated cells exhibited surface membrane blebbing with MoO₃ NPs apparent on the cell membrane surface. TUNEL positive cells were analyzed at X400 and positive apoptotic cells were counted in 20 random fields. Micrographs from cultures of RPMC’s treated with 400 µg/mL MoO₃ NPs for 24hrs. had a significant (p < 0.05) increase, 48%, in TUNEL-positive cells as compared to the control. An increase in ROS fluorescence was seen in micrographs in RPMCs treated with MoO₃ NPs when compared to the control. Caspase 3 and 9 levels showed an increasing trend in cultures treated with 400 µg/mL MoO₃ NPs. Results from this study indicate that exposure of RPMCs to 400 µg/mL MoO₃ NPs cause cytotoxicity as indicated by decreased cell viability. Increases in TUNEL positive cells and production of ROS with increasing levels of caspases suggest that MoO₃ NPs induced toxicity may be mediated through an apoptotic pathway.


2671 Toxicity of Molybdenum (VI) Oxide Nanoparticles in Rat Pleural Mesothelial Cells-CCL 216

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Molybdenum trioxide nanoparticles (MoO₃ NPs) are used as: an additive in paint, an ingredient in glass and ceramics, a coating in nanowires and plastics, and in the production of metal alloys. Nanoparticles may be produced as a bi-product of copper and tungsten mining and also as a consequence of industrial applications. MoO₃ NPs use in industrial settings raises the concern for possible adverse health effects as a result of occupational exposure. The purpose of this study was to investigate the toxicity of MoO₃ NPs in rat pleural mesothelial cells (RPMCs; CCL-16; ATCC). RPMCs were cultured in Ham’s F-12 Medium supplemented with 10% fetal bovine serum, L-glutamine, and penicillin-streptomycin and grown in 5% carbon dioxide at 37°C. Cultures were divided into two groups: a control group, exposed to vehicle alone, and an experimental group that was exposed to 400 µg/mL MoO₃ NPs for 24 hours. TUNEL positive cells were evaluated at X400 and Giemsa staining. Toxicity was assessed by the MTT assay, TUNEL assay, and ROS fluorescence. Caspase 3 and 9 levels showed an increasing trend in control and treated cultures. Cultures treated with increasing concentrations of MoO₃ NPs had a decrease in cell numbers from 13% to 100 µg/mL exposures to 73% for cultures exposed to 800 µg/mL as compared to controls. A concentration of 400 µg/mL was selected for further testing as this concentration gave approximately 66% survival rate. Treated cells exhibited surface membrane blebbing with MoO₃ NPs apparent on the cell membrane surface. TUNEL positive cells were counted in 20 random fields. Micrographs from cultures of RPMC’s treated with 400 µg/mL MoO₃ NPs for 24hrs. had a significant (p < 0.05) increase, 48%, in TUNEL-positive cells as compared to the control. An increase in ROS fluorescence was seen in micrographs in RPMCs treated with MoO₃ NPs when compared to the control. Caspase 3 and 9 levels showed an increasing trend in cultures treated with 400 µg/mL MoO₃ NPs. Results from this study indicate that exposure of RPMCs to 400 µg/mL MoO₃ NPs cause cytotoxicity as indicated by decreased cell viability. Increases in TUNEL positive cells and production of ROS with increasing levels of caspases suggest that MoO₃ NPs induced toxicity may be mediated through an apoptotic pathway.


2672 Nanotoxicity Evaluation of Doped Silicon Nanocrystals

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Recent discoveries of high yield synthesis by Kortshagen et al. producing doped silicon nanocrystals (DSNCs) via nonthermal plasma reactor have sparked great research interest. DSNCs are semiconducting materials that enable various applications from biosensing, bioimaging, optoelectronic, nanoelectronics, spectroscopic and chemical sensing to subwavelength microchips. This versatile semiconductor material has yet to interact with the environment where living organisms are found, so the environmental implications remain unknown. Hence, the proposed research entails the exploration of evaluating the nanotoxicity of DSNCs in model organisms, Shewanella oneidensis MR-1 (S.oneidensis). The drop plate assay was the method used to assess the toxicity of DSNCs. TEM, XPS, and hyperspectral imaging were used to characterize DSNCs. After experimentation, results regarding the potential toxicity of DSNC were inconclusive.


2673 Topography-Driven Toxicity in Response to Nano-Aluminum and Iron Oxide Exposures

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Substrate effects play a profound role in regulating biological outcomes. Topography can drive cell shape, organelle positioning, cytoskeletal structure, and function. Extracellular matrix (ECM) is secreted by the cell into a highly organized and fibrous biomaterial to maintain normal cell function and organ operations. Using photolithography, ridges of 10, 20, and 40 µm widths, and via electropinning, biomimetic random and aligned nanofiber (R-NF, A-NF) substrates, were produced to simulate physiologically relevant topography. A549 alveolar type-II ‘like’ cells were cultured, and toxicity was assessed in response to exposures of 10 nm aluminum, 30 nm iron oxide, and a 1:1 mixture of the two nanoparticle powders as compared to tissue culture polyethylene (TCPs). A multiplex flow cytometry Nexin reagent was utilized to assess viability, apoptosis, and necrosis after 8, 24, or 48 h following exposures of 0, 1, 5, 10, 20, and 100 µg/mL concentrations. Reactive oxygen species (ROS) was assessed after 48 h using a fluorescent probe, IL-8 secretion was measured by ELISA, and confocal imaging was conducted to evaluate cell shape, mitochondrial potential, and stress fiber formation. A549 cells displayed elongated extensions in multiple directions, while stress fibers were centrally localize on R-NF, 40 and 10 micron width ridges. Aligned nanofiber and 20 micron width ridges resulted in 42% more toxicity, five-fold more apoptotic cell death, and increased ROS production compared to R-NF, TCP, or the 40 or 10 micron width ridges. Confocal results indicated that cells on the 10 micron ridges were bridging multiple ridges resulting in R-NF morphologies. Ridges equal to the size of the cell diameter (14.5 microns), displayed A-NF cell morphologies indicating the cell’s ability to sense the underlying topography. In all cases, cell response deviates from TCPs, with aligned morphologies significantly altering viability and apoptosis. These results suggests future in vitro analysis should take into consideration substrate design to more accurately simulate, assess, and predict in vitro adherent cell toxicity. Distribution A. Approved for public release; distribution unlimited.


2674 Studies on Mitochondrial Dynamics under the Influence of Silver Nanoparticles Based on Surface Properties

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Silver nanoparticles (AgNPs) are increasingly used in products due to silver’s antimicrobial properties. Consumer use of such products can cause AgNP aerosolization and inhalation, potentially causing toxic effects. Nanoparticle (NP) toxicity is dependent on size, coating, shape, and charge. AgNPs increase reactive oxygen species (ROS) within cells and can trigger changes in mitochondrial membrane potential, critical for ATP formation and normal cell function. The mechanism behind AgNPs’ effect on mitochondrial structure and function remains unclear. As an inhalation model, we treated A549, an alveolar II-like cell line, with 50 nm AgNPs coated with citrate, polyvinylpyrrolidone (PVP), or branched polyethyleneimine (BPEI). The cells were evaluated using fluorescence microscopy and flow cytometry to investigate the impact of NP exposure on mitochondrial structural dynamics. To assess structural integrity impacting mitochondrial function, alterations of mitochondrial structural dynamics were correlated with elongation of individual mitochondria, changes in the interconnectivity of the mitochondrial network, and loss in mitochondrial membrane potential. The results demonstrated that the coated AgNPs increased the amount of mitochondon elongation compared to untreated cells. BPEI-coated AgNPs caused the most significant elongation at equivalent concentrations compared to the other NPs significantly and the interconnectivity of the mitochondrial network within the cell compared to untreated cells at concentrations below 50 µg/mL. Cells treated with citrate- and PVP-coated AgNPs showed less cell death and damage to mitochondrial membrane potential compared to naive cells, though the results were not statistically significant. Citrate-coated AgNPs caused significantly more damage to cells than PVP at higher concentrations. BPEI-coated AgNPs caused a dose-dependent loss in mitochondrial membrane potential and cell death when compared to naive cells. In summary, surface properties appear to impact mitochondrial structure, suggesting a possible mechanism for AgNP toxicity that could lead to new avenues of toxicological analysis.


2675 Ammonium-Functionalized Gold Nanoparticles Elicit Mitochondrial Dysfunction and Cell Death: A Transcriptomics and Proteomics Study Coupled with Functional Validation

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Systems biology is increasingly being applied in the field of nanosafety research for observing and predicting the biological perturbations inflicted by exposure to engineered nanomaterials. Here we used a combined transcriptomics and proteomics approach to assess the effects of Au nanoparticles (Au-NPs) of two different sizes (5 or 20 nm) on human
monocyte-like THP-1 cells. The role of surface functional groups was addressed by synthesizing ammonium-, carboxyl- or poly(ethylene glycol) (PEG)-modified Au-NPs. The Au-NPs were characterized using TEM, UV-Vis spectroscopy, DLS, and zeta potential measurements. We ensured that the NPs were endotoxin-free. Then, cytotoxicity screening was performed with THP-1 cells exposed to Au-NPs at doses up to 100 μg/ml using the Alamar Blue assay. Cell death was observed only for the ammonium-modified Au-NPs. Using TEM, the ammonium-modified NPs were found to be partly located in mitochondria. Next, we performed RNA sequencing and mass spectrometry-based proteomics analyses followed by pathway enrichment analysis. The importance of the surface modification, rather than primary particle size, was clearly demonstrated. Notably, the combined omics results suggested that mitochondrial function was deregulated in THP-1 cells upon exposure to ammonium-modified Au-NPs and subsequent experiments showed a pronounced drop in mitochondrial membrane potential and mitochondrial reactive oxygen species production in response to these Au-NPs. We also found that ammonium-modified, but not carboxyl-modified Au-NPs triggered cell death in *C. elegans*. These studies have disclosed cytotoxic effects of Au-NPs as a function of surface properties, and revealed the power of omics-based analyses.

### 2676 Lamellar Bodies and Mitochondria: Targets for FeO₄ and SiO₂ Nanoparticles Toxicity in A549 Cells

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Metal oxide nanoparticles (MNPs) are produced at high rates and occupational scenarios promote inhalation exposure. The generation of ROS has been described as a common mechanism of toxicity, however the interaction with relevant cell structures such as lamellar bodies (LB) and mitochondria represent a key target of toxicity. The aim of this study was to evaluate whether exposure to different MNPs, FeO₄ crystalline NPs and SiO₂ NPs of ~50 nm and 10–20 nm respectively, in human alveolar A549 cells alter LB and mitochondria. NPs dispersions were characterized by electron microscopy, dynamic light scattering, and laser doppler electrophoresis. Cell viability, cellular uptake, mitochondrial membrane potential and lysosomal damage were determined by MTT, transmission electron microscopy, JC-1 and lysotracker assay. Physicochemical characterization of NP dispersions with serum albumin bovine (BSA) showed hydrodynamic diameters in culture medium of 888 and 402.3 nm, for FeO₄ and SiO₂ respectively. Zeta potential was -8 mV for FeO₄ NPs and -13 mV for SiO₂ NPs. Images of transmission electronic microscopy (TEM) showed the internalization of both NP at 12 h by endocytosis with different localization. FeO₄-NP and SiO₂-NP were observed in acid compartments, mainly in LB. Also, SiO₂-NP were localized in delimited regions in the cytoplasm with morphological mitochondria changes. After 48 hours FeO₄-NP were still internalized by endocytosis NPs were not found inside lamellae. Interestingly we observed NPs near mitochondria and lipid droplets after exposure to both nanoparticles. There was no a statistically significant decrease in cell viability from the exposure to NPs. However, mitochondrial membrane potential (MMP) decreased at 6 and 12 h of exposure at the highest cell viability from the exposure to NPs. There was no a statistically significant decrease in mitochondrial membrane potential and lysosomal damage were determined by MTT, transmission electron microscopy, JC-1 and lysotracker assay. Physicochemical characterization of NP dispersions with serum albumin bovine (BSA) showed hydrodynamic diameters in culture medium of 888 and 402.3 nm, for FeO₄ and SiO₂ respectively. Zeta potential was -8 mV for FeO₄ NPs and -13 mV for SiO₂ NPs. Images of transmission electronic microscopy (TEM) showed the internalization of both NP at 12 h by endocytosis with different localization. FeO₄-NP and SiO₂-NP were observed in acid compartments, mainly in LB. Also, SiO₂-NP were localized in delimited regions in the cytoplasm with morphological mitochondria changes. After 48 hours FeO₄-NP were still internalized by endocytosis NPs were not found inside lamellae. Interestingly we observed NPs near mitochondria and lipid droplets after exposure to both nanoparticles. There was no a statistically significant decrease in cell viability from the exposure to NPs. However, mitochondrial membrane potential (MMP) decreased at 6 and 12 h of exposure at the highest concentrations of FeO₄ and SiO₂, respectively. This mitochondrial alteration seems to be preceded by an activation of the endosome-lysosome system only in exposure to SiO₂ NP. Mitochondrial damage and its accumulation in LB are potential targets of NPs toxicity, and could interfere with their metabolism and secretory functions leading to impairment of pneumocytes and thus respiratory disorders.

### 2677 Biodistribution of Inhaled Multiwalled Carbon Nanotubes in a Murine Mouse Model

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Synthetic Mimic of antimicrobial peptides (SMAMPs) are novel poly oxy-norbornene (PONs) based nanoparticle coatings that impart a positive charge on the nanomaterials. They are designed to imitate natural defense-host peptides that regulate the ability to act as an organism’s first line of defense within its immune system and simultaneously act as antimicrobial agents. In the absence of countercharged materials, positively-charged nanomaterials can cause a toxic response in a variety of aquatic species. PONs can be synthesized with a variety of different charge densities and therefore, in this study, our goal is to utilize gold nanoparticles (AuNPs) coated with SMAMPs in order to understand how differences in the charge of the coating will impact the model vertebrate organism, *D. rerio*. In order to alter the charge of the ligands, the SMAMPs were synthesized with different ratios of amine to propyl, either 50% amine or 75% amine. We then exposed *D. rerio* embryos for up to 5 days to various concentrations of either the ligands coated onto AuNPs or to the ligands alone. At lower concentrations, we saw catalytic metals that are often present during preparation. MWCNT 1020 was aerosolized using a linear feed dust-metering device to a particle attrition chamber and a single jet disperser. The aerosol was diluted to expose concentrations of 0.06, 0.2, or 0.6 mg/m³ with humidified air. Male B6C3F1/N mice were exposed for a total of 22 days over a period of one month. One week after exposure various organs were harvested, processed, embedded in paraffin, sectioned, and stained with Hematoxylin and Eosin. Examination of the lungs suggested minimal inflammation in the lungs at even the highest exposure level. The samples were analyzed by hyperspectral dark field microscopy using Cytovia to determine what tissues contained MWCNT and the relative amounts in those tissues. No particle was detected in the liver or kidneys. However, it was present in the lungs, brachial lymph nodes, mediastinal lymph nodes, and spleen. These results suggested that inhaled MWCNT spread primarily through the lymphatic system from the lungs. Funded by R2S ES022866 and R01 ES023209.
that the viability of the embryos was similar to the control despite the charge; however, there were drastic differences with the higher concentrations of ligands. For the ligand-coated NP solutions, the 75% amine ligands induced a lower viability; however, for the ligand-only solutions, the 50% amine ligands induced a lower viability compared to the 75% amine ligands. In addition, we also observed that some of the embryos exposed to either the ligand-coated NPs or the ligands-only hatched earlier than the controls. Gene expression indicated that exposure to the 50% amine at the lower and higher concentrations, as well as the 75% amine at the lower concentration instigated apoptosis, oxidative stress, or chorion degradation.

**2680 High-Throughput Hazard Assessment of a Diverse Suite of Precision-Engineered Nanomaterials Using Zebrafish**

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The biocompatibility of engineered nanomaterials (ENMs) is influenced by intrinsic physicochemical characteristics (e.g., surface chemistry). The systematic investigation of nano-bio interactions in vivo offers a powerful way to elucidate the relationships between ENM characteristics and biological outcomes. The objective of the current study was to evaluate the biocompatibility of well-characterized precision-engineered metal oxide, silica oxide, and silver silica nanomaterials using early-life-stage zebrafish (Danio rerio). All ENMs were synthesized and characterized by the HSPH-NIEHS Nanosafety Center at Harvard University, a member of the NIEHS Nanomaterials Health Implications Research consortium. ENMs were stably dispersed in ultra-pure water using a rigorous and standardized sonication protocol, which included monitoring hydrodynamic diameter and zeta potential over time. Zebrafish were dechorionated and statically exposed to each ENM (0, 2.32, 5, 10.7, 23.2, or 50 µg/ml; n=32/conc) from 6 to 120 hours post-fertilization (hpf). Two behavioral assays (at 24 and 120 hpf) and 22 developmental morphological endpoints were evaluated. Results showed that silver silica nanocomposites (4% or 16% [w/w]) yielded significant mortality at 24 and 120 hpf compared to controls. Moreover, exposure to aluminum oxide, cerium oxide, silica oxide, and iron oxide nanomaterials produced statistically significant abnormal behavior (e.g., hyper- or hypoactivity) at 24 and/or 120 hpf compared to control (p<0.05). Collectively, these results enhance our understanding of complex structure-activity relationships at the nanoscale and could contribute toward the rational design of safer nanomaterials. Future studies will investigate the role of oxidative stress and cell death in the acute toxicity of the silver silica nanocomposites and investigate the behavior of adult zebrafish that were only exposed to ENMs during early-life stages. These studies were supported by U01 ES027294.

**2681 Silica Nanoparticles Trigger the Blood Hypercoagulability and Thrombotic Effects Via JAK1/TF/PAR1 Pathway in Zebrafish**

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Silica nanoparticles (SiNPs) are widely applied in food sciences, industry and biomedicine, increasing the human exposure and potential health risk. However, the effect of SiNPs on circulatory system, especially on thrombus formation is largely unknown. This study explored the biological action of intravenous injection of SiNPs on thrombus effects in zebrafish (Danio rerio) and elucidated the underlying mechanisms. Using transgenic zebrafish, Tg(mpo:GFP) and Tg(fli-1:EGFP), the SiNPs-induced activation of neutrophil-mediated inflammation and impairment of vascular endothelium were assessed in the caudal vein. SiNPs significantly decreased blood flow and velocity measured by ZebraBlood analysis. In vivo particle dissolution is another important factor. Poorly soluble particles have longer retention half times in the body as compared to biosoluble particles. Here, we compare the biodistribution and retention of inhaled manganese oxide (MnOx) NPs with varying rates of dissolution in vivo. Male C57 mice (n=3-4, 14-19 g), were exposed to filtered air (FA), non-calcined MnOx NPs, or calcined MnOx NPs via whole body inhalation (WBI) for 5 hrs per day for 1 or 5 days. Organs were collected 24 hrs post-exposure and Mn content was analyzed using atomic absorption spectrometry (AAS). By day 5, retained lung Mn was higher in non-calcined MnOx (53 ± 135 ng/g) than calcined MnOx NPs (848 ± 76 ng/g), which was unexpected considering acellular dissolution measurements showed that the calcined MnOx NPs had a lower dissolution rate than non-calcined ones. The olfactory bulb (OB) had elevated Mn for both particle types. Elevated Mn was found in the hippocampus, striatum, and frontal cortex for calcined MnOx by day 5. To determine the role of solubility in the retention of NPs, C57 mice were exposed via WBI to FA, calcined MnOx NPs, or nebulized MnCl2 for 5 hrs. Organs were collected immediately, 6 hrs, 24 hrs, and 48 hrs post-exposure for measurements of Mn content. We hypothesized that upon deposition in the lungs, the MnOx particles were quickly cleared to other secondary target organs, such as the kidney (1681 ± 126 ng/g at 6 hrs vs FA, p=0.00245). The T1/2 in the lung of calcined MnOx was ~12 hrs, while that for MnCl2 was ~18 hrs. Elevations in OB tissue Mn occurred only in the MnOx-exposed mice starting 6 hrs post-exposure (1026 ± 64 ng/g). In contrast, there was no increase of measured Mn in the brains of MnCl2-exposed mice. This study demonstrates differences in biodistribution and retention of NPs of varying dissolution rates, which is a factor to consider when determining the potential toxicity of inhaled NPs. While the acellular studies did not predict well the lung retention for calcined MnOx NPs, this could potentially be explained by the binding of ionic Mn to constituents in lung tissue. Funded by NIH Grants R01ES020332, T32ES007026, and P30ES01247.

**2682 Biodistribution and Retention of Inhaled Manganese Oxide Nanoparticles in Mice: Role of Particle Solubility**

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Many factors can alter the translocation, biodistribution, and retention of inhaled nanomaterials (NPs): including size, shape, and surface properties. In vivo particle dissolution is another important factor. Poorly soluble particles have longer retention half times in the body as compared to biosoluble particles. Here, we compare the biodistribution and retention of inhaled manganese oxide (MnOx) NPs with varying rates of dissolution in vivo. Male C57 mice (n=3-4, 14-19 g), were exposed to filtered air (FA), non-calcined MnOx NPs, or calcined MnOx NPs via whole body inhalation (WBI) for 5 hrs per day for 1 or 5 days. Organs were collected 24 hrs post-exposure and Mn content was analyzed using atomic absorption spectrometry (AAS). By day 5, retained lung Mn was higher in non-calcined MnOx (53 ± 135 ng/g) than calcined MnOx NPs (848 ± 76 ng/g), which was unexpected considering acellular dissolution measurements showed that the calcined MnOx NPs had a lower dissolution rate than non-calcined ones. The olfactory bulb (OB) had elevated Mn for both particle types. Elevated Mn was found in the hippocampus, striatum, and frontal cortex for calcined MnOx by day 5. To determine the role of solubility in the retention of NPs, C57 mice were exposed via WBI to FA, calcined MnOx NPs, or nebulized MnCl2 for 5 hrs. Organs were collected immediately, 6 hrs, 24 hrs, and 48 hrs post-exposure for measurements of Mn content. We hypothesized that upon deposition in the lungs, the MnOx particles were quickly cleared to other secondary target organs, such as the kidney (1681 ± 126 ng/g at 6 hrs vs FA, p=0.00245). The T1/2 in the lung of calcined MnOx was ~12 hrs, while that for MnCl2 was ~18 hrs. Elevations in OB tissue Mn occurred only in the MnOx-exposed mice starting 6 hrs post-exposure (1026 ± 64 ng/g). In contrast, there was no increase of measured Mn in the brains of MnCl2-exposed mice. This study demonstrates differences in biodistribution and retention of NPs of varying dissolution rates, which is a factor to consider when determining the potential toxicity of inhaled NPs. While the acellular studies did not predict well the lung retention for calcined MnOx NPs, this could potentially be explained by the binding of ionic Mn to constituents in lung tissue. Funded by NIH Grants R01ES020332, T32ES007026, and P30ES01247.

**2683 Multiple Dose Metrics Reveal Shape and Charge-Dependent Toxicity of Au NPs**


Customizable Au nanoparticle (NP) platforms are driving innovations in drug discovery with massive therapeutic potential due to their biocompatibility, stability, and imaging capabilities; yet their development is outpacing our understanding of how their inherent properties impact their behavior and applicability. Systematic investigations of NPs whose differences are discrete must be used to identify the key parameters responsible for driving observed biological responses. Understanding biological responses is further complicated by lack of understanding with respect to the appropriate dose-metrics such as mass, surface area and particle count. Here, we investigated a suite of Au NPs comprised of two different shapes and two different surface functional groups to determine the relative contribution of shape and charge to toxicity. We compared spherical and star shaped Au NPs with either a positively charged surface functional group (branched polyethyleneamine, bPEI) or a negatively charged ionic stabilizer (citrate) and evaluated their uptake and toxicity in embryonic zebrafish using a variety of characterization methods and found that the most commonly used techniques of TEM and hydrodynamic diameter are not appropriate for irregularly shaped nanomaterials due to their assumption of spherical shape. Instead, we used measured 2D surface area and 2D perimeter from multiple TEM images to better estimate particle counts and surface area. We validated this method by comparing dose metrics calculated for spherical particles and found it to be in agreement with calculations from TEM diameter and hydrodynamic diameter. After comparing the dose metrics of mass, surface area, and particle count, citrate-stabilized AuNPs stars elicited the highest toxicity (LOAEL = 5 mg/L) followed by the anionic Au-Cit stars (LOAEL = 25 mg/L). Neither of the spherical particles elicited a response even at 50 mg/L. Our findings indicate the importance of considering both shape and charge in nanomaterial design. In addition, we show that commonly used nanomaterial characterization techniques can be modified to calculate important estimates of irregularly shaped nanomaterial dose metrics and demonstrate that an assumption of spherical shape for irregularly shaped particles is inappropriate.
Pulmonary exposure to carbon nanotubes or nanofibers (CNT/F), known to induce inflammation, toxicity, or tumorigenesis, is a concern due to increased production and dry powder handling. CNT/F represent a large class of materials if it is unclear if another similar toxicity. Our aim was to simultaneously test the pulmonary effects induced by CNT/F with variable physicochemical properties obtained from U.S. facilities. Characterization was done for seven different multiwalled CNT and two CNF selected based on nominal diameter ranging from 10-150 nm. Cytotoxicity, inflammation, and histopathology was assessed in mice 1, 7, 28, and 84 d following oral exposure to 4 or 40 nm CNT and CNF. Utilized doses and material preparation for in vivo dosing were representative to ongoing occupational exposures. Lactate dehydrogenase (LDH) activity, a marker of cytotoxicity, was dose-dependently increased in bronchoalveolar lavage fluid (BALF) resolving toward baseline by 84 d in all groups. In materials with a diameter greater than or equal to 50 nm, LDH was persistently increased. Polymeroma pericellular cell infiltration (%PMN), a marker of inflammation, was increased in all materials at 1 d post-exposure to 40 µg (50 nm: 31.1%, ≥50 nm: 37.1%). With exposure to materials less than 50 nm, PMN influx mostly resolved by 7 d while materials greater than or equal to 50 nm indicated persistent inflammation (7 d: <50 nm: 10.5%, ≥50 nm: 48.9%). For complement, inflammatory gene expression in lung tissue (e.g., Il1b, Il6, Ccl22, Cxcl12) and protein levels in BALF (e.g., Il1b, Il6, I5, Ccl22, Cxcl11), were elevated to a greater extent in materials with a nominal tube diameter greater than or equal to 50 nm. In contrast, microscopic evaluation of lung sections at 84 d post-exposure indicated that histopathology does not appear distinguishable between CNT/F using diameter. In conclusion, general cytotoxicity and inflammation exhibited a relationship with nominal diameter, with a threshold of sustained effects at approximately 50 nm and greater, which was dissimilar to histopathology. Ongoing research and modeling techniques will elucidate relationships between physicochemical characteristics and toxicities of various CNT/F.
Mitsui-7 MWCNTs (MWCNTs) are strong lung tumor promoters in B6C3F1 mice. B6C3F1 mouse lung tumors have many molecular and morphological similarities to human pulmonary tumors. In previous work, we demonstrated that exposure to inhaled MWCNTs following exposure to a DNA damaging agent caused potent promotion of lung tumors. To investigate a possible threshold for MWCNT-induced carcinogenesis, we exposed B6C3F1 mice to a single dose of either methylicholanthrene (MC, 10 µg/g BW, i.p.) or vehicle (corn oil). One week after i.p. injections, mice were exposed by inhalation to MWCNTs (5 mg/m³, 5 hours/day, 5 days/week) or filtered air (controls) for a total of 2, 5 or 10 days. At 17 months post-exposure, mice were euthanized and examined for lung tumor formation. Thirty percent of the filtered air controls, 33% of the MWCNT-exposed, and 47% of the MC-exposed, had a mean of 0.33, 0.33 and 0.4 tumors per mouse, respectively. By contrast, 94% of mice receiving MC followed by 10 days MWCNT had an average of 2.9 tumors per mouse while 81% of mice exposed to MWCNTs for 5 days had an average of 1.9 tumors per mouse, and 73% of mice exposed to MCNTs for 2 days had an average of 1.2 tumors per mouse. Additionally, mice exposed to MWCNTs or MC followed by MWCNTs had larger tumor volumes than their corresponding control groups. Preliminary data indicate a dose response in the percent of animals with tumors as well as the number of tumors per animal following exposure to MC and MWCNTs. In this study, mouse MWCNT lung burden approximates feasible human occupational exposures. Therefore, the results of this ongoing study indicate that caution should be used to limit human exposures to MWCNTs.

Inhalation of ambient ultrafine particles and engineered nanomaterials are associated with adverse airway responses, including allergic asthma. Generation of reactive oxygen species by inhaled nanoparticles (NPs) may activate immunological adjuvant pathways and enhance local sensitization to environmental and occupational allergens. To test the hypothesis that NP redox status is associated with adjuvant activity, we modified the redox activity of cerium dioxide (CeO2) NPs by incorporating increasing quantities of zirconium (Zr) into the crystalline structure of the CeO2 NPs. Female BALB/c mice were intranasally sensitized with ovalbumin (OVA) or OVA + 200 µg CeO2 (doped with 0%, 27% or 78% Zr) on days 1, 3, 6, and 8, and then challenged with OVA alone on days 22 and 23. Twenty-four hours later serum was collected to assess OVA-specific IgE and IgG1, bronchoalveolar lavage fluid (BALF) collected for quantitation of inflammatory cells, and lungs processed for detection and morphometric evaluation of eosinophils and intraepithelial mucous substanes (IM). OVA-sensitized and -challenged mice had minimal increases in serum IgE or IgG1 compared to non-sensitized mice (PBS control). However, marked increases in IgE and IgG1 were observed after co-sensitization with CeO2 (doped with 0%, 27% and 78% Zr) compared to OVA alone. OVA-sensitized and -challenged mice had increased BALF macrophages and eosinophils compared to controls. Sensitization with OVA + CeO2 (0% Zr) enhanced BALF total cells (2.3-fold increase), macrophages (2.8-fold), eosinophils (15-fold), and lymphocytes (3.8-fold) compared to OVA alone. Doping of CeO2 with 78%, but not 27% Zr further enhanced BALF total cells, macrophages, and eosinophils by 50-100% compared to OVA without Zr doping. Allergic inflammatory, epithelial, and mucous cell responses were minimal in lung tissues of OVA-sensitized and challenged mice, but marked eosinophilic alveolitis and bronchiolitis, and a modest increase in IM were induced by co-sensitization with OVA and all zirconium-doped CeO2 NPs. Mice sensitized with CeO2 (78%Zr) had more parenchymal eosinophils than CeO2 (0%Zr), but mucous and inflammatory cell responses were similar for all CeO2 NPs. Our results suggest that CeO2 can act as potent airway adjuvant for allergic sensitization, and that the redox activity of engineered NPs can affect the character and severity of allergic airway responses.
**2692** The Synthesis of Gum Arabic-Modified CdTe Quantum Dots with Low-Cytotoxicity for In Vivo Applications

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Gum Arabic (GA) is a natural glycoprotein polymer with low cytotoxicity and is often used in the food and pharmaceutical industries. Polymers are often used as capping agents in nanoparticles synthesis to prevent nanoparticle agglomeration and to reduce nanoparticle toxicity. CdTe quantum dots (QDs) have potential applications as probes for in vivo diagnostics. However, these nanoparticles have significant cytotoxicity. In this study we aimed to synthesise CdTe QDs with high luminescent properties for applications in in vivo bio-imaging. Two different synthesis methods were used. We capped the QDs with GA to reduce the cytotoxicity and improve the solubility of the nanoparticles. These QD-GA nanoparticles were characterized using Ultraviolet-visible (UV–vis) spectroscopy, Photoluminescence (PL) spectroscopy, High-Resolution Transmission Electron Microscopy (HRTEM) and Fourier Transform InfraRed spectroscopy (FTIR). Also, the hydrodynamic particle size, polydispersity index (PDI) and Zeta potential of GA capped QDs were also evaluated to ascertain their colloidal stability. The cytotoxicity of the QDs were evaluated on two human cancer cell lines, namely HeLa and PC-3 using the WST-1 assay. The PL intensity of the QDs synthesised using the two methods were 678nm and 675nm, respectively. HRTEM analysis showed that the average particle size were 3.45nm and 3.9nm, respectively. The average PDI and Zeta potential were 0.27 ± 0.02 and 0.35 ± 0.02. The cytotoxicity assays showed that GA capping reduced the cytotoxicity and improved the stability of the QDs. These nanoparticles can potentially be used for in vivo applications.

**2693** Involvement of ROS Generation in Copper Oxide Nanoparticles-Induced AP-1 Activation

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Occupational exposures to copper dusts or fumes have been reported to be harmful to human health, with possible risk of cancer among copper smelter workers. Copper (II) oxide (CuO) nanoparticles have not, to our knowledge, been extensively examined for potential carcinogenic or genotoxic effects. To investigate the mechanisms of CuO-induced pathogenesis, the effect of CuO on AP-1-MAPKs and ROS generation were investigated. The results indicated CuO caused a 2-fold increase in AP-1 activity in JB6 cells. The induction of AP-1 activity in cultured cell lines was time and dose-dependent. The signal transduction pathways for AP-1 activation were also investigated. Western Blot analysis demonstrated that CuO stimulates phosphorylation of p38 MAPK and ERKs. CuO also generated ROS when incubated with the cells as measured by electron spin resonance (ESR). Nano-sized CuO generated more ROS than the fine sized particles when incubated with the cells. Finally, co-incubation with a CD36 competitive ligand sulfo-N-succinimidyl oleate (SSO) or a CD36-specific antibody, uptake of GNPs was reduced. GNP exposure also induced mitochondrial membrane potential while pre-treatment with the CD36 antibody attenuated these changes. In addition, macrophages exposed to non-functionalized GNPs at concentrations of 0.5, 10, or 100 μg/ml for 1 or 3 h were harvested for metabolomic profiling and both metabolite and lipid fractions were examined. Principal component analysis showed all groups to be different from the control and one another. The number of compounds changed following exposure appeared to be both concentration- and time-dependent. Specifically, a total of 481 compounds < 0.01% altered following the exposure to 100 μg/ml of GNPs, whereas 291 compounds were altered following exposure to 50 μg/ml (Fold Change > 2; p<0.01). Compounds were associated with pathways such as spingolipid and cholesterol metabolism, glutathione synthesis, energy metabolism, inflammatory signaling and others. Lastly, a number of metabolic differences were found in common between cells exposed to the CD36 receptor ligand and GNPs suggesting CD36-dependent and independent responses. Together our data demonstrates the influence of functionalization on GNP-macrophage interactions, the role of CD36 in the cellular response, and metabolic pathways disrupted due to exposure.

**2694** Bismuth Sulfide Nanoparticle-Induced Nephrotoxicity and Autophagy-Associated Mechanisms

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Bismuth compounds have been widely used in electronics industry, alloy manufacture, cosmetics and medicine. Recently bismuth nanoparticles (BiNP) were synthesized for imaging and diagnostic purpose, while safety concern of bismuth cannot be ignored. Here, we prepared ultrasmall BiNP and showed an enhanced tumor CT imaging, but BiNP revealed a moderate nephrotoxicity in mice, including the elevated amounts of creatinine and blood urea nitrogen (BUN) in blood and urine. Pathologically, we found the increased amount of apoptosis in proximal tubule cells, indicating the injury of BiNP. In the meanwhile, the autophagy marker LC3II significantly increased in the kidney of the BiNP treated mice. The autophagy inducer rhamnycin can alleviate the kidney injury by BiNP, while chloroquine deteriorated the BiNP induced nephrotoxicity, as indicated by the creatinine, BUN, the kidney injury marker KIM-1, and pathological observations for the number of apoptotic cells. In vitro studies showed the cytotoxicity of BiNP on human embryonic kidney 293 cells (HEK293) compared to other cell types. The occurrence of monodansylcadaverine fluorescence staining and the amount of LC3II that can be inhibited by 3-MA indicated autophagy induced by BiNP. BiNP were capable of entering cells and localized in the cytoplasm observed by transmission electron microscopy with bismuth element confirmed by energy dispersive X-ray analysis. Mechanistically, we found that BiNP induced autophagy was through AMPK pathway but not P38 and MAPK pathway, followed by the inhibition of mTOR activity, with further increase of downstream protein expressions such as Beclin1, Atg12, and p62. With our novel finding of bismuth induced autophagy, potential approaches may be applied to reduce the nephrotoxicity by bismuth.

**2695** Alterations in Metabolite Profiles of Macrophages Exposed to Graphene Nanoplatelets

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Graphene nanoplatelets (GNPs) are novel 2D nanoparticles consisting of planar stacks of graphene with thickness of only 2-12 nm but width and length dimensions up to microns. GNPs are widely applicable due to their conductivity of electricity and heat, and their abundant surface area for drug delivery systems. Although human exposures are increasing, our knowledge regarding immune-specific responses to GNPs and mechanisms of interactions is lacking. Our current study utilized a metabonomic profiling approach to evaluate macrophage responses to GNPs. Further we assessed the potential role of the scavenger receptor CD36 in mediating these GNPs-induced responses. RAW264.7 macrophages were exposed to GNPs without functionalization or functionalized with cysteamine or amineylation at concentrations of 0, 25, 50, or 100 μg/ml for 1, 3, and 24h. Following exposure, no cytotoxicity was observed. Concentration-dependent changes in internalization were determined by alterations in side-scatter using flow cytometry. Non-functionalized GNPs internalized more than functionalized GNPs. Following incubation with a CD36 competitive ligand sulfo-N-succinimidyl oleate (SSO) or a CD36-specific antibody, uptake of GNPs was reduced. GNP exposure also induced mitochondrial membrane potential while pre-treatment with the CD36 antibody attenuated these changes. In addition, macrophages exposed to non-functionalized GNPs at concentrations of 0, 50, or 100 μg/ml for 1 or 3 h, or SSO for 1 h were harvested for metabolomic profiling and both metabolite and lipid fractions were examined. Principal component analysis showed all groups to be different from the control and one another. The number of compounds changed following exposure appeared to be both concentration- and time-dependent. Specifically, a total of 481 compounds < 0.01% altered following the exposure to 100 μg/ml of GNPs, whereas 291 compounds were altered following exposure to 50 μg/ml (Fold Change > 2; p<0.01). Compounds were associated with pathways such as spingolipid and cholesterol metabolism, glutathione synthesis, energy metabolism, inflammatory signaling and others. Lastly, a number of metabolic differences were found in common between cells exposed to the CD36 receptor ligand and GNPs suggesting CD36-dependent and independent responses. Together our data demonstrates the influence of functionalization on GNP-macrophage interactions, the role of CD36 in the cellular response, and metabolic pathways disrupted due to exposure.

**2696** Cytotoxicity of Copper (II) Oxide Nanoparticles in Rat Intestinal Cells: Effect of Simulated Gastrointestinal Fluids and Generation of Oxidative Stress


Metallic oxide nanoparticles (NPs) have applications in industry, medicine and commercial products. Exposure to NPs can occur by inhalation, dermal contact and oral ingestion. We have previously reported on the dose- and time-dependent cytotoxicity of CuO NPs (size < 50 nm) in rat intestinal cells (IEC-6) from the aspect of oral ingestion. This study assessed the effect of pretreating CuO NPs (1 mg/ml) with simulated gastrointestinal (GI) fluids (pepsin at pH 2 to 6, pancreatin at pH 7, bile salts at pH 7; incubated sequentially) on cytotoxicity in IEC-6 cells. The treated NPs were isolated by ultracentrifugation, suspended in media and probe sonicated before dosing the cells. Cells were exposed for 24 hr with the treated NPs (0.1 - 100 μg/ml) and cytotoxicity was assessed using a colorimetric method that measures mitochondrial activity. The zeta potential (ZP) and hydrodynamic diameter (HD) of similarly treated CuO NPs were measured after each incubation step. The ability of non-functionalized GNPs at concentrations of 0, 50, or 100 μg/ml for 1 or 3 h, or SSO for 1 h were harvested for metabolomic profiling and both metabolite and lipid fractions were examined. Principal component analysis showed all groups to be different from the control and one another. The number of compounds changed following exposure appeared to be both concentration- and time-dependent. Specifically, a total of 481 compounds < 0.01% altered following the exposure to 100 μg/ml of GNPs, whereas 291 compounds were altered following exposure to 50 μg/ml (Fold Change > 2; p<0.01). Compounds were associated with pathways such as spingolipid and cholesterol metabolism, glutathione synthesis, energy metabolism, inflammatory signaling and others. Lastly, a number of metabolic differences were found in common between cells exposed to the CD36 receptor ligand and GNPs suggesting CD36-dependent and independent responses. Together our data demonstrates the influence of functionalization on GNP-macrophage interactions, the role of CD36 in the cellular response, and metabolic pathways disrupted due to exposure.
minescent assay. Pretreatment of CuO NPs with GI fluids increased the cytotoxicity by 30% at 5 μg/ml relative to non-treated NPs. No differences were observed at the other concentrations. The lowest ZP (−3.4 mV) and highest HD (5104 nm) of the NPs was observed after incubation with pepsin at pH 2. Raising the pH stepwise one pH unit to pH 6 and incubating with pepsin increased ZP and decreased HD. Consistent changes in ZP and HD of CuO NPs were not observed at the other two steps of pretreatment. The 24-hr exposure of IEC-6 cells with pristine CuO NPs showed a dose-dependent increase in cellular H2O2 and a concomitant decrease in GSH. In summary, treating CuO NPs with simulated gastrointestinal fluids can alter their ZP and HD, but the modifications result in minimal alteration in cytotoxic effects of the particles. Pristine CuO NPs can generate oxidative stress within the cells, which is a potential mechanism of action for these particles. Consideration of the exposure scenario and mechanism of action of NPs are important aspects for the risk assessment of this emerging material. This abstract does not represent US EPA policy.

**2699** Assessment of Inflammatory and Oxidative Stress Response in Human Primary Bronchial Epithelial Cells Cultured in Air-Liquid Interface following Aerosolized Carbon Nanoparticle Exposure

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Carbon nanoparticles (CNPs) generated by incomplete combustion of diesel exhausts constitute an important component of the fine particulate matter (PM) related air pollution. CNPs are also used for the manufacturing of black ink, paint, plastic, and reinforcing agent in rubber goods. The widespread use of CNPs designated it as an evolving source of human exposure by inhalation exposure. Therefore, in this study we investigated the inflammatory and oxidative stress responses of NPs to assess their pulmonary toxicity by using a physiologically relevant in vitro human airway model combined with a newly developed exposure system. Human primary bronchial epithelial cells (PBECs) were cultured in an air-liquid interface. PBECs were exposed to 5, 17, and 25 μg/well aerosolized CNPs (Printem®) using the XposeALI® exposure system. After 24-hr exposure, cells were allowed to clean as control incubated in 5% CO2 at 37°C for 24h. Transcript expression of inflammation (IL6, CXCCL8, TNFα, NFKB), oxidative stress (HMOX1, SOD3, Gpx, GSTA1), and tissue injury (MMP9, TIMP1) markers were assessed using qRT-PCR. Protein levels of CCL8 and MMP-9 were measured using ELISA. P<0.05 was considered significantly different. An increased expression of tissue injury markers MMP9 and TIMP1 (p<0.05) were observed after exposure to the lowest CNP dose. An increased expression of tissue injury markers MMP9 and TIMP1 (p<0.05) were observed after exposure to 5 and 17 μg CNP / well. However, no significant changes of CCL8 and MMP-9 secretion were observed following exposure to CNP. Our data demonstrate induction of markers for inflammation and oxidative stress in association with alteration of tissue injury markers, which may be responsible for CNP-mediated pulmonary toxicity. Additionally these physiologically relevant in vitro models combined with XposeALI® exposure system is an optimal in vitro approach to assess air pollutant-induced human health hazards.
Engineered nanomaterials (ENM) are particles that are less than 100 nm in at least one dimension, fabricated for their unique properties applicable to medicine, cosmetics, chemistry, and other fields. One type of ENM, nickel oxide nanoparticles (NiO-nps) are particularly valuable in electronics, optics and batteries. Unfortunately, ENM demonstrate varying levels of bioactivity. Some ENM have been found to cause phagolysosome membrane permeability (LMP) in macrophages. This results in the release of lysosomal enzymes, such as cathepsins, into the cell's cytosol, resulting in inflammatory cytokine release and possible cell death.

To study the bioactivity of NiO-nps effect on cell membranes, we exposed two membrane models to NiO-nps, compared to well-studied titanium dioxide nanoparticles (TIO-nps). We hypothesized that NiO-nps would have similar bioactivity as TIO-nps. An LMP assay was performed by incubating bone marrow derived macrophages from Balb/c mice for 16 hrs. with both ENM at doses ranging from 12.5 to 50 µg/ml and measured levels of cytosolic cathepsin activity. Hemolysis assays and fluorescence lifetime imaging microscopy (FLIM) were performed on human red blood cells (RBC) as a surrogate for phagolysosome membranes. RBC were exposed to ENM at doses from 25 to 200 µg/ml for 4 hrs. FLIM was performed by using the fluorescence probe Di-4-ANEPPDHQ to determine ENM-induced changes in lipid membrane structure. Intensity weighted average lifetimes were determined using a PicoQuant MT200 time-resolved confocal microscope. Hemolysis assays results showed that TIO- induced hemolysis was significantly increased from the control at 50 and 100 µg/ml, while NiO induced hemolysis was significantly greater at 200 µg/ml. LMP data indicated that TIO- generated increased cathepsin release at 25 to 50 µg/ml compared to control. However, NiO resulted in no increase in cytosolic cathepsin activity. FLIM measurements of TIO- exposed RBC showed a significant increase in fluorescence lifetime at 50 and 100 µg/ml, while NiO did not affect the lifetime. We conclude that nickel oxide is not as bioactive in lipid membranes as titanium dioxide.

Nanoclay-enabled composite technology continues to expand based on incorporation of organomodified nanoclay (ONC), montmorillonite coated with different quaternary ammonium compounds, within polycarbonate, before the forecasts in the confluence of action. This study hypothesized that pre- and post-incinerated ONC exposed elicited differential modes of action on pulmonary cells compared to uncoated nanoclay, and that relevant human in vitro models correlate with in vivo effects. To assess ONC life cycle pulmonary toxicity, differentiated human monocytes (THP-1), fetal airways were dispersed into a miRQant MT200 (T. G. Kornberg1, 3. K. Fujita1, 2. S. Take2, R. Tani2, S. Endoh1, 2. J. Maru1, 2. S. Obara1, 2. and K. Honda1, 2). 1National Institute of Advanced Industrial Science and Technology, Tsukuba, Japan; and 2Technology Research Association for Single Wall Carbon Nanotubes (TASC), Tsukuba, Japan.

Graphene and related materials (GRM) have novel optical and thermal characteristics and are expected to be adopted for industrial applications; however, there are concerns with respect to the safety of carbon nanomaterials. In addition to direct interaction with the cell membrane, the proposed mechanisms of toxicity development include ROS generation and direct action on the DNA, but these remain to be clarified. In this study, (grant numbers ED481B 2016/190-0 and ED481B 2016/190-0) and the project NanoToxClass (ERA-SIINN/001/2013), N. Fernández-Bertolé was supported by an INDITEX-UDC fellowship, and F. Brandão by the grant SRFH/BD/101606/2014, funded by FCT (subsidized by national fund of MCTES).

Iron oxide nanoparticles (ION) have unique physicochemical properties, including superparamagnetism, which make them very promising for biomedical applications such as in magnetic resonance imaging, in hyperthermia-based cancer therapy, and in targeted drug/gene delivery. For all these uses, ION must be introduced in the organism, and previous studies showed that they can cross the intact blood-brain barrier. Besides, ION surface may be coated with different materials in order to increase their stability, prevent agglomeration and offer a platform for functionalization. Thus, although ION show in general a good biocompatibility, it is of paramount importance to deeply know how potential risks for the nervous system associated to ION exposure.

Hence, the main aim of this work was to assess genotoxic effects of silica-coated ION on glial cells (A172 astrocytes). Comet, 4’H2AX and micronucleus assays were performed, and experimental conditions involved 3 and 24 h incubations, 5-100 µg/ml dose range, and presence/absence of serum in the cell culture media. Results obtained showed slight induction of genotoxicity by ION, limited to long exposure times and highest concentrations, and independent on presence of serum in the cell culture medium. DNA double strand break production was only detected at the highest concentrations and longest time tested in comparison to untreated samples, probably related to the ION concentration at those conditions. No aneugenic effects were observed. This study contributes to increase the knowledge on the effects of ION on nervous system cells, and helps to provide a basis to define conditions to use ION in biomedical applications with minimal risk for patients' health. This study was supported by the New Energy and Industrial Technology Development Organization (NEDO), Japan.
**2705 Toxicity Responses of Various Fibrillar and Crystalline Nanocellulose Materials: Differences and Similarities**

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Extensive research of new nanostructured cellulose materials has resulted in advances in material properties and unique product applications. New technologies facilitated the creation of bacterial, nanocrystalline and micro/nano-fibrillar cellulose derived from natural sources that are easily integrated into bio-based and recyclable products. The aim of the current study was to compare five different nanocellulose (NC) particles using in vitro approaches to determine if several physico-chemical characteristics (i.e. size, shape, origin) yield specific cytotoxicity effects. Human lung epithelial cells (A549) were exposed to NC for 24 and 72 h to determine how variations in the properties contribute to cellular outcomes, such as cytotoxicity, oxidative stress, and cytokine secretion. Our results showed that nanofibrillated cellulose (NCF) induced stronger cytotoxicity and oxidative stress responses compared to cellulose nanocrystals (CNC). CNC, on the other hand, caused a significantly stronger inflammatory response compared to NCF. Additionally, immunostaining indicated that only CNC particles were taken up by the cells. Clustering analysis of the inflammatory cytokines/chemokines revealed a similarity of NCF to the carbon nanofibers response, while CNC was akin to that of chitin, a known immune modulator and activator of innate cells. Taken together, these results indicate that size and shape of NC particles are critical to determining their toxicity: CNC and NCF induce distinctly different patterns of toxic and inflammatory response in lung cells.

**2706 The Intracellular Fate of Multiwalled Carbon Nanotubes in Macrophages Using Laser Scanning Confocal Raman Microscopy**

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Both the production and use of multiwalled carbon nanotubes (MWN)s are rapidly increasing worldwide despite the possible adverse effects they may have on human health. The Biosciences Group at UT Dallas is interested in understanding the interactions of polymer- and protein-coated MWNTs with macrophages that are the first responders to invaders in the body. This information is relevant for improving the biomedical efficacy of MWNT therapeutics, for understanding mechanisms of MWNT biocompatibility, and for designing methods to ameliorate MWNT toxicity. To better understand potential mechanisms of MWNT toxicity, it is important to know whether MWNTs physically enter cells and where they locate in cells. We have developed three methods to measure the subcellular location of MWNs and reconstruct 3D images of cell-associated MWNs using confocal Raman microscopy. 3D images of cells are reconstructed with stacks of optical sections from confocal planes to place the subcellular MWN locations in the context of the intact cell. The results at 37 °C show that polymer- and protein-coated carboxylated-MWNs (C-MWNs) are within punctate vesicles, most likely in the endosomal/lysosome system. Conversely, at 4 °C, C-MWN signals are only found at the periphery of the cells around the membrane, suggesting that the uptake of C-MWNs is through an energy-dependent receptor-mediated endocytosis pathway. Future work will involve cluster analyses and live-cell imaging to access whether MWNs that induce cytokine release can be correlated with damage to the lysosomal membrane and redistribution of MWNs to the cytoplasm. If successful, this will result in a relatively rapid technique that can be used to determine whether lysosomal damage has occurred because of MWN exposure, and could also be applied to many other types of Raman-active nanoparticles that may induce pro-inflammatory responses.

**2707 Differences in Multi- and Single-Walled Carbon Nanotube-Induced DNA Methylation: Alterations in Association with Nuclear Deposition**

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Subtle DNA methylation alterations mediated by carbon nanotubes (CNTs) exposure might contribute to pathogenesis and disease susceptibility. In order to understand the epigenetic toxicity, in particular DNA methylation alterations, of single-walled (SW)CNTs and short multiwalled (MW)CNTs, we performed genome/gene-wide, gene-specific DNA methylation and RNA-expression analyses after multiwalled bronchial epithelial cells (16HBE14- cell line). In addition, the presence of CNTs on/in the cell nucleus was evaluated in a label-free way using femtosecond pulsed laser microscopy. Generally, a higher number of SWCNTs, compared to MWCNTs, was deposited at the cellular and nuclear level after exposure. Nonetheless, both CNT types were in physical contact with the nuclei. No global (S-mC) DNA methylation alteration was observed for both CNTs. After exposure to MWCNTs, 2398 genes were hypomethylated (at gene promoters), and after exposure to SWCNTs, 589 Cpg sites (located on 501 genes) were either hypo- (-493 Cpg sites) or hypermethylated (96 Cpg sites). Cells exposed to MWCNTs exhibited a better correlation between gene promoter methylation and gene expression alterations. Differentially methylated and expressed genes induced changes (MWNTs > SWCNTs) at different cellular pathways, such as p53 signalling, DNA damage repair and cell death. On the other hand, SWCNT exposure showed hypermethylation on functionally important genes, such as SKI proto-oncogene (SKI), glutathione S-transferase pi 1 (GSTP1) and shroom family member 2 (SHROOM2) and neurofibrillarosis type 1 (NFI), which the latter is both hypermethylated and downregulated. After exposure to both types of CNTs, epigenetic alterations may contribute to toxic or repair responses. Moreover, our results suggest that the observed differences in the epigenetic response depend on particle type and differential CNT-nucleus interactions.

**2708 Cytokine Production by Rat Mesothelial Cells Exposed to Carbon Nanotubes as a Means of Assessing Long-Term Risks for Mesothelioma**

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Multiwalled carbon nanotubes (MWNTs) are a type of highly durable engineered nanomaterial with many industrial applications but are structurally similar to asbestos fibers. It has been shown previously that MWNTs cause fibrosis and inflammation in the lungs of mice. Prolonged exposure to MWNTs could potentially cause neoplastic transformation of mesothelial cells in the pleural lining of the lungs leading to mesothelioma. However, it is not known whether MWNTs cause mesothelioma in humans. A biomarker of human mesothelioma is osteopontin (OPN), a cytokine that produced by mesothelial cells that mediates cell migration. We hypothesize that long-term exposure to MWNTs would cause transformation of normal rat mesothelial cells to mesothelioma cells in vitro that can be predicted by an increase in OPN. Normal rat mesothelial cells (NRMs-2) were grown in six-well cell culture plates and exposed to t(6)- or r(5)-MWNTs for 45 weeks. With each new cell passage, MWNTs were reintroduced to the cell medium for a final concentration of 0.1 μg/ml. Expression of the mRNA encoding OPN was measured using real-time qPCR, the concentration of OPN secreted protein in vitro was measured using an ELISA, and cell invasion assays were used to analyze the ability of cells to exhibit neoplastic characteristics. Rat pleural mesothelioma cells (ME1) were used as a comparison to NRM2 cells. Mesothelioma (ME1) cells spontaneously produced high levels of OPN whereas untreated control NRM-2 cells did not. There was a significant increase in OPN mRNA expression in NRM-2 cells after chronic exposure to r-MWNTs but not t-MWNTs. In the invasion assay, ME1 cells spontaneously showed increased invasion compared to untreated NRM2. Surprisingly, chronic exposure to t-MWNTs but not r-MWNTs caused increased invasion of NRM2 cells. Induction of OPN mRNA does not correlate with increased invasion of MWNT-treated mesothelial cells. Further study is needed to assess reliable biomarkers of mesothelial cell transformation to predict the carcinogenicity of MWNTs. Funding: Supported by NIEHS grant RO1-E502089 and NIEHS P30-E5025128.
Neutrophils are a circulating leukocyte found in the blood that migrates into tissues during inflammation. They are highly phagocytic, and due to the presence of cytotoxic granules, they are known to be very important microbialicidal cells. Since it has been demonstrated that tissue-resident macrophages secrete pro-inflammatory cytokines following exposure to silver nanoparticles (Ag-NP), which activate the inflammatory response, we wanted to determine how the neutrophils would respond to the presence of Ag-NPs since they will likely migrate into exposed tissues. 10 nm PVP-coated Ag-NPs were incubated with primary neutrophils isolated from human blood, and it was noted using microscopy that at doses greater than 10 micrograms per milliliter (μg/mL), the neutrophils were completely destroyed within an hour. Even a 1 hour incubation with 10 μg/mL resulted in a 20% reduction in neutrophil cell viability. Neutrophil activity was assessed at sub-toxic doses to determine the full extent at which these cells are being affected by Ag-NPs. At 1 and 10 μg/mL doses with 1 hour of exposure, a significant decrease in myeloperoxidase activity was observed, which suggests a defect in bactericidal activity of these cells. Although we do not observe any detectable Ag-NPs in our TEM images, we hypothesize that they were taken up by these active phagocytes, and dissociated quickly into silver ions that impacted neutrophil cellular viability and function. Since neutrophils are an essential cell in the clearance of microbial pathogens, it is important to assess whether the use of Ag-NPs as antimicrobial agents will impact the removal of infectious agents.

NiO and NiOH Nanoparticles Induce Oxidative Stress-Mediated Cellular Signaling Deregulation

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Nanomaterials have become increasingly popular in the production of a wide range of products including cosmetics, pharmaceuticals, cancer research, printer toners, food, electronic manufacturing, and many other products. In this study, we examined toxicity of Ni in two different forms, NiO and NiOH. Crystalline structure analyses revealed that NiO has a cubic shape and NiOH is hexagonal. Measurement from the Brunauer–Emmett–Teller method indicated that the specific surface areas of NiO and NiOH are 73.45 m²/g and 103.17 m²/g, respectively. The point-of-zero charge analyses showed that surface charge of NiO is neutral in cell media while NiO is slightly negatively charged. Surface charge of nanoparticles may influence bioavailability. Exposure to NiO and NiOH induced oxidative stress in A549 cells, which produced consequential alterations in cell functions. Caspase 3 activity was increased upon exposure to NiO or NiOH (N=3, Ps < 0.05). Semi-qualitative staining demonstrated a dose-dependent loss of mitochondrial membrane potential by NiO or NiOH. Exposure to NiOH caused a higher degree of apoptosis in A549 cells than exposure to NiO. Suppression of cell proliferation in A549 cells occurred upon exposure to NiO at concentrations above 25 μg/mL, and all tested concentrations of NiOH (N=3, Ps < 0.05). NiO caused an increase of cells in the S and G2 phases while NiO resulted in an increase of cells in the S phase. The collective outcome was reflected in differential cell viability. NiO produced a statistically significant (N=3, Ps < 0.05) higher reduction in cell viability than NiO in concentrations of 50 μg/mL. NiO dissolution from NiO and NiO and particle morphology may play crucial roles in toxicological outcome.

Fibroblast to Myofibroblast Differentiation in Response to the Mechanical Properties of Engineered Nanomaterials (ENMs) and Asbestos

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The mechanisms responsible for initiating fibroblast differentiation and impacting wound healing efficacy following exposure to ENMs and asbestos are currently underevaluated. To broaden our understanding of the fibroblast-driven wound healing response, I aim to evaluate a model of fibroblast to myofibroblast differentiation following exposure to ENMs and asbestos fibers. I hypothesize that exposure to high aspect ratio, stiff ENMs drives fibroblast to myofibroblast differentiation, and that mechanical and paracrine signaling is responsible for cytoskeletal organization and increased extracellular matrix (ECM) expression. I utilize IMR90 cells (human normal, lung, embryonic fibroblasts) exposed to particles/fibers with a variety of physical properties and geometries, including carbon black spherical particles (M120), crocidolite asbestos fibers, Mitsui multi-wall carbon nanotubes (MWCNT-7), and flexible MWCNTs (flex-2), to evaluate material properties that drive fibroblast to myofibroblast differentiation. Using α-smooth muscle actin (α-SMA) as a myofibroblast marker, the data supports that MWCNT-7 and asbestos induce significant a-SMA expression after 72 hours. The increase in a-SMA corresponds to significant upregulated protein expression involved in calcium signaling, cytoskeletal organization, and ECM production. In addition, ENMs and asbestos elicit significant upregulation of epidermal growth factor receptor, a response previously reported following asbestos exposure. In the future, we hope to further clarify mechanisms that drive myofibroblast function and modify the model to include relevant substrate stiffness to healthy and fibrotic lung microenvironments. This research is supported by the NIEHS Grant T32 ES057272 and the NIEHS Superfund Research Program P42 ES05660. Proteomics data were collected with the help of Drs. Ahsan and Solomon through the COBRE CCRD Proteomics Core - SP30GM110759.

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Nickel (Ni) compounds are classified as carcinogenic to humans but the underlying mechanisms are still poorly understood. Furthermore, effects related to nanoparticles (NPs) of Ni have not been fully elucidated. The aim of this study was to investigate genotoxicity and mutagenicity of Ni and NiO NPs and compare the effect to soluble Ni from NiCl₂. We employed different models; i.e. exposure of 1) human bronchial epithelial cells (HBEc) by DNA strand break analysis (comet assay and γ-H2AX staining); 2) six different mouse embryonic stem (mES) reporter cell lines (ToxTracker) that are constructed to exhibit fluorescence upon the induction of various pathways of relevance for (geno)toxicity and cancer; and 3) mES cells followed by mutagenicity testing (Hprt assay). The results showed increased DNA strand breaks (comet assay) for the NiO NPs and at higher concentrations also for the Ni NPs whereas no effects were observed for Ni ions/complexes from NiCl₂. By employing the reporter cell lines oxidative stress was observed as the main toxic mechanism and protein unfolding occurred at higher doses for all three Ni-containing materials. Oxidative stress was also detected in the HBEc cells following NP-exposure. None of these materials induced the reporter related to DNA damage and stalled replication forks. A weak, mainly not statistically significant increase in mutations was observed for all Ni-containing materials. We conclude that the NPs show more pronounced genotoxic effects compared to Ni ions/complexes, indicating more serious health concerns. The mechanism appears to be oxidative stress-driven rather than direct DNA binding.

Cytotoxic Effect of Mycosynthesized Silver Nanoparticles: A Mechanistic Perspective Study

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Silver nanoparticles (AgNPs) have emerged as most widely used nanomaterial especially in healthcare and medicine. Physically and chemically synthesized AgNPs are already being preferred for such applications, while fungal mediated synthesis of AgNPs, termed as mycosynthesised AgNPs are gaining attention in the recent era. With the rise in applications, the reports of their toxic effect on cells of human origin are also increasing. Hence, there is a pressing need to study their nanotoxicological aspect. Past studies mainly focused toxicity of AgNPs prepared by chemical and physical methods. On the contrary, the toxicity of mycosynthesised AgNPs remained a neglected issue. As far as human exposure is concerned, these nanoparticles can enter the human body through oral, dermal and nasal exposure. Hence present study utilized the representative cell lines of these routes viz. IEC6, HaCat and A549 respectively representing intestine, skin and lung. The exposure study utilized AgNPs synthesized by Fusarium oxysporum. The rationale behind using this fungus is that many studies reported it to synthesize AgNPs rapidly and at low cost. After synthesis, they were characterized by various methods. The visual observation of change in color from colorless to brown gave the primary confirmation of synthesis. Further characterization by UV-Vis Spectrophotometry shown the characteristic peak around 420 nm, FTIR analysis confirmed the capping of nanoparticles by fungal proteins, X-ray diffraction study confirmed the presence of elemental composition to be silver, Nanosight LM20, and Zeta potential analysis showed the presence of polydisperse and stable nanoparticle respectively. Transmission Electron Microscopy showed the nanoparticle size ranging from 5-25 nm. During in vitro toxicity assessment studies, MTT assay revealed their IC50 value to be 8 µg/mL. Lactate dehydrogenase leakage assay concluded that they cause damage to the cell membrane, leading to leakage of cellular content. Through Reactive Oxygen Species assay, it was found to induce accumulation of reactive radicals, damaging cellular materials including the DNA which was confirmed by DNA fragmentation assay. In summary, the mycosynthesized AgNPs shown the mechanistically similar mode of action to damage all the three cell lines, showing the similar damage to cell irrespective of the organ of origin.

Nanoceria Dissolved in Acidic Environment Is Stabilized by Some Carboxylic Acids


Nanoceria is an abrasive used to prepare integrated circuits by chemical mechanical planarization and is also a diesel fuel additive, among other applications. It has therapeutic potential for multiple conditions with an oxidative stress/inflammation component including cancer, radiation damage, bacterial infection, sepsis, wounds, stroke-induced ischemia, and retinal degeneration. The objective was to determine conditions that contribute to its dissolution and to identify carboxylic acids that stabilize it under aqueous conditions. Citric acid is a stabilizing agent for hydrothermal nanoceria syntheses and for nanoceria dispersions. Citrate-coated nanoceria (primary particle size ~4 nm, hydrodynamic diameter of agglomerates ~14 nm) was introduced into 2 kD MWCO Slide-A-Lyzer™ dialysis cassettes immersed in iso-osmotic media. Samples were repeatedly withdrawn over 28 weeks from within the cassette and media surrounding the cassette for cerium quantitation by ICP-MS and high resolution electron microscopy. Dissolution-induced nanoceria transformation occurred at pH 4.5, increasing cerium in media surrounding the cassette and decreasing primary particle size. Citric, malic, and lactic acids and ammonium maintained the ~14 nm agglomerates, which grew to ~1 micron in media containing 7 other carboxylic acids. Nanoceria transformation occurred over 3 phases. 1) Formation of superstructures and initiation of pH-dependent nanoceria dissolution. 2) Oxidative nanoceria dissolution and reduction of primary particle (but not secondary structure) size. 3) Appearance of porous structured agglomerates, assuming a skeletal appearance. In contrast, nanoceria was stable for months in iso-osmotic citrate at pH 7.2, water, and in the presence of horseradish peroxidase and hydrogen peroxide at pH 6.1 (a condition that degrades carbon nanotubes). By extension of these observations, acidic environments, as found in phagolysosomes, may degrade nanoceria by dissolution. Carboxylic acids form coordination complexes with metal ions. Formation of cerium-chelate complexes after nanoceria dissolution may enable cerium redistribution within and across the cell, and uptake into plant roots. Ligands that enable nanoceria dissolution in acidic environments may greatly affect nanoceria’s fate (dissolution rate and size). Supported by 1R01GM109195.

Influence of Titanium-di-Oxide Nanomaterial Agglomeration on Toxicity and Biological Responses In Vitro

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Nanoparticles (NPs) of size 1-100 nm can be potentially toxic due to their very high surface area compared to their bulk counterparts. Agglomeration and Aggregation (AA) of NPs reduces their toxicological impact, but their toxicological relevance is not fully elucidated. The rise in applications, while fungal mediated synthesis of AgNPs, termed as mycosynthesised AgNPs are gaining attention in the recent era. With the rise in applications, the reports of their toxic effect on cells of human origin are also increasing. Hence, there is a pressing need to study their nanotoxicological aspect. Past studies mainly focused toxicity of AgNPs prepared by chemical and physical methods. On the contrary, the toxicity of mycosynthesised AgNPs remained a neglected issue. As far as human exposure is concerned, these nanoparticles can enter the human body through oral, dermal and nasal exposure. Hence present study utilized the representative cell lines of these routes viz. IEC6, HaCat and A549 respectively representing intestine, skin and lung. The exposure study utilized AgNPs synthesized by Fusarium oxysporum. The rationale behind using this fungus is that many studies reported it to synthesize AgNPs rapidly and at low cost. After synthesis, they were characterized by various methods. The visual observation of change in color from colorless to brown gave the primary confirmation of synthesis. Further characterization by UV-Vis Spectrophotometry shown the characteristic peak around 420 nm, FTIR analysis confirmed the capping of nanoparticles by fungal proteins, X-ray diffraction study confirmed the presence of elemental composition to be silver, Nanosight LM20, and Zeta potential analysis showed the presence of polydisperse and stable nanoparticle respectively. Transmission Electron Microscopy showed the nanoparticle size ranging from 5-25 nm. During in vitro toxicity assessment studies, MTT assay revealed their IC50 value to be 8 µg/mL. Lactate dehydrogenase leakage assay concluded that they cause damage to the cell membrane, leading to leakage of cellular content. Through Reactive Oxygen Species assay, it was found to induce accumulation of reactive radicals, damaging cellular materials including the DNA which was confirmed by DNA fragmentation assay. In summary, the mycosynthesised AgNPs shown the mechanistically similar mode of action to damage all the three cell lines, showing the similar damage to cell irrespective of the organ of origin.
USPION exhibit physicochemical properties that are advantageous for theranostic biomedical products, such as intravenously administered medical imaging contrast agents; however, the toxicity mechanisms are not completely elucidated. Therefore, the goal of this study was to evaluate cellular interactions of USPION (PVP-coated) on human coronary artery endothelial cells (HCAECs) as a vascular cell model. USPION had an average size of ~20 nm, were negatively charged, and of spherical shape as assessed by transmission electron microscopy (TEM), dynamic light scattering, and zeta potential analyses, respectively. Using the alamar blue (AB) assay, cells treated with 25 or 200 µg/mL exhibited decreased cell viability to ~80% and 50% of controls, respectively, after 6 h of USPION exposure. Cellular uptake was evaluated by TEM after 3 h exposure of HCAEC to 25 µg/mL USPION. To evaluate the role of reactive oxygen species (ROS) production on cytotoxicity, cells were incubated with DCFDA dye, exposed to 25, 50, 100 or 200 µg/mL of USPION for 6 and 16 h, and fluorescence emission was evaluated by fluorescence spectrophotometry and microscopy. To further determine the role of ROS, HCAEC were pretreated with the antioxidant N-acetyl-cysteine (NAC) for 1 h prior to USPION (100 µg/mL) treatment, and cell viability was assessed by AB. Internalization of USPION in secondary lysosomes and perinuclear localization was observed as early as 3 h of exposure. No ROS production was apparent when using spectrophotometric DCFDA assay and fluorescence was detected in only a few cells exposed to 200 µg/mL USPION, with signal interference caused by particle overload in the cytoplasm. Our results indicate that there is no evidence of increased ROS production after USPION exposure nor increased cell viability by pretreating HCAEC with NAC prior to USPION exposure; thus, the toxicity mechanism appears unrelated to ROS production under the experimental conditions used.

**2720 DHA-Supplemented Diet Reduces Nanoparticles-Induced Lung Inflammation with Methylation Changes**

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Nanoparticles such as multi-walled carbon nanotubes (MWCNT) have many benefits and are used for many different purposes, such as medical and personal products. Exposure to MWCNT has been found to cause inflammation and lung disease, but the mechanism is still uncertain. It is becoming increasingly evident that dietary and environmental influences can result in physiological changes through epigenetics. Epigenetic alterations are known to hold substantial potential as biomarkers for environmental exposures; this, in turn, may provide insight into mechanisms of environmentally related diseases and allow for a better understanding of disease etiology. Therefore, we utilized a murine model to determine the effect of dietary docosahexaenoic acid-supplemented diet (DHA-SD) on MWCNT-induced inflammation, lung disease, and epigenetic changes. Balb/c mice were fed with normal diet or 50µM DHA-SD for 4 weeks, then both normal and DHA diet-mice were exposed to either dispersion media, 50 µg FA-21 (high Ni-MWCNT), or silica (SiO2) as a positive control via oropharyngeal instillation. One week post-exposure to particles, lung and blood were harvested for analyses and DM controls. No significant BBB changes were observed using SCIP assay on mouse brain endothelial cells (MBEC) and 2) myography using naiv thoracic aorta from male C57BL6 mice incubated with 1% serum from exposed mice to evaluate vasodilatory changes. Analyses of BALF inflammatory cytokine levels revealed dose-dependent increases in IL-1β, IL-4, IL-5, TNF-α and KC/GRO. Maximal neutrophil influx was observed 1d following the 3 µg dose, but at 3d following the 10 µg dose. A significant increase in TNF-α gene expression was observed with serum containing the exosomal fraction when compared with exosome depleted fractions and protein controls. No significant changes were observed using sodium fluorescein cerebral uptake assay. Further evaluations of BBB integrity will be done via immunohistochemistry. Evaluation of other systemic parameters are ongoing. These preliminary findings suggest that MWCNT pulmonary exposure induces inflammatory activation in the lungs of C57BL6 mice in a dose- and time-dependent manner and may produce serum bioactivity via exosomal delivery.
Engineered nanoparticles are used throughout industry and consumer products; however, we are just now beginning to mechanistically understand how inhalation exposure to nanoparticles may impact human health. Most research efforts have focused on pulmonary disease endpoints such as fibrosis, allergic-type reactions, and cancer, while the area of viral pathogen susceptibility remains less well explored. Previous work in our group has determined that respiratory epithelial cell (SAECs) to single-walled carbon nanotubes (SWCNTs) increases host susceptibility to Influenza A virus (IAV) infection. To better understand the molecular mechanisms that contribute to these observations, we investigated how SWCNTs modulate the host innate immune response, with a focus on lipid metabolism enzymes such as sphingosine-1 phosphate lyase (SPL) and sphingosine kinase 1 (SK1), and expression of interferon-stimulated genes (ISGs), specifically interferon-induced proteins with tetratrico peptide repeat (IFITMs) and interferon-induced transmembrane proteins (IFITMs). We hypothesized that SWCNTs are inhibiting expression of the lipid metabolism enzyme, SPL, resulting in decreased expression of ISGs, and thus increased viral titers. For our approach, we exposed SAECs to 20 μg/ml pristine SWCNTs for 24 hours, followed by exposure to IAV for 24 hours. We quantified viral infection with viral titers and the mRNA expression of SPL, SK1, IFITM genes via quantitative real-time polymerase chain reaction (qRT-PCR). Gene expression analysis showed that SWCNTs did not alter the expression of ISGs alone, but did significantly repress IAV-induced expression of several specific ISGs. Lipidomic analysis via LC/MS-MS demonstrated significant changes in sphingolipid and ceramide species. The importance of the balance between lipid metabolism enzymes, rather than their individual levels was also demonstrated. Future work will focus on the impacts of inhibition or over-expression of SPL and SK1 on the interaction between SWCNTs and IAV in SAECs. These studies highlight the important role that lipids play in the immune response, as well as the importance of assessing pathogen susceptibility when determining the environmental health and safety of nanomaterials.

Carbon Nanodots (CNDs) are a new class of carbon nanomaterials at the forefront of research for inherent therapeutic and biological applications. Their fluorescence and stability permit biological labeling, bio-imaging, and drug delivery. In this study, we studied the effects of CNDs on human NADPH oxidase-1 (NOX1), glutathione S-Transferase (GST) and glutathione reductase (GR) on human EA.hy926 endothelial cells. Our results indicated that CNDs at various concentrations showed no adverse effects on EA.hy926 cells. Further, there was a significant increase in the levels of NAD(P)H: quinone oxidoreductase-1 (NOX1), a vital cellular defense against oxidative stress and overproduction of reactive oxygen species (ROS). We also significantly reduced TNF-α-induced expressions of IL-8, monocyte chemotactic protein 1 (MCP1) and Interleukin Molecule 1 (ICAM-1), three important markers of vascular inflammation. Overall, these results suggest that CNDs could serve as a viable option for the treatment of inflammatory diseases such as atherosclerosis.
ACD response. Interestingly, we have identified that 20 nm silica NPs, a common NP in consumer goods, suppress the late phase inflammation and edema in a mouse model of ACD. Upon examination of the skin tissue, it appears as though 20 nm silica NPs reduce inflammatory cytokines (IL-6, IL-1β, and KC) and mast cell degranulation 24 hours after NP and DNPβ application. The suppressive effects of silica NPs also appear to be both size- and charge-dependent. Furthermore, NPs and aminated NPs fail to suppress the ACD response. Preliminary evidence suggests a direct effect of the NPs on skin, rather than a change in the bioavailability of DNPβ due to NP binding. When applying both 20 nm and 400 nm at a constant surface area, only the 20 nm silica NPs suppress the ACD response. Future studies will include looking at activation signals for the TLR4 downstream pathway including MyD88, TAK-1, IKK complex, and NF-κB in BEAS-2B cells stimulated with NiNP and LPS. This response was specific to NiNP as no other MNPs were able to increase IL-6 and IL-8 expression. Also, the synergistic effect of NiNP on LPS-induced cytokine production was only observed in human bronchial epithelial cells (BEAS-2B). NiNPs selectively increase LPS cytokine expression in human BEAS-2B cells to enhance IL-6 and IL-8 production at both mRNA and protein levels suggesting that they are able to promote further inflammation in the lung. Further study will help to elucidate the mechanism of adjuvant effect of NiNPs on LPS-induced lung inflammation and will include looking at activation signal for the TLR4 downstream pathway including MyD88, TAK-1, IKK complex, and NF-κB in BEAS-2B cells stimulated with NiNP and LPS. Supported by NIH Grant R01-ES020897, NIEHS Training Grant T32ES007046, and NSF Grant 15-022.

**2726 Requirement of STAT6 in Exacerbation of Allergic Lung Inflammation in Mice by Multi-Walled Carbon Nanotubes**


Multi-walled carbon nanotubes (MWCNTs) have numerous applications in electronics, engineering and medicine. There is increasing evidence that MWCNTs can have harmful effects upon inhalation. Of particular susceptibility are individuals with asthma, an allergic lung disease characterized by elevated levels of IgE, immune response, airway hyper-sensitivity, mucus cell metaplasia and airway remodeling. Signal transducer and activator of transcription 6 (STAT6) is a transcription factor with roles in TH2 type inflammation. With this study we sought to examine the role of STAT6 in MWCNT-induced asthma exacerbation. Furthermore, we hypothesized that STAT6 is required for exacerbation of allergen-induced lung inflammation by MWCNTs. Male C57BL6 and STAT6-/- mice were dosed via intranasal aspiration on days 0, 2, 4, 14, 16 and 18 with either vehicle, HDM, or a combination of HDM and tMWCNT; n=41. Necropsy was performed by NIEHS Grant R01ES020897 (JCB), NIEHS Training Grant T32ES007046, and NSF Grant 15-022 (JCB).

**2727 Selectively of Nickel Nanoparticles in Enhancement of LPS-Induced Pro-Inflammatory Cytokine Production in Human Bronchial Epithelial Cells In Vitro**


Nickel nanoparticles (NINPs) are commonly used as a catalyst for the production of multiwalled carbon nanotubes (MWCNTs). Nickel serves as an allergen that can directly activate the Toll-Like Receptor 4 (TLR4). TLR4 is also activated by Lipopolysaccharides (LPS), a ubiquitous component derived from gram-negative bacteria that is known to induce lung inflammation by causing secretion of pro-inflammatory cytokines (e.g., IL-6, IL-8). Recently, it was found that metal nanoparticles (MNPs) enhanced allergy in the presence of LPS in mice. Since major route of exposure for NiNPs is through inhalation, we hypothesized that NINPs would exacerbate LPS induced lung inflammation through amplification of downstream signaling intermediates (e.g., NF-κB, MyD88) to enhance IL-6 and IL-8 production. Human bronchial epithelial cells (BEAS-2B) were stimulated with NINPs (4 ug/cm²), LPS (50 ng/ml), or both. Cell supernatants and mRNAs were collected after 6, 24, and 48hr after the exposure to measure IL-6 and IL-8 protein secretion and mRNA expression levels, respectively. Different cell lines including human monocytic cells (THP-1) and mouse bronchial epithelial cells (E10), and different MNPs (cobalt, silver, and gold) were used to determine the species specificity and selectivity of nanoparticles on enhancement of LPS-induced cytokine production. NINPs synergistically increased LPS production of IL-6 and IL-8 both at mRNA and protein level in BEAS-2B cells. This response was specific to NiNP as no other MNPs were able to increase IL-6 and IL-8 expression. Also, the synergistic effect of NiNP on LPS-induced cytokine production was only observed in human bronchial epithelial cells (BEAS-2B). NiNPs selectively increase LPS cytokine expression in human BEAS-2B cells to enhance IL-6 and IL-8 production at both mRNA and protein levels suggesting that they are able to promote further inflammation in the lung. Future research will continue to identify the mechanism of action of these small negatively charged silica NPs.

**2728 Pulmonary Engineered Nanomaterial Exposure during Late Gestation Attenuates Plasma Estrogen**


Maternal exposure to engineered nanomaterials (ENMs) during gestation is associated with uterine vascular impairments as well as endothelial disruptions that may lead to altered gestational outcomes. We have previously shown that nano-titanium dioxide (nano-TiO2) inhalation impairs endothelium-dependent arterial dilation in uterine arterioles of pregnant rats. However, the mechanism underlying this dysfunction has not been fully determined. One possibility is alterations in the steroid hormone estrogen (E2), which is elevated during pregnancy. Furthermore, E2 is critical for synthesis of the hormone kisspeptin (Kiss), which is a potent vasconstrictor. Therefore, we examined how circulating E2 and Kiss are involved in nano-TiO2-induced vascular dysfunction. Pregnant (gestation day (GD) 10) Sprague-Dawley (SD) rats were exposed to nano-TiO2 aerosols (11.3 ± 0.1 mg/m3/hr, 5 hr/d, 8.2 ± 1 day; n = 6) or sham exposed (n = 6) to evaluate endocrine consequences of maternal exposure. Plasma was collected on GD 20 to evaluate circulating levels of E2, progesterone (Pg), luteinizing hormone (LH), follicle stimulating hormones (FSH), prolactin (PRL) and corticosterone (CORT). In a separate set of experiments, virgin or pregnant rats were exposed via intra-tracheal instillation to nano-TiO2 (100 µg) suspended in 200 µL of vehicle (normosol and 5% fetal bovine serum, n = 7) or sham controls (n = 5). 24 hours later thoracic aorta was dissected and multiple 2-3 mm segments were cut and mounted in each wire myograph chamber (DMT 620M) containing physiological salt solution (PSS) at 37°C. After 60 min of equilibration, the maximum contractile response was determined using high-potassium PSS (KPSS). Vessels were then washed with PSS and allowed to relax until initial tension returned. Contractile responses were determined via addition of a single dose of 50 µL phenylephrine (1 x 10−6), norepinephrine (50 µL of 0.1 x 10−4 to 1 x 10−1 M), and plasma E2, which was added to the PSS. Plasma E2 was significantly decreased at GD 20 in exposed (11.08 ± 2.5 %) vs. sham controls (94.7 ± 10.53%). These studies represent the first evidence pulmonary ENM exposure perturbs the normal gestational endocrine vascular axis via a Kiss dependent mechanism of dysfunction. Supported by NIH ES015022.

**2729 Nanoparticles Exposure and Health Effect Markers in Frozen Exhaled Breath Condensate**

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Exhaled breath condensate (EBC) could be an ideal biological matrix, as it could provide information about the subsequent biological effects of an inhalation exposure and as a biomarker of lung disease. The purpose of this study is to examine whether biomarkers in EBC could be correlated with nanoparticle concentration in EBC. 18 indium tin oxide (ITO)-exposed nanomaterial workers and 23 non-exposed controls were recruited from nanotechnology factories. A questionnaire was used to collect detailed information about life style, personal habits, disease history and occupational history. EBC was collected by an ECoScreen turbo (Viasys GmbH, West Germany).
Höchberg, Germany). The particle size-number distribution of ultrafine particles (PM0.1) were assessed NanoSight LM10 HS system (Nanosight Ltd., Salisbury, UK) using the Nanoparticle Tracking Analysis (NTA). The biomarkers measured for each aspect of toxic endpoints included: 1. Inflammation and oxidative damage markers, such as Clara cell protein (CC16), nitric oxide (FeNO), and 8-hydroxydeoxyguanosine (8-OHdG). 2. Cardiovascular biomarkers, such as fibrinogen, vascular cell adhesion molecule (VCAM), intercellular adhesion molecule-1 (ICAM-1), high-sensitivity C-reactive protein (hsCRP), and heart rate variability (HRV). 3. Lung function test. We found plasma Gpx (glutathione peroxidase) level was significantly higher in high particles concentration workers (log concentration≧8.1) than low particles concentration workers (log concentration<8.1). 8-isoPGF2alpha in EBC was not correlated with concentration (x10^8 #particles/mL), % of UFP and particles number of UFP. Particles concentration (x10^8 #particles/mL) was also not associated with 8-OHdG in urine, plasma and WBC as well as SOD in plasma. The possible reasons for the correlation between particles concentration and elevated levels of both Th1 and Th2 cytokines over control are increased levels of CXCL1/KC, LDH and protein in the BALF. The lung inflammation induced by Nano-Ni was confirmed by histological examination, which showed inflation of a large amount of polymorphonuclear (PMN) cells and macrophages in the alveolar space, alveolar septa, peribronchial and peribronchiolar areas. Nano-Ni also caused increased MMP-2/9 protein levels and activity in the BALF. By six weeks after exposure, Nano-Ni-exposed mice developed chronic lung inflammation as evidenced by infiltration of neutrophils and enlarged foamy macrophages and extensive pulmonary fibrosis. Nano-Ni-exposure caused acute biomarkers and chronic inflammation in BALB/c mice, but at a much lesser degree. Nano-Ni-P caused a moderate acute neutrophilic inflammation, but mostly resolved by six weeks. Nano-Ni-C only caused mild acute and chronic inflammation. Our results suggest that Nano-Ni exposure can cause severe acute and chronic lung inflammation and injury while surface modification such as carbon coating can alleviate Nano-Ni-induced lung injury.

2730 Surface Area- and Mass-Based Comparison of Lung Toxicity and Allergic Exacerbation in an Ovalbumin Asthma Model following Pulmonary Exposure to Fine and Ultrafine Nickel Oxide

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The correlation of specific nanomaterials' physico-chemical properties with toxicological responses is an area of growing interest with implications for the selection of appropriate dose metrics in nanotoxicity studies. In this study, the role of nickel oxide mass and surface area in the induction of pulmonary inflammation and exacerbation of respiratory allergy was explored. To address this concept, 181 nm fine (NiO) and 90 nm ultrafine (NiONP) particles were characterized and incorporated into an in vivo time course study and ovalbumin (OVA) asthma model. Particle toxicity was compared at equal masses of 40 micrograms and at equal surface areas of 1.92 mm^2. For the time course study, female BALB/c mice were exposed once to particles or vehicle control by oropharyngeal aspiration and euthanized 1, 10, 19, or 29 d post-exposure, which represent critical time points in the OVA model. For the OVA model, mice were exposed with particles on d 0, sensitized to OVA via IP injection on d 1 and 10, challenged with OVA by aspiration on d 19 and 28, and euthanized on d 29. In the time course study, exposure to mass-normalized doses of particles resulted in significantly elevated lactate dehydrogenase levels, lung neutrophil number, and mediastinal lymph node size in mice exposed to NiONP, which persisted to 29 d post-exposure. However, normalization of doses for surface area mitigated all differences between particles, suggesting that NiO surface area drives pulmonary inflammation. In the OVA model, exposure to equal masses of NiO and NiONP induced differential mechanisms of immune cell infiltration. Exposure to NiO caused increased pH over allergy controls and higher levels of Th2 cytokines in the lavage fluid. Exposure to NiONP resulted in significantly increased lymph node size over all other groups and elevated levels of both Th1 and Th2 cytokines over control animals, but reduced serum OVA IgE levels. Interestingly, normalization of doses for surface area in the OVA model mitigated differences in serum IgE and Th2 cytokine levels, but not eosinophil influx to the lung and Th1 cytokines. Overall, findings suggest that although surface area of NiO dictates pulmonary injury and inflammation, it may not be the only physico-chemical property responsible for modulation of immune responses in the lung.

2731 Comparative Mouse Pulmonary Toxicity of Nickel Nanoparticles with Different Surface Modification

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With the development of nanotechnology, a large amount of nanoparticulates are being produced or will be produced. Due to their specific physical and chemical properties, nanoparticulates have been increasingly used in the fields of engineering, information technology and biomedicine. Nickel nanoparticles, because of their low melting point, high magnetism, high surface area, and high reactivity, have found applications in various areas including industry, environment, and biomedicine. Thus occupational and non-occupational exposure to nickel nanoparticles are increasing. It is necessary and urgent to study the adverse effects of nickel nanoparticles on individuals as well as to find ways to alleviate their toxicity. In this study, we examined and compared the short-term and long-term effects of three kinds of nickel nanoparticles with different surface modification on mouse lungs. Mice were intratracheally instilled with nickel nanoparticles without surface modification (Nano-Ni), nickel nanoparticles partially passivated with oxygen (Nano-Ni-P), or nickel nanoparticles coated with a thin layer of carbon (Nano-Ni-C). Control mice were instilled with normal saline. Our results showed that Nano-Ni exposure caused severe acute lung inflammation at day 3 after exposure, which was reflected by increased number of neutrophils and increased levels of CXCL1/KC, LDH and protein in the BALF. The lung inflammation induced by Nano-Ni was confirmed by histological examination, which showed infiltration of a large amount of polymorphonuclear (PMN) cells and macrophages in the alveolar space, alveolar septa, peribronchial and peribronchiolar areas. Nano-Ni also caused increased MMP-2/9 protein levels and activity in the BALF. By six weeks after exposure, Nano-Ni-exposed mice developed chronic lung inflammation as evidenced by infiltration of neutrophils and enlarged foamy macrophages and extensive pulmonary fibrosis. Nano-Ni-exposure caused acute biomarkers and chronic inflammation in BALB/c mice, but at a much lesser degree. Nano-Ni-P caused a moderate acute neutrophilic inflammation, but mostly resolved by six weeks. Nano-Ni-C only caused mild acute and chronic inflammation. Our results suggest that Nano-Ni exposure can cause severe acute and chronic lung inflammation and injury while surface modification such as carbon coating can alleviate Nano-Ni-induced lung injury.

2732 Lung Deposition and Retention of Multi-Walled Carbon Nanotubes after 28-Day Inhalation and 28-Day Post Exposure in SD Rats

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The newly revised OECD inhalation toxicity testing guidelines 412 and 413 require measurement of lung deposition and retention of poorly soluble nanomaterials in order to evaluate their clearance and biopersistence. With this aim, male SD rats were exposed to MWCNT at 0, 0.257, 1.439 and 4.255 mg/m^3 for 28 days (6h/day, 5day/week, 4week). After 28 days of exposure, the rats were sacrificed at post-1, 7, and 28 days and bronchoalveolar lavage (BAL) fluids were obtained to evaluate changes in inflammatory cells and markers. Blood biochemistry, hematology and histopathology examinations of the lungs were also conducted. The lung deposition and retention of MWCNT were evaluated by elemental carbon (EC) content in the lungs after digestion of lung cells (PMN) and LDH in the BAL fluids increased in a significant concentration-dependent manner after 28 days of MWCNT exposure and at 7 and 28 days post exposure compared with the controls. Lung deposition and retention of MWCNT in the lungs showed concentration and time dependency after 28 days of MWCNT exposure and post-exposure 7 and 28 days. Based on these endpoints examined a NOAEL was identified at 0.257 mg/m^3 for 28 day subacute inhalation of MWCNT. (1159).

2733 Role of Toll-Like Receptor 5 in Multi-Walled Carbon Nanotube-Induced Lung Injury

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Innate immune responses play a significant role in mediating environmental lung injury. Toll-like receptors (TLRs) recognize exogenous (pathogen-associated) or endogenous (danger-associated) molecular patterns and shape innate immune responses. Bacterial Flagellin is the only known ligand for TLR5. However, we have recently demonstrated a significant contribution of TLR5 in sterile lung injury induced by either ozone or short fragment hyaluronan, suggesting that TLR5 may be involved in lung injury responses. The contribution of TLR5 in nanomaterial-induced lung injury is completely unknown. We aimed at investigating the role of TLR5 in multi-walled carbon nanotube (MWCNTs)-induced lung injury. We exposed wild type and TLR5 KO mice to well characterized tangled or rod-like MWCNTs (single oropharyngeal aspiration of 2 mg/kg) and studied lung injury at days 1 and day 21 post exposure. Lung lavage analyses and histology were employed to assessed lung injury and inflammation. Measurement of total lung collagen and morphometry was used to evaluate pulmonary fibrosis. Both forms of MWCNT induced significant lactate dehydrogenase and cytokine release in bronchoalveolar lavage fluid at day 1 and day 21. Lavage fluid from TLR5 KO mice had higher amounts of total proteins and LDH at day 21 post exposure. Inflammatory and fibrotic mediators such as (osteopontin, platelet derived growth factor-aa and transforming growth factor-beta) were elevated at days 1 and 21 after both forms of nanotube
exposure. However, the magnitude of inflammatory response was significantly higher in rigid rod-like MWCNT exposed TLRS KO mice. In conclusion, these results demonstrate a potential role of TLRS in mediating MWCNT induced lung injury. Further studies are underway to explore the mechanistic basis of observed responses.

2734 Mouse Pulmonary Dose- and Time Course-Responses Induced by Exposure to Nitrogen-Doped Multi-Walled Carbon Nanotubes

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Multi-walled carbon nanotubes (MWCNT) can be modified by doping them with other elements. These modifications change the intrinsic properties of MWCNT and may also alter their bioactivity. In this study, we compared the in vivo bioactivity of nitrogen doped multi-walled carbon nanotubes (NDMWCNT) to pristine MWCNT to test the hypothesis that nitrogen doping would alter their bioactivity. First, characterization of the MWCNT and NDMWCNT was conducted. High resolution TEM confirmed the multilayer structure of MWCNT with an average layer distance of 0.36 nm, which was not altered by nitrogen doping. TEM analyses indicated the nanomaterials had similar widths (NDMWCNT=53 nm, MWCNT=49 nm) and lengths (NDMWCNT=4.73 µm, MWCNT=3.86 µm). Comparison of MWCNT and NDMWCNT XPS spectra demonstrated presence of a N1s peak, while FTIR spectra indicated the presence of N-H bonding only in the NDMWCNT sample. For in vivo studies, male C57BL/6J mice received a single dose of either dispersion medium (DM; vehicle control), 2.5, 10, or 40 µg/mouse of MWCNT, or 40 µg/mouse of NDMWCNT. Animals were euthanized at 1 and 7 days post-exposure for whole lung lavage (WLL) studies. NDMWCNT caused time- and dose-dependent pulmonary inflammation. However, on an equivalent mass basis, it is less than that caused by MWCNT. Activation of the NLRP3 inflammasome was assessed by qPCR in lung mRNA. A significant increase in NLRP3 transcripts was observed in NDMWCNT-exposed mice relative to MWCNT-exposed mice. At 56 days post-exposure, histopathological analyses determined lung fibrosis in MWCNT-exposed mice was greater than that determined for NDMWCNT-exposed mice. These data indicate that nitrogen doping of MWCNT decreases their bioactivity, and that lower activation of the NLRP3 inflammasome by NDMWCNT relative to MWCNT may be responsible.

2735 Multi-Walled Carbon Nanotube Inhalation Alters Expression of SP-D and Inflammation in the Rat Lung


When aerosolized, multi-walled carbon nanotubes (MWCNT) pose an inhalational hazard. Previous studies have shown that rats exhibit a dose dependent acute inflammatory response without altering mucous disbursement and that MWCNT are not retained in the lungs for up to 30 days. Surfactant protein-D (SP-D) is an airway epithelial cell-derived lung collectin, an important modulator of the innate and adaptive immune response, and a regulator of apoptotic cell clearance in the lung. The objective of this study was to examine the effects of long term MWCNT exposure via inhalation, on levels of SP-D expression. Male Sprague Dawley rats were exposed to aerosolized MWCNT at 0.06, 0.2, and 0.6 mg/m3 for a total of 22 days over the course of one month. Bronchoalveolar lavage (BAL) fluids were taken 5 days post-exposure to obtain BAL differential cell counts and total protein concentration. Adherent lung samples were analyzed for SP-D gene expression and accumulation of neutrophils and macrophages. Native gel electrophoresis and Western blot analysis for SP-D levels in cell-free BAL fluid were performed and BAL cell counts and SP-D levels were correlated by regression analysis. Exposure to MWCNT’s induced a significant change in BAL neutrophil counts and SP-D protein expression. Rats exposed to 0.2 mg/m3 of aerosolized MWCNTs had significantly lower levels of SP-D than control rats exposed to ambient air. However, rats exposed to a concentration of 0.6mg/m3 MWCNTs expressed significantly more SP-D when compared to the control group. The increase in SP-D level in this group coincided with a significant increase in numbers of neutrophils found in BAL cell differentials. Additionally, there was a negative correlation between numbers of macrophages in the BAL of this high concentration exposure group and the levels of SP-D in BAL supernatant. This data suggests that chronic exposure to MWCNT contributes to increased expression of SP-D which may alter macrophage uptake of MWCNT and attenuation of inflammatory cell accumulation in BAL. Supported by: P30 ES023513, T32 ES007059, U01 ES021027.

2736 Kinetic Time Courses of Inhaled Silver Nanoparticles in Rats

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Silver (Ag) NPs are emerging priority substances closely monitored by main health and safety agencies. Despite their extensive use, there is a lack of data on their in vivo behavior in the body, in particular following inhalation, the predominant route of exposure in the workplace. We thus evaluated the toxicokinetics of Ag NPs after inhalation in rats exposed nose-only to 20 nm Ag NPs during 6 h at a concentration of 15 mg/m3 (21834 ± 85512 particles/cm3). The generated aerosol showed a uniform size distribution of Ag agglomerates with a geometric mean (± SD) of 791 ± 1.888 µm. The time courses of Ag concentration in lungs, blood, tissues and excreta were established over 14 days following the onset of inhalation. All samples were analyzed using inductively-coupled plasma mass spectrometry (ICP-MS). Elimination profiles revealed that feces was the dominant excretion route and represented on average (± SD) 5.1 ± 3.4 % of the inhaled exposure dose compared with urinary excretion, which amounted to 0.01 ± 0.006 %. In the lungs, highest percentage of inhaled dose was observed at the end of the 6-h inhalation and reached 1.9 ± 1.2 %. Blood concentrations increased progressively post-inhalation, reached a maximum at 168 h and decreased thereafter, but values remained 80 times lower than lung levels. In extrapulmonary organs, such as liver, spleen, and kidney, concentrations were also much lower than lung concentrations, representing less than 0.2 % of dose at all times and peak levels were reached at t = 72 h followed by a progressive decrease over the 14-day sampling. Results show that only a small percentage of inhaled dose reached the lungs - the majority of dose most plausibly remained in the upper respiratory tract - and that Ag NPs also poorly translocated to the systemic circulation.

2737 Short-Term Inhalation Study of Graphene Oxide Nanoplates

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Graphene oxides possessing unique physicochemical properties are expected to be applied within industrial science for electronics, pharmaceuticals and medicine. However, the toxicity of graphene oxide exposure has not been clarified. Therefore, a short-term inhalation toxicity study of graphene oxide was conduct by using a nose-only inhalation exposure system for male Sprague-Dawley rats. A total of four groups (15 rats per group) were exposed: (1) control (fresh air) (2) low concentration (0.76 ± 0.16 mg/m3) (3) moderate concentration (2.60 ± 0.19 mg/m3) (4) high concentration (9.78 ± 0.29 mg/m3). The rats were exposed to graphene oxide for 6 h/day for 5 days followed by recovery 1, 3, and 21 days. The concentration of graphene oxide did not change body weight, and organ weight of the rats after the short-term exposure during the recovery period. Minimal differences were observed in the level of lactate dehydrogenase, alkaline phosphatase, total protein, and albumin between the exposed and control groups suggesting minimal liver and kidney damage. In addition, no significant difference was observed in the bronchoalveolar lavage cell differential such as lymphocytes, macrophages, and PMN. Graphene oxide ingested alveolar macrophages were observed in the lungs of all concentration groups from post 1 day to post 21 days. Thus, these short-term inhalation studies provide important initial information about toxicities between graphene and graphene oxide following nose-only exposures in lung and lack of toxicity in other organs.
Comparison of the Toxicological Effects of Multi-Walled Carbon Nanotubes and Nitrogen-Doped Multi-Walled Carbon Nanotubes on Rat Lung Function

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The toxicological effects of multi-walled carbon nanotubes (MWCNT) have been widely investigated. Outcomes can range from initiating pulmonary inflammation and fibrosis to cardiovascular and central nervous system effects in animal models. Previous studies in our laboratory have shown that inhalation of MWCNT (Mitsui-7) results in airway hyperactivity to methacholine (MCh) in rats. Nitrogen-doped multi-walled carbon nanotubes (N<sub>2</sub>-MWCNT) are MWCNT that have been functionalized to incorporate nitrogen. They are utilized in applications such as lithium batteries and as matrix fillers in composite materials. N<sub>2</sub>-MWCNT are shorter and more brittle than their MWCNT counterparts, which may reduce toxicity. The purpose of this study was to determine if exposure to N<sub>2</sub>-MWCNT results in alterations in lung function that are comparable to those observed after MWCNT exposure. Sprague-Dawley rats were administered MWCNT or N<sub>2</sub>-MWCNT (25, 50, or 250 µg) in dispersion medium by intratracheal instillation. Lung resistance (R<sub>L</sub>), dynamic compliance (C<sub>dyn</sub>), and reactivity to MCh aerosol were examined at 1 and 7 days after treatment with 25, 50, and 250 µg MWCNT. Additionally, baseline R<sub>L</sub> was decreased at 7 days after treatment with 50 µg MWCNT without a corresponding change in reactivity to MCh. The only alteration observed in N<sub>2</sub>-MWCNT exposed animals was a decrease in baseline R<sub>L</sub> at 7 days after 250 µg N<sub>2</sub>-MWCNT exposure, with no corresponding change in reactivity to MCh. These results suggest that N<sub>2</sub>-MWCNT are less toxic than MWCNT with respect to lung function.

Pulmonary Toxicity and Gene Expression Changes in Response to Multi-Walled Carbon Nanotube Exposure in Rats


Carbon-based nanomaterials have significant commercial and industrial applications and, therefore, human exposure to them potentially resulting in adverse health effects should be anticipated. Understanding the molecular mechanisms underlying the toxicity induced by carbon nanotubes has merit in the prevention of the adverse health effects potentially resulting from their exposures. Currently, a rat toxicity model was employed to investigate the molecular mechanisms underlying the pulmonary toxicity induced by exposure to multi-walled carbon nanotubes (MWCNT). Rats were exposed, by whole-body inhalation exposure, to air or an aerosol containing MWCNT particles (5 mg/m<sup>3</sup>, 6 hours/day, 3 days). Toxicity and global gene expression profiles were determined in the lungs of the rats, 16-hours following the last exposure. MWCNT particles, often associated with alveolar macrophages (AMs), were detected in the lungs of the exposed rats. Lung histological changes resulting from MWCNT exposure were mild and consisted mainly of interstitial accumulation of macrophages. Bronchoalveolar lavage (BAL) toxicity parameters such as lactate dehydrogenase activity, number of AMs and PMNs, oxidant production by phagocytes, and levels of multiple cytokines (IL-1β, IL-10, MCP-1, MIP-2, and TNF-α) were significantly altered in response to inhalation exposure to MWCNT in the rats. Global gene expression profiling by next generation sequencing identified a large number of significantly differentially expressed genes (fold change >1.5 and FDR p value <0.05) in the lungs of the MWCNT exposed rats, compared with the controls. Bioinformatics analysis of the gene expression data identified significant enrichment of several disease/biological functions (for example, cancer, organismal injury and abnormalities, cell cycle, respiratory disease, cellular movement, and inflammatory response) and canonical pathways (for example, LXR/RXR activation, granulocyte and agranulocyte adhesion and diapedesis, complement system, acute phase response, and atherosclerosis signaling). Taken together, the data provided insights into the molecular mechanisms underlying the pulmonary toxicity induced by inhalation exposure of rats to MWCNT.

Characterization of Pulmonary Toxicity following Graphene Inhalation

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Graphene is a 2D nanomaterial with unique physicochemical properties making it highly marketable for applications in numerous industries. Our previous studies have shown that toxicity varies depending on size and oxidative form of graphene following a single bolus-dose aspiration in mice, with exposure to reduced graphene oxide (rGO) resulting in the greatest degree of lung injury and inflammation. The goal of this study was to characterize pulmonary toxicity following subacute inhalation of rGO. C57BL/6J mice were exposed to rGO (2.41 µm MMAD, 2.69 GSD) by whole body inhalation at 5.0 mg/m<sup>3</sup> for 5 h/d for 19 d, 0.5 mg/m<sup>3</sup> for 5 h/d for 19 d, or 0.5 mg/m<sup>3</sup> for 0.5 h/d for 19 d to achieve depositions with a two order of magnitude range. Controls were exposed to filtered air. Lung burden of rGO, parameters of lung toxicity and inflammation, and histopathology were analyzed 0, 3, 7, and 14 days post-exposure. Lung burden at 0 d was 0.78, 6.47, and 34.4 µg per lung for the low, middle, and high dose. Following high dose exposure, lactate dehydrogenase (LDH) in lung lavage fluid (LLF, lung injury) and total lavage cells (inflammation) were increased up to 1 m compared to control. The cellular increase was due primarily to macrophage influx. Neutrophils were also significantly increased; however, this cell population accounted for less than 1% of the total cells. Inflammation and injury resolved by 3 m. Inflammatory and tissue remodeling proteins in LLF followed a similar pattern. Pathologic analysis indicated increased macrophages in the lungs at 0 and 3 d as minimal, resolving over time. Particle burden in macrophages was also observed to decrease over time. Following middle dose exposure, total lavage cells increased at 3 d, due primarily to macrophage influx, and resolved by 1 m. Significantly fewer inflammatory proteins were elevated in LLF following exposure to the middle dose compared to the high dose, with resolution by 1 m. There was no difference in lung cellularity following low dose exposure. No lung pathology or increased LDH in LLF were observed following middle or low dose exposures. Exposure to rGO led to an acute dose-dependent increase in lung injury and inflammation, which resolved over time, with 0.5 mg/m<sup>3</sup> causing a minimal degree of inflammation representing the low observable effect level.

Developmental Toxicity Assessment of Four Different Preparations of Multi-Wall Carbon Nanotubes in Mice after Repeated Intratracheal Instillation

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Some studies have reported that maternal exposure to manufactured nanomaterials, including carbon nanotubes, may induce reproductive and developmental toxicity, such as teratogenicity. In order to initiate grouping and read across for filling data gaps in the developmental toxicity of carbon nanotubes via airway exposure, we conducted repeated intratracheal instillation studies of various preparations of multi-walled carbon nanotubes (MWCNTs) in pregnant mice. Four types of MWCNT dispersions (bulk, heat-treatment, single dispersion by Taquann method, and heat-treatment after single dispersion) were repeatedly administered to pregnant Crlj:CD1(ICR) mice on gestational days 6, 9, 12, and 15 at dosages of 4.0 mg/kg/day. Ten pregnant mice per group were dissected on gestational day 17, and then developmental toxicity was evaluated. The body weights of the heat-treatment MWCNTs exposed mice significantly decreased. In the other 3 groups, the body weights of MWCNTs exposed mice also decreased, although the changes were not statistically significant. Body weight of fetuses was significantly decreased in the bulk, the heat-treatment, and the heat-treatment after single dispersion MWCNTs exposed group. External malformations of fetuses were observed in the bulk and the heat-treatment MWCNT exposed groups. No statistically significant difference was observed between the control group and all MWCNT exposed groups in the numbers of corpora lutea, number of implantations, and placental weights. These results suggested the teratogenic effects caused by the intratracheal exposure of MWCNTs are dependent on their dispersion methods. Further examinations are needed to clarify the mechanism of the developmental toxicity by MWCNTs.
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The inhalatory route is considered their main form of entry of nanoparticles (NPs) and the lung is a vulnerable target organ given its direct interaction with the environment, being the alveolar region more susceptible. Exposure to metallic NPs, titanium dioxide (TiO2) and zinc oxide (ZnO), widely used in the manufacturing of diverse products (textiles, food products, electronics, etc.), leads to reactive oxygen species (ROS) generation and cytotoxicity. Alveolar cells produce pulmonary surfactant, constituted by lipids and surfactant proteins (A, B, C and D), which main functions are to maintain pulmonary homeostasis and the immune response; and have been reported to be affected by exposure to cigarette smoke and particulate matter. Our work aims to evaluate the relationship between the exposure to TiO2 and ZnO NPs and the expression of surfactant proteins in A549 human lung epithelial cells. Propidium iodide and MTS assays showed that the exposure after 12, 24 and 48 h to TiO2 and ZnO NPs in different concentrations (2.5, 5.0, 10, 15 and 20 µg/mL of ZnO; 25, 50, 100, 150 and 200 µg/mL of TiO2) do not affect cell viability. The production of ROS determined at 3, 12 and 24 h; showed an increase in the generation of superoxide anion (O2·−) after 12 and 24 h and hydrogen peroxide (H2O2) after 24 h of exposure to TiO2; in the treatment with ZnO an increase in O2·− was observed after 24 h, in a concentration dependent manner, respectively. In regard to the surfactant proteins, after 12 h of treatment with TiO2 (25, 50 and 100 µg/mL) a slight decrease of SP-A and SP-D was observed, while the exposure to ZnO (5.0, 10 and 15 µg/mL) induced an increase; for SP-B, in both NPs treatments an increase of surfactant proteins was observed. We conclude that the exposure to non-cytotoxic concentrations of TiO2 and ZnO NPs induced ROS and reduced of SP-A and SP-D, possibly from the increase in pro-inflammatory cytokines, deriving in tissue damage and a possible alveolar collapse.


An increasing number of engineered nanomaterials (ENMs) has been used in many industrial sectors or consumer products, thus an inhalation exposure to these materials may occur either in occupational environments during their production or consumer use. Citrate capped gold nanoparticles (Au NPs) in suspension (50 µg/mL) and silver (~7 nm) supported on silica (~10 nm) nanospheres (15%Ag@SiO2) were provided by the ENM Resource and Coordination Core (ERCC) with full physicochemical and morphological characterization. The nanomaterials were used as supplied without further modification. Toxicity assessment was performed using an in vitro air-liquid interface method exposure to these materials at 15%Ag@SiO2. Exposure to Au NPs did not affect cell viability. The production of ROS determined at 3, 12 and 24 h, in a concentration dependent manner, respectively. In regard to the surfactant proteins, after 12 h of treatment the expression of surfactant proteins, after 12 h of treatment with TiO2 (25, 50 and 100 µg/mL) a slight decrease of SP-A and SP-D was observed, while the exposure to ZnO (5.0, 10 and 15 µg/mL) induced an increase; for SP-B, in both NPs treatments an increase of surfactant proteins was observed. We conclude that the exposure to non-cytotoxic concentrations of TiO2 and ZnO NPs induced ROS and reduced of SP-A and SP-D, possibly from the increase in pro-inflammatory cytokines, deriving in tissue damage and a possible alveolar collapse.
2745 Toxicity Assessment of Aerosolized Engineered Nanoparticles following In Vivo Respiratory Exposure

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With the ever increasing use of engineered nanomaterials (ENMs) in modern technology, there is a corresponding increase in risk of exposure (both occupational and consumer) and a need to evaluate the health effects these materials may have on the body. Here, gold nanoparticles (Au NPs; 18.4 nm diameter) in suspension (50 µg/mL) and silver supported on silica nanoparticles (15%Ag@SiO2; 17.2 nm diameter) were evaluated. Toxicokinetic assessment was performed in vivo on mice in a nose-only inhalation system (inExposure, Scireq, Emka Tech.). Generated Au NP aerosol concentrations were 764 µg/m³. Particle size distribution yielded a geometric mean diameter of 42 nm with a geometric standard deviation of 1.75. Controls were exposed to the Au NP suspension fluid where the particles were removed by multiple high-speed centrifugations. Aerosol concentration for 15%Ag@SiO2 exposure was 3.5 ± 1.1 mg/m³. Particle size distribution yielded a geometric mean mobility diameter of 91 nm. Shams were exposed to HEPA filtered laboratory air. For both materials, mice were necropsied immediately (0 wk) or 3 weeks (3 wk) after the last exposure. In Au NP exposed mice, bronchoalveolar lavage (BAL) analyses of total and differential cell counts, cytokines, total protein, and lactate dehydrogenase (LDH) did not show significant differences compared to sham-exposed mice. Additionally, pulmonary mechanics measurements and histopathology evaluations showed no significant differences. Whereas sub-acute exposure to 15%Ag@SiO2 caused a significant increase of total cell recruitment into the lungs (measured in BAL fluid) at 0 wk post exposure (1,760 ± 10³ cells/mouse) compared to shams (47 ± 10³ cells/mouse). At 3 wk post exposure, cell numbers returned to normal (40 ± 10³ cells/mouse). The percentage of neutrophils in BAL fluid was 51% at 0 wk and 7% at 3 wk post exposure compared to 1.6% in shams. Additionally, there was a significant difference in cytotoxicity between sham and 15%Ag@SiO2-exposed males. Histopathological analysis of lung tissues showed marked increases in lymphocyte and neutrophil presence in several exposed samples.

2746 Safety of Novel Antimicrobials Based on Silver Nanoparticles Assessed by Methods In Vivo

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Due to antimicrobial properties, nano silver has been traditionally used in a variety of applications such as cosmetics (toothpaste), health care (treatment of burns, chronic wounds), textile coatings, and food packaging. Increased dermal application of silver nanoparticles is connected with another concern, namely potential adverse health effects. The high toxicity of single layer graphene and multi-layer graphene is usually attributed to its high surface to volume ratio providing more efficient contact of nanoparticles with microorganisms. The aim of the currently performed project research ALTERBIO is to identify and select innovative antimicrobial agents, based on silver nanoparticles (AgNPs) able of covalent bond within a polymeric system and without undesirable effects on human health and the environment. Within the project, promising agents with proved efficient and stable antimicrobial effects were subjected to a battery of toxicological tests to avoid local and systemic toxicity hazard. In compliance with the current European legislation restricting the use of experimental animals, the toxicological methods employed comprise exclusively in vitro procedures based on cellular and tissue models either of human origin or mimicking human tissues such as 3D models EpiDermTM (OECD TG 439), EpiOcularTM (OECD TG 492) and organotypic HET-CAM. The tests performed so far showed that AgNPs bound to montmorillonite are not irritant to skin or eye including mucosa. None of the tested samples showed endocanal disruption potential in the XenoScreen YES/YAS Assay (based on transfected Saccharomyces cerevisiae strains) and in the estrogen receptor transactivation assay (OECD TG 455). Although the results of chromosomal aberration test (OECD TG 471) showed a distinct degree of mutagenicity, the skin penetration/absorption test (OECD TG 428) did not prove permeation of silver nanoparticles through the skin barrier. Newly developed antimicrobial silver-based nanomaterials were proved as safe for dermal applications. The study was supported by TE02000006 Centre for alternative environment friendly high effective polymer antimicrobial agents for industrial applications (ALTERBIO).

2747 Platox In Vitro and In Vivo Investigations (28-Day Inhalation) to Generate Valid Toxicity Data for Risk Assessment of Carbon-Based Nanoplatelets

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Carbon-based nanoplatelets (CNP) represent a new class of 2-D nanostructures in multiple variants and with interesting functional properties (e.g. material enforcement and electrical conductivity). A very high toxicity among members of the carbon family is not expected for CNP platelets, however, for other CNP architectures. Commercial CNP candidates (ACS Material, USA) were selected, covering single layer or multi layer graphene, carbonyl graphene, single layer graphene oxide, and graphite oxide. Technical soot (Printex 90) served as particulate, non-platelet reference. The CNP were analyzed on sterility and endotoxin content; morphology (SEM pictures) and the specific surface area (BET method) were evaluated. As in vitro screening models both, primary rat alveolar macrophages (AM) and MRC-5 human lung fibroblast cells were analyzed on membrane damage (LDH release) and metabolic activity (AlamarBlue test). Interestingly, the two single layer graphene samples induced marked concentration-dependent membrane damage in AM after 24 h of incubation, with a BMD30 of 3.2 and 2.5 µg/cm², whereas no such effect was observed for MRC-5 cells. Some LDH release was also observed for single layer graphite oxide (BMD30: 39.3 μg/cm²). The other materials were nearly inactive. Significant effects on metabolic activity were not observed. In AM, single layer graphene CNP additionally induced direct DNA damage and release of PGE2. In conclusion, single layer graphene showed a geno toxic potential in vitro in AM, but not in lung fibroblasts. Based on the in vitro screening data and for validation, a single layer (highest) and a multilayer (lowest toxic potential) were selected for in vivo investigations. The dose range finding (DRF) test, with oral gavage instillation (0.02 and 0.2 mg/rat, each) single layer graphene was confirmed as the most inflammogenic sample in bronchoalveolar lavage fluid (BALF) inducing the recruitment of neutrophils and eosinophils. In the subsequent 4-week nose-only inhalation study with the same total dose (predicted by MPPD model) the inflammatory response of single layer graphene was weaker and no eosinophils were detected in BALF. Histopathological examination is underway. PLATOX funding: FP7- ERA-NET SIINN.

2748 Naknowbase: A Nanomaterials Relational Database Populated with Extant Data from the US EPA Office of Research and Development (ORD)


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The continued emergence and growth of nanotechnology, including nanomaterials (ENM) in industry and commerce, raises the possibility of environmental releases of ENM, and the need to predict potential environmental consequences. A substantial amount of research has been published on this topic, but the information is difficult to assimilate and synthesize for broader scientific inquiry. In 2016, a relational data base (“Naknowbase”) was developed featuring parameters relevant to the physical and chemical features of ENM and their potential actions in environmental and biological systems. This year, the database was populated with relevant data from peer-reviewed articles published by ORD authors. To do so, an initial search of internal EPA publication records yielded approximately 400 articles responsive to terms such as “nanoparticles”, “nanomaterials” and major ENM constituents (Ag, TiO2 etc). From those articles, literature reviews, articles concerning nanomaterial synthesis or green chemistry, and articles unrelated to nanomaterials and their environmental effects, were excluded. 68 manuscripts dealing with the release of nanomaterials into the environment, environmental fate transport or transformation, exposure or effects on ecological or biological systems were identified as high priority. Authors of the articles who were current EPA employees were contacted and requested to provide the original data in a formatted data entry template. Where data from original authors was unavailable, data were extracted from electronic versions of the papers by trained curators. To date, data have been collected from about 50 of the 68 high priority manuscripts. The database is currently available within the Agency as a downloadable SQL file with corresponding schema. Future efforts, as priorities allow, will include continued data curation, high priority manuscripts. The database is currently available within the Agency as a downloadable SQL file with corresponding schema. This abstract does not reflect US EPA Policy.
In this study, the in vitro dermal penetration method in OECD428 Skin Absorption: In Vitro Method using Franz (diffusion) cells was used to determine percutaneous absorption levels and distribution of zinc oxide in human skin from a formulated cosmetic product (sunscreen) containing nanoparticles of ZnO (14% in weight). The diffusion cell contained a donor compartment and a jacketed receptor compartment maintained at 37°C by a thermostatic jacket, separated by a membrane (human skin) that was in contact with the receptor fluid. Test item was spread on the skin membrane (exchange surface: 0.64cm²) and the skin disks were exposed to the test item for 24h. Receptor fluid samples were collected at 0 and 24h post application and then any remaining test item was collected. Two samples of stratum corneum (collected by tape stripping) were used to evaluate distribution between the upper and the lower stratum corneum layers. The stratum corneum, epidermis and dermis were analyzed by ICP-MS for quantification of total zinc. Absorption is considered to be the relative percentage of the dose recovered in the skin layers and receptor fluid after 24h skin contact versus the total amount applied on the skin surface (as recommended by OECD428). The average mass balance was 114±12% (EMA guideline acceptance criteria: ±15% for precision and accuracy). The concentration of zinc oxide in receptor fluid was lower than the limit of quantification (1.00 µg/mL; i.e. <0.5% of the applied dose). The absorbable amount in skin (following washing) was 1.1% for the last pool. This indicates that the compound reaches the inner level stratum corneum that is in contact with the epidermis. Zinc was also found in the exposed skin (epidermis and dermis) at 1.4% of the dose applied. The absorbable amount in skin (following washing) was 9.7% of the applied dose. Dermal absorption is primarily a diffusion-driven process, therefore test substance in the lower layers of the stratum corneum should be assumed to form a reservoir that may contribute to AD pathogenesis.

Alzheimer’s disease (AD) is a debilitating progressive neurodegenerative disease that usually starts slowly and worsens over time. Though AD etiology is believed to be multifactorial, there is considerable evidence showing the role of copper exposure in beta-amyloid (βA) aggregation and AD pathogenesis. Owing to the fact that βA aggregation takes place in the synaptic cleft where copper is found in high concentrations, copper exposure especially in the form of nano particles can be more detrimental. Though copper-induced oligomeric forms of βA accumulate and significantly damage the brain cells, inducing cognitive dysfunctions, the mechanism that initiates and promotes βA oligomerization remains unknown. We hypothesized protein radical formation as an initiating mechanism for βA oligomerization. Therefore, we used the highly sensitive immuno-spin trapping technique to investigate protein radical formation as a possible mechanism of βA oligomerization. Here, we have investigated copper ions and nano particles induced βA radical formation. The results clearly showed that both variants of copper nano particles (25 nm and 70 nm sizes) significantly produced βA radical in presence of hydrogen peroxide. To further validate copper nano particle-induced protein radical formation in biological context, we exposed IMR-32 hippocampal neuroblastoma cells expressing βA to both variants of copper nano particles and analyzed protein radical formation using confocal microscopy. Confocal microscopy results showed protein radical formation in IMR-32 cells exposed to either variant of copper nano-particles in presence of hydrogen peroxide. Taken together, these results suggest that both variants of copper nano particles induce βA radical formation which can lead to βA oligomerization, and therefore contributes to AD pathogenesis.
Nanoparticles (NPs) upon entry into a physiological environment, accumulate a coating of biomolecules or biocorona (BC) altering NP properties and cellular interactions. Association of the BC is governed by NP properties, time, and the physiological environment. Variability in the formation of the NP-BC between individuals and the effect of lifestyle changes remains unknown. We hypothesized that incubation of NPs in plasma from different human subjects would form distinct BCs that exercise would result in alterations in the NP-BC. Blood samples were collected from non-exercising individuals prior to and following a 7-day exercise routine. Fe3O4 NPs (20 nm and PVP coated) were incubated in plasma for 8 h and a proteomics approach was utilized to evaluate NP-BCs. Fe3O4 NPs were evaluated due to their use in biomedical applications. The analysis of all pre-exercise NP-BCs revealed the association of distinct proteins. The NP-BCs that formed from the 10 pre-exercise samples had 99 proteins in common. Quantitative analysis demonstrated that these 99 proteins absorbed on the NP in differing amounts. Association of apolipoprotein-C1, complement C4B, and transthyretin with NPs trended with cholesterol levels. No unique proteins were found to associate with NPs based on sex. Comparisons of the NP-BC that formed prior to and following the exercise regiment demonstrated alterations. In general, the BC formed following exercise was more complex or unique as determined by increased numbers of distinct proteins that bound. Although many proteins were shared between these samples, differences in abundance were found. Specifically, NP association with apolipoproteins, complement proteins, coagulation factors, and others were reduced, whereas association of immunoglobulins, fibrinogen, insulin growth factor-2 and others were increased following exercise. To determine the biological impact of these BCs, human macrophages were exposed to Fe3O4 NPs with BCs at 25 µg/ml for 24 h and variations in cytotoxicity, internalization, and inflammatory response (gene expression of TNF-α, IL-6, and IL-8) were examined. BCs that bound to inflammatory cells influenced and modulated the inflammatory response by cells. These findings demonstrate that humans will likely form unique BCs on NPs and that lifestyle changes such as exercise can influence NP-biomolecule interactions. NIEHS ES024392.
of erenumab to birth mothers during gestation. Postnatal evaluation included: external, visceral and skeletal evaluations and behavioral assessments. Pregnant monkeys were assigned to receive vehicle (n=20) or erenumab (n=30) at 50 mg/kg. Subcutaneous administration of erenumab to pregnant cynomolgus monkeys at 50 mg/kg Q2W was well tolerated. The mean maternal/infant serum erenumab concentrations on postpartum days (PPD) or birth days (BD) 14, 28, and 91 were 66/117 µg/mL, 23/46 µg/mL, and 0.009/0.188 µg/mL, respectively. Erenumab concentrations were generally below the LLOQ after PPD91 in adult females and after BD91 in all of the infants. There were no erenumab-related changes in clinical signs, food consumption, body weights, gestation length, or pregnancy/postpartum outcomes (i.e., fetal or infant losses). Overall fetal loss was 3 of 20 (15.0%) in the control group and 4 of 23 (17.4%) in the erenumab group. Fetal losses in the erenumab group (both total and per trimester) were comparable to the control group and less than historical control values at the Testing Facility (average fetal loss of 22.5% and range of 38.9%). There were no erenumab-related effects on gestation length or on infant sex ratio. The overall incidence of infant loss (2 of 17 or 11.8% for controls, 3 of 19 or 15.8% for erenumab) was comparable between the 2 groups and within the historical control range of the Testing Facility for 18 previous ePPND studies (range of 0% to 20% infant loss; average of 10.1%). There was no difference, subcutaneous administration of erenumab to pregnant cynomolgus monkeys at 50 mg/kg Q2W from GD20-22 until parturition was well tolerated. No maternal toxicity was observed, and there were no effects on fetal/infant losses or growth and development through 6 months postpartum. This study supports the safe use of erenumab in women of child-bearing potential who experience migraines.

2758 Perinatal Prozac Exposure and Tryptophan Manipulation Reduces Pup Survival and Sex-Dependently Increases Autism Related Behaviors in Mice

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Autism is a developmental disorder with increasing incidence that now affects 1 in 68 US children, primarily males. Genetic and environmental studies show that positive or negative aberrations in serotonergic signaling during development can contribute to autism etiology. Controversial literature also suggests maternal use of selective serotonergic signaling during development can contribute to autism etiology. The vertebrate zebrafish (Danio rerio) embryo is an emerging model for screening teratogenic compounds. We aim to understand the developmental toxicity effects of environmental pollutants by using zebrafish embryos to identify compounds that negatively affect neumast of the lateral line, a system of tactile sense organs in fishes. The zebrafish neuromasts are mechanoreceptors that respond to the flows of water, and are structurally and functionally similar to human inner ear hair cells. Neuromasts are positioned along the zebrafish body through migration of the neuromast primordium formed near the otic vesicle. Transgenic zebrafish (TgTg expressing fluorescence in neuromasts) were used in a primary high throughput screen (HTS) of 294 compounds from EPA’s ToxCast phase I chemical inventory, primarily consisting of pesticides and antimicrobials. This generated a list of 48 potential hits that altered neuromast development and/or migration. These hits were re-screened with a higher number of replicates to confirm and characterize effects, and to determine the lowest effect levels (LELs). This resulted in 22 confirmed neuromast disruptors. A univariate analysis was performed on the confirmed hits to identify ToxCast in vitro assays that significantly correlated with the identified in vivo neuromast disruptors. Several assays for serotonin signaling were retained. In conclusion, our results identified several environmental pollutants with neuromast disrupting capacity. This suggests that the developing zebrafish embryo is an efficient in vivo model that can be used for rapid identification of compounds interfering with neuromast development and has the potential to be developed into a predictive toxicity model. This abstract does not necessarily reflect US EPA policy.

2759 Embryonic-Only Arsenic Exposure Alters Skeletal Muscle Satellite Cell Function in Killifish (Fundulus heteroclitus)

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Arsenic is a contaminant found worldwide in drinking water and crops. Epidemiological studies have correlated arsenic exposure with reduced weight gain and improper muscular and neuronal development. In vitro studies have also shown that arsenic impacts myogenic and neurogenic differentiation. The purpose of this study was to determine if embryonic-only arsenic exposure permanently reduces the population or function of muscle satellite cells, using killifish as our model organism. Killifish embryos were exposed to 0, 50, 200, or 800ppb AsIII until hatching, and fish were raised in clean water for 28, 40, or 52 weeks. To assess baseline cell populations, trunk skeletal muscle sections were collected at each time point. To assess cellular function, skeletal muscle injuries were induced at each time point by injection of cardiotoxin into the trunk of the fish. Muscle sections were collected at 3, 7, and 10 days post-injury. Collagen expression was examined to assess the level of fibrosis. PCNA and myogenin expression were quantified to compare proliferating cells and newly formed myoblasts. At 28 weeks, baseline collagen levels were 2.0- to 2.2-fold greater in the fish embryonically exposed to 200 or 800ppb arsenic. Although no significant differences were seen at 40 weeks, fish were increased by 2.1- to 2.2-fold at 52 weeks. After cardiotoxin injury, collagen levels remained higher in 28 week old arsenic exposed fish even after 10 days of recovery. At 40 and 52 weeks, there were still slight increases in collagen levels during the recovery period, but this was not significant. Baseline PCNA levels were higher in exposed fish at 40 and 52 weeks following cardiotoxin injury, PCNA is statistically reduced in exposed groups at day 3 of the recovery period. By day 10, a slight reduction in PCNA expression remains, but this is not significant. Based on these results, arsenic exposure during early life stages may be impairing muscle satellite cells, leading to reduced skeletal muscle regeneration after tissue injury.

2760 Developing the Zebrafish Neuroumat as a Predictive Toxicity Model

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Pyriproxyfen is a pyridine based larvicide categorized as a juvenile hormone analog and is widely used to control agricultural, veterinary and human health pests. It is also used in drinking water sources for mosquito control in Brazil, which lead to the debate that exposure to pyriproxyfen may cause microcephaly. We used the vertebrate zebrafish model to evaluate the effects of pyriproxyfen on the neuronal development. Zebrafish embryos were treated with increasing concentrations of pyriproxyfen and were imaged and assessed for morphological malformations. We identified three different malformations in the head region: narrow head, short head and small eyes. Quantification of the effects showed that both the length and width of the head were significantly shorter in pyriproxyfen treated embryos at 10 µM and higher concentrations. Next, we used the transgenic zebrafish line Ngn1:GFP to visualize the neuronal system. Two malformations were observed in the brain region after pyriproxyfen exposure: a small midbrain and a neural tube closure defect in the hindbrain region. Pyriproxyfen altered the expression of the neuronal marker syn2a and glial cell markers gfab and mbp, as determined by qPCR. However, the expression of the neuronal marker genes ngn1 and elavl3 was not changed. Furthermore, transcrip-
tomic analysis was performed to identify the molecular mechanisms altered by pyriproxyfen exposure, which potentially could lead to the observed phenotype. In conclusion, our results show that pyriproxyfen exposure affects the morphology of the brain and alters the expression of glia-specific genes during early embryonic development in zebrafish.

**2762 Developmental Regulation of Nuclear Factor Erthroid-2 Related Factors (nrf/s) by AHR1b**

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Interactions between regulatory pathways provide additional mechanisms for organisms to both adapt to their environment and respond to endogenous stress. One interaction recently identified in adult mammals, cell cultures, and fish embryos occurs between the aryl hydrocarbon receptor (AHR) and members of the nuclear factor erthroid-2 related factor (nrf) family. Each transcription factor regulates numerous downstream genes involved in the cellular response to toxicants and oxidative stress; they each also play a role in normal development. The zebrafish model was chosen to explore the role of AHR regulation of nrf genes during development and in response to toxicant exposure, as there are three ahr and six nrf genes in the zebrafish genome. We have previously shown that each nrf gene in zebrafish has several anabolic response elements (XRE): cis-promoter elements that are theoretically AHR binding sites. To determine whether an AHR protein, AHR1b, is responsible for transcriptional regulation of nrf genes during development, a loss-of-function assay using a morpholino knockdown approach was conducted followed by a Choromatin Immunoprecipitation (ChIP) direct binding study. The expression of nrf1a was dependent on AHR1b and its expression was directly regulated through site-specific XREs in its cis-promoter at 24 hours post fertilization. However, nrf1a expression was not altered by exposure to TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin), a toxic ARH receptor agonist. The expression of nrf2a and nfe2 were induced by TCDD, and AHR1b directly regulated their expression by binding to specific cis-XRE promoter elements. Lastly, nrf2b and nrf3 were neither induced by TCDD nor regulated by AHR1b. Taken together, these results show that AHR1b transcriptionally regulates nrf genes in a site-specific manner via binding to cis-promoter XREs during development. These data provide a clearer picture of the importance of this interaction during the sensitive embryo life stage, and provide a better understanding of how combinatorial molecular signaling protects embryos from potentially embryotoxic events following toxicant exposure. R01ES006272, R01ES16366, F32ES019832, P20GM103423.

**2763 Developmental and Reproductive Toxicity Studies in Sprague Dawley Rats and New Zealand White Rabbits with Palbociclib**

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Palbociclib (IBRANCE®) is a selective inhibitor of the cyclin-dependent kinase (CDK) 4 and 6, approved for the treatment of breast cancer. Fertility and early embryonic development toxicity studies were conducted in male and female rats via oral gavage at doses up to 100 and 300 mg/kg/day, respectively. Males were dosed for 4 cycles (each cycle comprises 3 weeks consecutive daily dosing followed by a 1 week non-dosing period), and paired with untreated females during the last two weeks of the 15 week dosing period. Females in a separate study were dosed once daily beginning 15 days before cohabitation, during cohabitation and continuing until gestation day (GD) 7. In the males, there were no effects on functional fertility at any dose, but there were microscopic changes in the testes with secondary findings in the epididymides at ≥30 mg/kg; 100 mg/kg also resulted in lower epididymal and testicular weights, sperm density, and sperm motility. There were no effects in females following doses up to 300 mg/kg. Developmental toxicity studies were conducted in rats and rabbits with daily doses up to 300 and 20 mg/kg, administered from GD6-17 and GD7-19, respectively. In the presence of maternal toxicity (lower body weight gain and food consumption compared with control), low fetal body weights and relative organ weights were observed at 100 mg/kg in rats and small forepaw phalanges were noted in a few fetuses at 20 mg/kg in rabbits. Palbociclib was not teratogenic in rats or rabbits. Fetal skeletal variations in rats (increased incidence of cervical ribs at ≥100 mg/kg) and rabbits (increased incidences of 13 ribs at ≥10 mg/kg) were considered palbociclib-related but non-adverse since both are considered.

**2764 Endodermal Ossification in Developing Long Bones of F1 Offspring following Administration of Andecaliximab to F0 Dams during Gestation and Lactation**

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Matrix metalloproteinase 9 (MMP9) has been shown to be related to trabecular bone architecture. In a pre/postnatal development study, F0 dams were administered an anti-MMP9 antibody andecaliximab (andeca) every 3 days IV at 10, 30 or 100 mg/kg/dose or vehicle GD 6 through LD 21. Skeletal bone radiographs, bone length, bone densitometry (pQCT), and histopathology of selected bones were assessed in F1 offspring on Days 21, 49, and 105 pp. Andeca was associated with increased with increasing dose (milk 10-20% of serum conc.). F1 serum exposure increased with dose, and F1: F0 serum ratios ranged from 2% at 10 mg/kg/dose to 60% at 100 mg/kg/dose on LD 11, and were < 1% by LD 21. F1 survival, clinical condition, body weight and behavioral and reproductive function were unaffected. At 100 mg/kg/dose, animals presented persistent slight (< 9%) decreases in femur length. At Day 21 pp, these findings were associated with widespread changes in long bone physis, including increased thickness, metaphysis enlargement and/or increased radiographic bone correlating microscopically with physeal hypertrophy and increased trabecular metaphyseal bone, which were consistent with mild increases in metaphysis bone mineral content and density. Radiographic and microscopic changes observed at Day 21 pp in long bone epiphysis and metaphysis were fully resolved by Day 49 pp. Bone densitometry changes at Day 49 pp consisted of increased total area at the femur distal metaphysis, associated with increased bone mass persisting to Day 105 pp and radiographic enlargement of the femur distal metaphysis. Additional findings at Day 105 pp included a few bent radii (varus), and radiographic bone loss in the proximal humerus occasionally associated with a luxation and/or osteophytes and/or enlargement of the proximal epiphysis. These results indicate that andeca exposure when administered to F0 dams during gestation and lactation, and support the pharmacologic role of MMP9 in endochondral ossification of developing long bones. Additional studies may be required for further validation.

**2765 Sensitivity to Monoamine Uptake Inhibitors is Modified by Methylmercury and d-Amphetamine Exposure in Adolescence**

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During adolescence, monoamine neurotransmission is continuously developing and may be more vulnerable to early exposure to the environmental neurotoxicant, methylmercury (MeHg). To test this hypothesis, we examined sensitivity to the dopamine, norepinephrine, and serotonin reuptake inhibitors d-amphetamine (d-AMP), desipramine, and clomipramine (respectively) following exposure to MeHg during adolescence. Water containing 0 ppm and 3 ppm MeHg was given to two groups of twenty-four male C57BL/6 mice during adolescence (postnatal days 21-60). Within each group, mice were also given i.p. injections of saline or d-AMP (1 mg/kg/day) from postnatal days 28 to 42. This produced four treatment groups (n = 10-12/group): Control, d-AMP, MeHg, and d-AMP + MeHg. As adolescent mice lever pressed under fixed-ratio schedules of reinforcement (FR 1, 15, 30, 60, and 120). Following baseline, acute intraperitoneal injections of d-AMP (0.3 - 1.7 mg/kg) desipramine (5.6 - 30 mg/kg), and clomipramine (5.6 - 30 mg/kg) were administered, with each dose-response determination separated by a week. Rates of lever pressing were analyzed using Mathematical Principles of Reinforcement, a model that describes operant (voluntary) behavior in terms of motivational, motoric, and memorial factors. Adolescent d-AMP and d-AMP + MeHg exposure enhanced sensitivity to acute d-AMP and desipramine. Adolescent d-AMP and MeHg exposure also enhanced...
sensitivity to acute clomipramine; though, this enhanced sensitivity was blocked by combined exposure to d-AMP + MeHg in adolescence. These data demonstrate that there are long-term alterations in monoamine sensitivity following adolescent exposure to methylmercury and d-amphetamine.

Using novel in situ placental perfusion methodology we were able to conclusively identify and quantify ENM translocation from the maternal to fetal compartment. Collectively, our results reported using this model may allow for its future use in ENM screening prior to therapeutic application during pregnancy.

Obesity rates have increased significantly over the past 150 years. Mounting evidence suggests that exposure to certain environmental chemicals may alter lipid metabolism, accumulation and promotion of adipogenesis leading to weight gain. Tetrabromobisphenol A is a brominated flame retardant commonly used throughout the world. TBBPA has been shown to promoting lipid accumulation and weight gain, in zebrafish, following exposure (10 mM-1 μM) during several stages of development and to induce lipid accumulation in murine 3T3-L1 cells through activation of PPARy. This study was conducted to determine the effects of early life exposure to TBBPA on metabolic endpoints. Wistar rats (n=15 litters per dose) were exposed by oral gavage to 0, 0.1, 0.25, 250 mg TBBPA/kg BW from gestation day 6 to postnatal day 90. Daily body weights, monthly body mass composition, and glucose, insulin, leptin, and adiponectin serum levels data (weaning, peripubertal, postpubertal and 1 year timepoints) were determined in male and female pups. A subset of animals was aged out to 1 year to investigate any persisting effects due to developmental exposure. Glucose tolerance challenge and an insulin sensitivity test were performed on male and female pups at 9 months and 1 yr of age (5-6 per dose). Multivariate analysis of variance (MANOVA) analysis showed that females in the high dose group were leaner later in life compared with controls (68.45±1.30 % lean, 62.41±2.26 % lean, respectively) at beginning at PND 242. There were not any significant body weight differences, but fat mass gain of treated females plateaued whereas control females continued to gain fat throughout life. These effects were absent in males. Microarray analysis of 1 yr old female livers showed gene expression changes in the high dose group related to adipogenesis pathway and metabolic disease. Further investigation is being conducted but some genes of interest are FABP7, CSRP2, and HSPP1. Overall, findings from this study shows that TBBPA has persistent effects on numerous metabolic endpoints.
consumption values compared to non-dosed rabbits. Mean total body weight gains of animals administered CO or DI were 20 and 11% lower, respectively, than rabbits that were not gavaged. These results clearly demonstrate that feed fiber content and vehicle selection can affect maternal health parameters typically used in identifying a MTD.

2770 Harm-Reduction Tobacco Inhibits Osteogenesis Via Misregulation of Survival Signaling Networks


While conventional smoking habits are on the decline in the United States, other forms of tobacco usage have risen to the forefront of public health concern. Of particular interest is the trend of pregnant women turning to purported harm-reduction tobacco products (HRTPs). Women who struggle with nicotine addiction may turn to these products as they are advertised to pose less harm to users than conventional cigarette smoking. However, initial studies suggest that use of HRTPs during pregnancy does not reduce adverse pregnancy outcomes and may have adverse effects on the fetal skeleton. Though reproductive and developmental toxicity has been well established for conventional tobacco use during pregnancy, the biological processes responsible for how HRTPs impact early bone development remains unclear. Here we provide evidence that HRTPs, snus, smokeless tobaccos, and reduced tar/nicotine cigarettes are teratogenic to the developing skeleton using embryonic stem cell (ESC) and mouse models. To assess in vitro embryotoxicity, ESCs were differentiated into osteoblasts during concurrent exposure to snus or reduced tar/nicotine cigarettes. HRTP exposure was found to have a teratogenic effect that decreased osteoblast differentiation at subtoxic levels in vitro. Mouse embryos exposed in utero to HRTPs demonstrated abnormal craniofacial and long bone development. Teratogenic doses were also found in vitro to possess increased cellular reactive oxygen species levels and reduced nuclear FOXO1 and FOXO3, two transcription factors that drive free radical scavenging enzymes response. Investigation of upstream signaling kinases identified elevated AKT activation following HRTP exposure while co-administration of AKT inhibitor was found to rescue osteogenesis. Overall, our results suggest that HRTP exposure during development misregulates survival kinase signaling and creates a state of oxidative stress. Together, these events may drive the ultimate disruption of normal skeletal development.

2771 Evaluation of DART Data Pertaining to Reclassification of Chloroform as a Proposition 65 Reproductive Toxicant


California’s Office of Environmental Health Hazard Assessment (OEHHHA) recently reconsidered chloroform for listing as a reproductive toxicant under Proposition 65. This analysis evaluated the animal developmental and reproductive toxicity (DART) data for chloroform to determine if the weight of evidence supported its continued classification as a reproductive toxicant. A total of 14 animal studies that addressed developmental endpoints were considered in the evaluation. Data were tabulated and no observed adverse effect levels (NOAELs) identified. NOAELs tended to be substantially higher in the gavage oral dosing studies compared to the inhalation studies. Among the 8 in utero developmental toxicity studies, consistent findings of maternal toxicity (including statistically significant decreased maternal body weight gains and/or body weight gains and decreased food consumption) were associated with both gavage oral and inhalation exposures. High dose exposures (≥200 mg/kg/day) were also associated with very early (peri-implantation) losses. Based on the body weight data, the NOAEL for maternal toxicity was established in inhalation studies at 3 ppm (4 mg/kg/day). Statistically reduced fetal weights as well as alterations/delays in ossification were also consistently observed in the in utero developmental toxicity studies and occurred at doses equal to or above those at which maternal toxicity was seen. Examination of these data indicated that the offspring effects of chloroform were likely secondary to maternal toxicity and not a direct effect of exposure. The NOAEL for offspring effects was established in inhalation studies as 10 ppm (12 mg/kg/day). Findings among the 4 reproductive studies were consistent with those from the in utero developmental toxicity studies, and NOAELs from these studies (which did not involve inhalation exposure) were generally higher than those from the in utero developmental toxicity studies. Findings from 2 in vitro developmental toxicity assays provided limited useful information in the overall evaluation. For the purposes of human health risk assessment, we conclude that chloroform is not a direct developmental toxicant and that doses that are protective of the maternal animal should also be protective of developing offspring. Given this information, chloroform does not warrant listing as a reproductive toxicant under Proposition 65.

2772 Transcriptomic Analysis of Vascular Disruptor Effects in Embryonic Zebrafish

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Zebrafish is an effective model for studying vascular perturbation during embryonic development because of its small size and rapid vascular development, which can be followed using transgenic fish expressing GFP in vascular endothelial cells. Using this model, we previously identified 22 new vascular disruptors. Here, we present a transcriptomic analysis of gene expression changes after exposure to four of the zebrafish vascular disruptors: trifluorotributin, rotenone, pyrroclastin, and pyridaben. At two days post exposure, RNA sequencing of the whole transcriptome was performed, following pathway analysis using Ingenuity Pathway Analysis (IPA). We specifically focused on expression changes that were common between the four vascular disruptors. All vascular disruptors altered expression of genes involved in the canonical pathways relating to vascular development, like vasculogenesis, angiogenesis, endothelial cell development, cardiovascular development and other related functions. We found that expression of HIF1AN, EGR1, CTGF, CYR61, TCAP were downregulated by exposure to vascular disruptors, indicating that angiogenesis, vasculogenesis, and endothelial cell development were affected. The results also showed that apoptosis was downregulated by exposure to these four compounds, while genes involved in proliferation had an increased expression. We also looked into factors that affect mitochondrial pathways and found that the expression of several genes in the cytochrome C oxidase pathway were altered by vascular disruptor exposures. Expression of ATPF1 and HSPD1, known to be involved in mitochondrial membrane potential and mitochondrial protein functions, were induced by all exposures. We are now performing experiments to further investigate the findings from the IPA analysis.

2773 Potentiation of Dioxin-Induced Edema by Oxidative Stress in Developing Zebrafish


Previously, we reported the involvement of cyclooxygenase type 2b - thromboxane pathway and oxidative stress in causing edema response by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in early larval zebrafish. We recognized that edema by lower concentration of TCDD was abolished by either thromboxane receptor (TP) antagonist or antioxidants. In the present study, we studied the possible interaction of aryl hydrocarbon receptor (AhR) pathway and oxidative stress. Paraoxon, an oxidative stress inducer itself did not affect edema formation, but it augmented edema by lower concentration of TCDD at 55 hpf (pre-cardiac edema). TCDD-induced edema was abolished by antioxidants in the presence or absence of paraoxon. Paraoxon also potentiated edema caused by U46619, a TP agonist. Different to TCDD, antioxidants only inhibited the potentiation effect of paraoxon. TCDD-induced edema and potentiation of paraoxon to U46619 or TCDD were augmented in Nr2a2a morphants. Sulforaphane, an activator of Nr2f, suppressed TCDD-evoked edema regardless of the presence of paraoxon in both 55 and 72 hpf larvae. Sulforaphane also abolished potentiation effect of paraoxon to U46619 without affecting U46619-evoked edema. Taken together, these results suggest the enhancing role of oxidative stress in edema by TCDD and TP activation in developing zebrafish. 1) Teraoka et al., 2014. Aquatic Toxicol.
Influence of Pregnancy and Fasting on Metabolome Plasma Analysis in a Rat Prenatal Toxicity Control Study

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BASF and metabolomics established a database (MetaMapTox) for the detection of toxicological modes of action (MOA) based on metabolic profiles in rat plasma of > 750 compounds. Approximately 250 different endogenous metabolites are measured and more than 100 toxicological MOAs can be differentiated. The database was built with plasma from non-pregnant, fasted rats and lacks information on the metabolite profile of non-fasted, pregnant rats. In the present prenatal toxicity control study (OECD 414), Wistar rats received no more than drinking water by gavage, non-pregnant rats were treated equally. Plasma metabolic profiles of pregnant and non-pregnant Wistar rats under fasting and non-fasting conditions were compared. The influence of fasting conditions on the embryonic and fetal development was examined. Fasting resulted in a moderate body weight reduction compared to controls (90% in pregnant, 94% in non-pregnant rats). All reproductive and embryo-fetal parameters were not altered. A principal component (PC) analysis was applied to a separate if FLX affects groups. The first principal component (PC1, largest possible variance) was pregnancy, the second largest possible variance (PC2) was fasting. We noted an interaction component between fasting and pregnancy which was not observed in fasted, non-pregnant rats. Metabolites typically altered in Progesterone, 17α-Estradiol and Androstenedione in pregnant rats and Hydroxybutyrate and Adrenaline in fasted rats. The gained knowledge will improve characterization of maternal toxicity by metabolomics.

Fluoxetine Affects the Differentiation of Dopaminergic Neurons In Vitro

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Three recent meta-analyses suggest an association between prenatal exposure to the antidepressant drug fluoxetine (FLX) and an increased risk of autism in children. This study aimed to investigate if FLX affects processes involved in dopaminergic neuronal differentiation as a possible mechanism underlying this link, possibly via interference with the estrogen system. Mouse neuronal precursors (wild-type (WT) and estrogen receptor β knock-out (ERβKO)) were differentiated to midbrain dopaminergic progenitor cells (mDPCs) and concomitantly exposed to therapeutically relevant FLX concentrations. Dopaminergic progenitors were then evaluated for expression of differentiation and stemness markers, as well as of nuclear estrogen receptors (ERs), using qPCR. In WT cells, FLX treatment led to a significant increase in early regional specification markers Orthodenticle homeobox 2 (Otx2) and Homeobox engrailed-1 and 2 (En1 and En2). On the other hand, two transcription factors essential for mDA neurogenesis, LIM Homebox transcription factor 1 alpha (Lmx1a) and Paired-like homeodomain transcription factor 3 (Ptx3) were significantly down-regulated by FLX treatment. Finally, the stemness marker Nestin (Nes) was significantly increased and the neuronal differentiation marker β3-tubulin (Tubb3) significantly decreased in WT mDPCs. Additionally, the expression of both ERα and ERβ was significantly down-regulated in WT cells after FLX treatment, suggesting an involvement of these receptors in the observed effects. Indeed, in ERβKO cells, FLX had no or even opposite effects on the genes involved in mDPCs specification, on Nes and on Tubb3. These findings suggest that FLX increases induction of dopaminergic precursors at the expense of differentiation to serotonergic neurons, yet decreases the maturation of dopaminergic neurons. These effects seem to be partly ER-dependent as no or even opposite effects were observed in ERβ-deficient cells. Further studies are needed to link these molecular events to development of the dopaminergic system and address if these findings could partly underlie the association between prenatal FLX exposure and increased autism risk in children.

Embryonic Exposures to Perfluorobutanesulfonic Acid (PFBS) Impair Growth and Pancreatic Organogenesis in the Zebrafish, Danio rerio

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Since phase-out of more persistent perfluorosulfonates from American non-stick and stain-resistant products in the early 2000s, perfluorobutanesulfonic acid (PFBS) has replaced these compounds as a primary surfactant. Compared to other longer chain perfluorosulfonates which have human half-lives of 5-10 years, PFBS has a half-life in the human body of just over 1 month. Environmental, ecological, and human concentrations of PFBS have been steadily rising in recent years, raising concerns about potential negative health effects. We have previously found that embryonic exposures to a related and more persistent compound, perfluorooctanesulfonic acid (PFOS), decreased pancreas length and insulin-producing islet area in zebrafish embryos (Danio rerio). The objective of this study was to identify the extent to which embryonic PFBS exposures disrupt pancreatic organogenesis and to compare these findings with historical PFOS toxicity data. Zebrafish embryos were exposed to 0 (0.01% DMSO), 16, or 32 µM PFBS daily beginning at 1 day post fertilization (dpf) until microscopic examination at 4 and 7 dpf. Embryos from two different transgenic fish lines (Tg(lnsulin:GFP) and Tg(ptf1a:GFP)) were examined using fluorescent microscopy for islet area and total exocrine pancreas length, respectively. Embryos exposed to PFBS had increased incidence of tail and spinal deformities, delayed inflation of the swim bladder. PFBS embryos were shorter than control embryos, and had 5-13% shorter pancreata. Islet area decreased by 12-13% due to PFBS treatment, but to a lesser degree than historical PFOS data. Overall, this work suggests that developmental exposures to PFBS can perturb embryonic development and pancreatic organogenesis, and that further risk assessment is warranted. Funding for this work was provided by the National Institutes of Health R01ES025748 and R01ES0282801 to AT-L, and F32ES028085 to KES.

A 3D Microphysiological Model of Sonic Hedgehog Signaling for Toxicity Testing

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Human development is orchestrated via secretion and sensing of small molecules through the tissue microenvironment. Sonic Hedgehog (SHH) signaling in the developing facial processes are a prototypic example, whereby SHH ligand is produced, processed, and secreted from the ectoderm. In turn, the ligand is shuttled through the extracellular matrix where it binds mesenchymal receptors, initiating a gradient of transcriptional response with high proximal to low distal activity. Genetic or chemical disruption in any part of the pathway can lead to orofacial clefting (cleft lip, cleft palate), serious birth defects. Modeling this complex cellular interplay in vitro has many applications for toxicity testing and beyond. Using the adverse outcome pathway framework as a guide, we constructed a 3D microphysiological model of epithelial-mesenchymal SHH signaling. Using microscale physical phenomenon, we created and epithelial monolayer which covers a 3D extracellular matrix where it binds mesenchymal receptors, initiating a gradient of transcriptional response with high proximal to low distal activity. Recapitulating a hallmark pathway response, a live cell dual luciferase reporter assay shows SHH ligand produced from the epithelium generates a reproducible gradient of pathway activity in the adjacent mesenchyme, with high proximal activity decreasing to no activity 500 microns into the gel. Endogenous SHH pathway activation was efficiently inhibited in dose-response by small molecule inhibitors of epithelial secretory (SHH production/cholesterol modification/palmitoylation), ECM transport (SHH ligand) and mesenchymal sensing targets (Smoothened, Gli1) at IC50s comparable to in vivo. Supporting the utility of this approach for high-content chemical screening, microscale devices are molded into commercial microtiter plates (40 devices/plate), are viable for at least 30 days, recover after wash-out and can be re-dosed, and are well suited for luminescent, fluorescent and high-content imaging readouts. Together, these findings demonstrate a novel and practical microphysiological model with broad utility for investigating epithelial mesenchymal interactions and disruptions in development.
Human obesity is a complex metabolic disorder disproportionately affecting people of lower socioeconomic strata, and ethnic minorities, especially African Americans and Hispanics. Although genetic predisposition and a positive energy balance are implicated in obesity, these factors alone do not account for the excess prevalence of obesity in lower socioeconomic populations. Therefore, environmental factors, including exposure to pesticides, heavy metals, and other contaminants, are agents widely suspected to have obesogenic activity, and they are also spatially correlated with lower socioeconomic status. To investigate whether a causal relationship between exposure to the heavy metal, cadmium (Cd), and obesity in a cohort of children exists, we analyzed an extensive collection of first trimester maternal blood samples obtained as part of the Newborn Epigenetics Study (NEST) for the presence of Cd, and these results were cross analyzed with the weight-gain trajectory of the children through five years of age. Next, the role of Cd as a potential obesogen was analyzed in an in vivo zebrafish model. Our results indicate that the presence of Cd in maternal blood during pregnancy is associated with increased risk of juvenile obesity in the offspring, independent of other variables, including lead (Pb) and smoking status. This effect was recapitulated in a zebrafish model, in which exposure to Cd at a level approximating those observed in the NEST study was associated with increased juvenile abdominal adiposity. In conclusion, our findings identify Cd as a potential human obesogen. Moreover, these observations are recapitulated in a zebrafish model, suggesting that the underlying mechanisms may be evolutionarily conserved, and that zebrafish may be a valuable model for uncovering pathways leading to Cd-mediated obesity in human populations.

The development of new pharmaceuticals benefits from continuous advances in biomedical research for both adults and children. Safety evaluation of new pediatric medicines is performed by the conduct of toxicology studies using juvenile animals. The minipig is now considered as a useful alternative non-rodent species for safety testing of pharmaceuticals. Human parallels in many features of its anatomy, physiology and biochemistry make the minipig a good model for man. For use in juvenile toxicology studies, the development of main organs or systems (including immune system) of the minipig still requires further characterization. In addition, the immune system could evolve towards gene expression and biological pathways in the zebrafish vertebrate model system depending on carbon chain length. The zebrafish model is ideal for developmental and molecular toxicity studies. Zebrafish were exposed to control or 4, 40 or 400 ppb of the PFCAs throughout embryonic development. Global gene expression profiles for each PFCa were then determined. Categories of gene function and diseases were identified for altered genes using Ingenuity Pathway Analysis. Cancer was the top disease identified among all the PFOA treatments unlike PFHxS and PFBA. However, most of the PFHxS and PFBA treatments did identify gastrointestinal diseases as one of the top diseases similar to PFOA. One of the top pathways identified among all PFBA treatments was FXR/RXR activation, which plays a role in numerous metabolic pathways. These findings further the comparison between the PFCAs and provide guidance on additional molecular and disease pathways to target for a more thorough understanding of the potential adverse health outcomes of an embryonic exposure to PFOA, PFHxS, or PFBA.

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Confined parking garages with insufficient ventilation can create hazardous environments for people breathing the air. Some possible health effects from inhalation of gasoline-related compounds (for example, BTEX, benzene, toluene, ethylbenzene, and xylenes) include eye, nose and throat irritation, headaches, loss of coordination, nausea, damage to liver, kidney and central nervous system. This study provides a preliminary assessment of the air quality of an underground parking garage in downtown Washington, DC. It focuses on VOCs (volatile organic compounds) in the air that can impact public health. In June 2017, samples were collected and exposed in 15-minute intervals once a week during lunch hours ranging from 11 am to 1 pm. For each sampling interval, three solid-phase microextraction (SPME) fiber coatings were exposed in the parking garage, and analytes with a high affinity for the sorbent were selectively adsorbed. Humidity and temperature were noted since they are known to influence VOC volatility and absorbance onto the SPME fiber. The analytes were thermally desorbed from the fiber into a gas chromatograph – mass spectrometer (GC-MS) to identify volatile organic components. During the same sampling interval, a photoionization detector (PID) was used in assessing total VOCs in the garage. Total VOC concentrations ranged from 100 to 600 ppb, measured in isobutylene equivalents. The chemical composition captured by the SPME/GCMS consisted of BTEX and other aromatic/organic compounds. Some compounds were acetone, propylbenzene, ethanediol, and alkane. Known sources for these compounds include gas emissions, automobile exhaust, cleaning products and tobacco smoke. This study indicated that locations such as underground parking garages associated with these sources can have a complex mixture of volatile organic compounds at levels as high as a few hundred ppb.
Commuting by car exposes drivers and passengers to pollutants such as VOCs (volatile organic compounds) that can affect one’s health. The VOCs benzene, toluene, ethylbenzene, and xylene (BTEX), are particularly harmful for those who suffer from respiratory and cardiovascular diseases. This study was done to examine the identity and levels of VOCs inside the cabin of a vehicle, during a normal commute through the city of Washington D.C. (from Arlington, VA to North East, D.C.). Ten tests were run over a five-week period. A MiniRAE3000 monitored total VOC concentrations, and SPME (solid-phase micro extraction) field samplers collected samples from air. These SPME samples were later analyzed for chemical composition by GC/MS (gas chromatography mass spectrometry). In general, total VOC levels were higher in downtown DC and decreased in suburban areas of Arlington. Chemical compositions of the VOCs collected by SPME in downtown DC were similar for all ten tests. The most prominent VOCs included BTEX. High exposure to these toxins can cause damage to the overall health of the driver and passengers. The National Institute of Occupational Safety and Health short-term exposure limit is 1.0 ppm (3.2 mg/m³) for benzene and 151 ppm (560 mg/m³) for toluene. The time-weighted average recommended exposure limits for 10-hour workdays during a 40-hour workweek are ~0.3 ppm in isobutylene equivalents; given published correction factors, 0.1 ppm benzene is equivalent to 0.2 ppm in isobutylene equivalents. While further calibration is necessary to determine precise benzene concentrations, BTEX levels during the commute were well below short-term exposure limits but could approach exposure limits for those spending 10-hour workdays in traffic areas with consistently high levels of BTEX.
Polycyclic aromatic hydrocarbons (PAHs) play a central role in the carcinogenic effects of combustion-derived pollution, but have also been linked to CVD. Pyrene is among the most abundant PAHs on CPMs, but has received little toxicological attention due to its low carcinogenicity. However, our recent results suggested that pyrene triggered calcium influx ([Ca2+]i) in human endothelial cells (HMEC-1), and potentiated proinflammatory responses in bronchial epithelial cells induced by other agents. Here we explored the mechanisms of pyrene-induced ([Ca2+]i) in HMEC-1. Pyrene induced a prolonged ([Ca2+]i) that seemed to be initiated by Ca2+-release from intracellular stores and followed by a later phase of extracellular Ca2+-influx. Use of pharmacological inhibitors indicated that the initial phase was linked to store operated calcium channels (SOCE)/endoplasmic reticulum (ER), possibly involving inositol 1,4,5-trisphosphate receptor (IP3R). However, a role of transient receptor potential channels (TRPC) could not be excluded. The late phase seemed more linked to plasma membrane and calcium release-activated channels (CRAC). Pyrene also changed the membrane microstructure, rendering the membrane more fluid. Both the pyrene-induced ([Ca2+]i) and membrane alterations appeared to depend on activation of aryl hydrocarbon receptor (AhR)-signalling. Furthermore, addition of cholesterol inhibited the increase of membrane fluidity and ([Ca2+]i) triggered by pyrene, indicating that membrane alterations at least partly preceded the Ca2+-entry. By contrast, pyrene did not induce genomic alterations by pyrene, indicating that membrane alterations at least partly depended on activation of AhR-signalling as indicated by lack of CYP1A1/-1B1 gene expression. Instead, pyrene suppressed CYP1-expression by the prototypical carboxylic acid receptor (CAR)-signalling. Pyrene also induced membrane wrinkle formation of extracellular Ca2+. These responses seem to be initiated by Ca2+-release from extracellular stores and followed by a later phase of extracellular Ca2+-influx. Use of pharmacological inhibitors indicated that the initial phase was linked to store operated calcium channels (SOCE)/endoplasmic reticulum (ER), possibly involving inositol 1,4,5-trisphosphate receptor (IP3R). However, a role of transient receptor potential channels (TRPC) could not be excluded. The late phase seemed more linked to plasma membrane and calcium release-activated channels (CRAC). Pyrene also changed the membrane microstructure, rendering the membrane more fluid. Both the pyrene-induced ([Ca2+]i) and membrane alterations appeared to depend on activation of aryl hydrocarbon receptor (AhR)-signalling. Furthermore, addition of cholesterol inhibited the increase of membrane fluidity and ([Ca2+]i) triggered by pyrene, indicating that membrane alterations at least partly preceded the Ca2+-entry. By contrast, pyrene did not induce genomic AhR-signalling as indicated by lack of CYP1A1/-1B1 gene expression. Instead, pyrene suppressed CYP1-expression by the prototypical carcinogenic PAH, benzo[a]pyrene. We propose that pyrene is an AhR-ligand that specifically triggers non-genomic signalling, involving IP3R/SOCE-mediated Ca2+-release from ER, followed by CRAC- and possibly also TRPC-mediated entry of extracellular Ca2+. These responses seem to be initiated by AhR-mediated membrane remodeling. Thus, pyrene and other PAHs could possibly affect vascular tone and contribute to development of CVs by distorting endothelial ([Ca2+]i).-
expressions of cleaved-caspase 3 and cleaved-caspase 9 at 4 hr were the highest ones. In conclusion, the autophagy was firstly induced, then the apoptosis was mediated via the intrinsic apoptotic pathway, and finally the necrosis was caused in BEAS-2B cells exposed to low concentrations of crotonaldehyde.

### 2790 Crude Oil Vapor Effects upon Airway Epithelial Ion Transport and Lung Function in the Rat
J. A. Thompson, NIOSH, Morgantown, WV.

Crude oil vapor (COV) is a mixture of hydrocarbon vapors and volatile organic compounds (VOCs). Workers in the oil and gas industry are potentially exposed to COV while conducting routine tasks such as manual sampling, gauging and filling crude oil storage tanks from oil tanker trucks. The effects of COV inhalation exposure on the pulmonary system are unknown. Previously, we found there were no significant changes in pulmonary function or airway epithelial ion transport after acute inhalation exposure to COV (300 ppm total VOCs, 6 h/d, 1 d). In the current study, the effects of a sub-chronic inhalation exposure of COV on lung function and epithelial ion transport were investigated. Rats in whole body chambers were exposed to 300 ppm total VOCs for 28 d. Experimental endpoints were measured at 18 h, 28 h and 90 d post-exposure. Total VOCs, benzene, toluene, ethylbenzene, and xylene concentrations were monitored and regulated during exposures to maintain concentration constancy. Transepithelial potential difference (Vt), transepithelial resistance (Rt), and short-circuit current (isc) were measured in tracheae mounted in Ussing chambers and treated with the ion transport inhibitors amiloride (Na+ channel blocker; apical), 5-nitro-2-(3-phenylpropylaminol)benzoic acid (NPPB; Cl− channel blocker; apical), and ouabain (Na+K+ pump blocker; basolateral). Compared to air-breathing controls, the isc response to NPPB was increased significantly at 28 d post-exposure, indicating an increase in CI transport in the airway epithelium. There were no changes in Vt or Rt at 18 h or 28 d post-exposure, although the dynamic conductance (Gdyn) and resistance to inhaled methacholine (MC1) were measured in anesthetized rats. COV significantly increased basal Rt compared to air-breathing controls at 90 d post-exposure. There was no effect of COV on basal Cdyn or reactivity to inhaled MC1 at any time point. Our results indicate that sub-chronic exposure to COV changes airway ion transport and pulmonary function.

### 2791 Three Dimensional (3-D) Printer Emission-Induced Cell Toxicity
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Three dimensional (3-D) printing with polymer thermoplastics is known to emit millions to billions of ultrafine particles (diameter <100 nm) and volatile organic compounds that are formed by thermal decomposition and/or vaporization and condensation of the filament. There is a paucity of data on potential toxicity of these emissions. As such, we generated emissions from a commercially available 3-D printer while operating with acrylonitrile butadiene styrene (ABS) or polycarbonate (PC) filaments in a 0.5 m³ environmental chamber. Particles were collected into serum free cell culture media using a liquid impinger sampler and the size and number concentration determined using nanoparticle tracking analysis. The mean sizes of PC and ABS-emitted particles in cell culture media were 160 ± 57 nm and 181 ± 21 nm, respectively. To determine the cytotoxicity of 3-D printer emissions, human small airway epithelial cells (SAEC) were seeded in a 96-well plate at a density of 1.5 × 10⁴ cells per well and treated for 24 h with 1 × 10⁴ particles/ml from PC and ABS emissions, followed by the cellular viability assays. The results showed that the exposures of PC and ABS emissions to SAEC significantly decreased the cell viability by 67% and 31%, respectively. Furthermore, it was found that the exposure of the PC emission significantly induced more toxicity than that of the ABS emission. Our preliminary data indicate that the emissions generated by 3-D PC and ABS filaments induce a toxic response in SAEC, and the emission of the PC filaments is significantly more toxic than that of the ABS filaments. Further studies are in progress to evaluate the mechanisms of 3-D printer emission-induced cellular toxicity.
bacterial clearance, and diminished Th2 responses. Cessation of SHS exposure for 21 d restored previously suppressed responses, including phagocyte recruitment, IgA secretion, and mucous cell metaplasia. However, in contrast with FA-Tg + mice, the SHS-Tg + mice had pronounced epithelial necrosis, alveolar space consolidation, and lymphoid hyperplasia; indicating lagged unfavorable effects of early postnatal SHS exposure in life. Taken together, these data suggest that early exposure to SHS may predispose muco-obstructive disease patients to exacerbated bacterial infections.

2795 Electronic Cigarette Chemicals Transfer from a Vape Shop to a Nearby Business in a Multi-User Building

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Electronic cigarettes (EC) are nicotine delivery devices that produce aerosol without combustion of tobacco and therefore they do not produce sidestream smoke. Nevertheless, users exhale large clouds of aerosol that can result in passive exposure of non-users. The exhaled aerosol settles on indoor surfaces where it can produce a residue analogous to thirdhand smoke from conventional cigarettes. We refer to this residue as EC exhaled aerosol residue (ECEAR). The objective of this study was to determine if exhaled EC aerosol transferred from a vape shop to a mini-mall, where it was produced, to a nearby business (field site) where it could deposit as ECEAR. The buildup of ECEAR was evaluated in commonly used fabrics (cotton and paper towels) placed inside the field site across from the vape shop. Fabrics were subjected to short (days) and long-term (months) exposures. Flavor chemicals, nicotine, nicotine alkaloids, and tobacco-specific nitrosamines (TSNAs) were identified and quantified in unexposed controls and field site samples using analytical chemical techniques. Several flavor chemicals present in EC refill fluids and aerosols were detected in ECEAR samples from the field site. Nicotine, nicotine alkaloids, and TSNAs were detected in extracts of paper and cotton towel fabric from the field site. The most abundant marker of EC aerosol contamination (highest concentration found = 23,260 ng/g of fabric). Nicotine and nicotine alkaloids were detected after 1 day of exposure in the field site (154 ng/g of nicotine/g of paper), and these chemicals generally increased as exposure times increased. TSNAs, which have been linked to carcinogenesis, were also detected in short- and long-term-exposed samples from the field site. In a multiuser mini-mall, chemicals in EC aerosol traveled from a vape shop into an adjacent business where they deposited forming ECEAR. Tenants occupying multi-user buildings and regulatory agencies should be aware of this potential environmental hazard.

2796 Subacute Acrolein Exposure-Induced Inflammation in the Larynx

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Acrolein exists abundantly in cigarettes and contributes to the most non-cancer toxicity in the respiratory system among components of cigarette smoke. Symptoms commonly complained by smokers pertain to hoarseness and lowered fundamental frequency of voice. However, the mechanism of the voice impairment induced by smoking remains elusive. This study was designed to investigate if subacute inhalation exposure to acrolein in rats caused an inflammatory reaction in larynxes. Sprague Dawley rats (male, 4-month old) were exposed to either 3-ppm acrolein (treatment) or filtered air (control) in a whole-body exposure chamber for 5 hr/day, 5 days/week, for 4 weeks. Laryngeal tissues were dissected at 4 hr after the last exposure. Pathological changes of inflammatory cells and tissue damage were evaluated in the true vocal folds. The qPCR analysis showed that the expressions of cytokines and chemokines were increased in acrolein-exposed tissues compared to control tissues. Furthermore, the expression of metalloproteinases was upregulated in acrolein-exposed tissues. These findings suggest that exposure to acrolein induces inflammation in larynx, which involves NF-κB regulation and alteration of gene expressions of proinflammatory cytokines. Our data builds the groundwork for further mechanistic investigation of inflammation induced by acrolein in larynx structure and its role in the dysfunction of vocal folds during cigarette smoke. Supported by NIH/NIDCD R01DC011759.

2797 Nicotine Characterization of an In Vitro Inhalation Exposure System Using Conventional and Next-Generation Tobacco Products: A US/UK Inter-Laboratory Comparison

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Comparative analysis of data from interlaboratory studies utilizing different exposure systems is an important step in standardizing in vitro testing of next generation tobacco and nicotine products (NGPs). Dosimetric characterization can align different systems and add confidence to the biological data obtained following exposure, allowing for a better dose-response assessment. Three in vitro aerosol exposure systems (Borgwaldt RM205, Virocell VC10 and VC1) were compared in two laboratories - US and UK - with three different types of nicotine delivery products - 3R4F reference cigarette (at ISO and HCl regimes), an e-cigarette (Vype ePen) (at CRM81 regime) and a tobacco heating product (glo) (at modified HCl regime). Cambridge filter pad (CFP) air was used to generate aerosol allowed 24h perfusion of cells, exposure products, exposure systems and laboratories. CFP aerosol was extracted with solvent, spiked with an internal nicotine standard and quantified by UPLC/MS MS. The cigarette at ISO was 0.080 mg/puff in both labs, and at HCl was 0.193 mg/puff in the UK and US respectively. The e-cigarette was 0.069 mg/puff in both labs and the glo was 0.048 and 0.050 mg/puff in the UK and US respectively. A General Linear Model ANOVA demonstrated that nicotine profiles were characteristically different between products and puffing regimes (p=0.000, where <0.05 was considered statistically different) but were the same within product category across different laboratories, exposure systems and puffing regimes. Such information is especially important when establishing new inhalation exposure facilities and characterizing exposure systems to demonstrate consistency with established inhalation exposure results. Interlaboratory studies help to demonstrate transferability and reliability when aligning different in vitro methods, laboratories and products. Furthermore, thorough dosimetric characterization provides confidence in the in vitro response to NGPs compared to traditional combustible cigarettes. The interlaboratory data further suggest that the nicotine quantification method employed is an appropriate approach to compare different exposure systems in different locations.

2798 Reversibility of In Vitro Biological Effects in Cigarette Smoke-Exposed 3D Lung Tissue Models Following Switching to a Tobacco-Heating Product

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Tobacco heated products (THPs) potentially offer a safer alternative to combustible cigarettes. Recent in vitro studies have shown reduced biological effects of THPs compared to 3R4F reference cigarette smoke. Existing in vitro data, however, has been generated performing acute, single exposures not reflective of consumer use. Furthermore, the reversibility of the biological effect of cigarette nicotine quantity switching to THPs has not been extensively studied in vitro. A feasibility study was conducted to assess the potential of using MucilAir™ tissues in a 4 week repeated exposure study. Tissues were exposed to 3R4F smoke (15 mins x3 times a week) for 2 weeks after which the cohort were split into three conditions - further 2 week repeated exposure and switching to THP or a switch to air. The Borgwaldt RM205 generated whole aerosols at the Health Canada Intense smoking regime. Endpoints assessed included cytotoxicity, tight-junction integrity and cytokine expression (panel of 33 cytokines). The results were compared to a continuous air exposure control at week 4. During the 4 week repeated exposure the case remained below 10% for all tested conditions and TEER above 500 Ω/insert, indicative of tissue integrity. After two weeks 3R4F repeated exposure, an increase in cytokine expression was observed (14 FC>1.5, p<0.05), however following 4 week 3R4F repeated exposure, a strong differential cytokine expression was demonstrated, with 14 responsive cytokines in the culture media including MMP-9, IL-6, IL-4, IL-1α, VEGF at p<0.05, FC>1.5. However, tissues that were switched to THP aerosols for 2 weeks following 3R4F repeated exposure, demonstrated lower cytokine expression with only eotaxin-3 and MMP-9 remaining.
2799 An Approach to Testing Undiluted E-Cigarette Aerosol In Vitro Using 3D Human Airway Epithelium


With the increasing emergence and subsequent popularisation of electronic nicotine delivery systems (ENDS), suitable and fit-for-purpose in vitro test methods are required to enable relevant pre-clinical ENDS assessment. Current in vitro exposure systems have complex aerosol generation and dilution principals that may not be practicable when assessing less toxic aerosols, such as those from e-cigarettes. In this study, 3D human airway tissues (MucAir™), were exposed to cigarette or e-cigarette aerosols at the air-liquid interface (ALI) using a modified Vitrocell VC10 to deliver undiluted aerosols, thereby fixing the dose and varying the exposure duration (mins). Following exposure, nicotine in the cell-culture media and cellular functional endpoints, including TEER, cilia beat frequency and cytotoxicity, were assessed. Using 3ARF scientific reference cigarettes two smoking regimes, ISO and HCl, were initially tested and resulted in IC50s of 5.2 and 2.1 mins respectively with equivalent cell media nicotine concentrations of 1458 ng/mL and 1640 ng/mL. Using a high aerosol delivery, open tank e-cigarette device (Vype eBox), and blended tobacco (18mg/mL nicotine) liquid in this modified VC10 system (CRM 81 regime), a cytotoxicity dose-response curve was obtained having an IC50 30 mins with corresponding nicotine 10957 ng/mL. Functional endpoints tested following e-cigarette exposures indicated a loss in mucociliary clearance and cell tissue integrity observed at exposure durations >20 mins. To further test the aerosol generation of the modified exposure system, a positive control aerosol was tested in parallel. Addition of 0.025% cinnamaldehyde to the e-liquid formulation increased cytotoxicity of the delivered undiluted aerosol, inducing 80% cytotoxicity after 20 mins of exposure, compared to 0% cytotoxicity with regular e-liquid at the same exposure duration, further substantiating the aerosol delivery technique. Nicotine dosimetry assessments further demonstrated that adaptations made to the VC 10 did not adversely affect performance, enabling the generation of consistent and significantly higher delivery of e-cigarette aerosols. This study has enabled the delivery of undiluted cigarette and e-cigarette aerosols to in vitro 3D human reconstituted lung epithelia at the air-liquid interface, which in turn has reduced exposure times from hours to minutes.

2800 Comparison of In Vitro Cytotoxicity and Genotoxicity of Condensates Derived from E-Vapor Products and Combustible Cigarettes

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The FDA draft guidance (2016) on premarket tobacco application for e-vapor products recommends full toxicity assessment including in vitro genotoxicity and cytotoxicity. Herein, we collected the condensates from e-vapor products (MarkTen™ with menthol and non-menthol flavors) and reference (3RF) cigarettes, on a Cambridge filter followed by an impinger filled with ethanol at 0°C. The condensates were then tested using standard in vitro assays: Neutral red uptake (NRU) for cytotoxicity, Salmonella mutagenicity (Ames), and micronuclei (MN) for genotoxicity. E-liquids used for e-vapor products contained aerosol formers (propylene glycol [PG] and glycerol), water, nicotine (up to 2.5%) and flavor mixtures. The condensates (up to 47 mg total particulate matter (TPM)/mL) were analyzed for key formulation components and carbonyls, stored frozen at -70° C, tested in vitro with 48h of collection, and assessed for stability up to 8 weeks in storage. The 3RF condensate tested positive in the NRU assay (IC50 of 0.048±0.004 mg/mL TPM), whereas the e-vapor condensates showed viability >80% (IC50 could not be estimated). The 3RF condensate tested positive in the Ames assay in strains TA1537 and TA98, whereas the e-vapor condensates did not in any of the five strains tested. The 3RF condensate also tested positive in the MN assay but no significant effect was observed with the e-vapor condensates. Key formulation components as well as carbonyls were detected in e-liquid condensates and were stable for up to 8 weeks at -70° C. In summary results from this study are consistent with the literature findings that e-vapor product aerosols have substantially lower biological activity than combustible cigarettes.

2801 An Impact of Exposure to Aerosols from Nebulized E-Liquids on Human Organotypic Airway Cultures


E-cigarettes (e-cig) comprise a variety of electronic devices used to heat an e-liquid. The e-liquids are typically composed of nicotine and varying flavors in propylene glycol and glycerol-based solutions. More than 7000 e-liquids can be found on the market. Some flavors have been categorized as Generally Recognized as Safe (GRAS), by the US FDA when used as food ingredients but their potential toxicity when inhaled is largely unknown. With the increasing use of e-cigs and variety of e-liquids, robust toxicity evaluations are needed. This study showed that nebulizing e-liquids to generate aerosols is a promising testing approach to assess their potential toxicities. Organotypic airway cultures were exposed to nebulized aerosols of two different e-liquids: 1) a propylene glycol and glycerol solution containing nicotine without flavors (PG/G/NIC), and 2) a propylene glycol and glycerol solution containing nicotine without flavors (PG/G/NIC/Flavors). The flavor mixture contains a selection of 28 flavors representing their respective FDA when used as food ingredients but their potential toxicity when inhaled is largely unknown. With the increasing use of e-cigs and variety of e-liquids, robust toxicity evaluations are needed. This study showed that nebulizing e-liquids to generate aerosols is a promising testing approach to assess their potential toxicities. Organotypic airway cultures were exposed to nebulized aerosols of two different e-liquids: 1) a propylene glycol and glycerol solution containing nicotine without flavors (PG/G/NIC), and 2) a propylene glycol and glycerol solution containing nicotine without flavors (PG/G/NIC/Flavors). The flavor mixture contains a selection of 28 flavors representing their respective chemical groups according to relevant toxicological data; their concentration was derived from the maximum use levels. Tissues exposed to air or nebulized cigarette smoke (CS)-bubbled phosphate-buffered saline (PBS) were used as benchmark comparisons. Culture morphology, secreted proinflammatory mediator profile, and Affymetrix array-based whole transcriptome were analyzed after exposure. Exposure characterization included measurements of the deposited PG, G, and NIC concentrations in exposure chambers. Culture morphology was not different across the groups. Concentrations of secreted proinflammatory mediators following exposure to PG/G/NIC aerosol were not different from the air-exposed controls. However, concentrations of interleukin (IL6 and IL8 higher in samples exposed to PG/G/NIC/Flavors aerosol compared with those in the air-exposed samples. Changes in the gene expression profiles were detected; the number of the altered genes after exposure to PG/G/NIC or PG/G/NIC/Flavors aerosols were substantially lower than those exposed to aerosolized CS-bubbled PBS.

2802 Withdrawn by Author

2803 The Use of Human 3D Reconstructed Bronchial Tissue to Study the Effects of Cigarette Smoke and E-Cigarette Aerosol on a Wide Range of Cellular Endpoints

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In 2015, Public Health England concluded e-cigarettes are around 95% less harmful than smoking tobacco cigarettes and in 2016 the Royal College of Physicians stated e-cigarettes should be promoted widely as a substitute for smoking. However, some recent data has indicated that e-cigarette aerosol can potentially produce reactive oxygen species which may give rise to inflammation, DNA damage and reduced cell viability. In this study, biological endpoints were investigated in the EpiAirway™ 3D in vitro model (MatTek Corp), a highly differentiated human airway culture derived from primary tracheal/bronchial epithelial cells. EpiAirway™ tissue was exposed at the air-liquid interface to whole smoke from a conventional tobacco cigarette and aerosol generated from an e-cigarette device using two different e-liquids (commercial and experimental e-liquid both with 2.4% nicotine). Smoke or aerosol was generated using a VITROCELL™ VC 1 smoking machine following Health Canada Intense and CRM81 regime, respectively. The integrity of the epithelial barrier of tissues, was measured before and 24 hours after exposure using trans-epithelial electrical resistance. Tissue viability was assessed using the MTT assay. Conditioned media were collected 24 hours after the exposure, to determine tissue secretion of the pro-inflammatory cytokines IL-6 and IL-8. The 8-Isoprostane assay evaluated oxidative stress response. Tissue morphology was assessed by H&E staining. The TUNEL assay was used to detect apoptotic cells;
2804 Toxological Assessment of Aerosol from Flavored E-Liquids in Sprague Dawley Rats: A 90-Day Sub-Chronic Inhalation Study

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Relatively few in vivo inhalation studies were performed to evaluate the toxicity of flavor compounds commonly added to e-liquids. The toxicity of chemical groups of flavor substances was characterized in a 90-day sub-chronic inhalation study according to the OECD 413 testing guidelines using a selection of 28 flavors, which represented their respective chemical groups according to the relevant toxicological data. Sprague-Dawley rats were exposed for 6 h/day, 5 d/week for 13 weeks to aerosols of e-liquid (propylene glycol (PG), vegetable glycerin (VG) and nicotine), with three concentrations of flavor compound mixture, or VG with medium concentration of flavor compound mixture. The target test atmosphere concentrations of nicotine, PG and VG were 23 µg/L, 1520 µg/L and 1890 µg/L, respectively. The lowest concentrations of the 28 flavors tested in this study corresponds to the current maximum use levels in tobacco products. Rats exposed to the nicotine-containing aerosols had gender-specific changes to body weight—lower in males, higher in females. Subtle changes of the respiratory physiology parameters indicated a mild irritancy of the flavor mixture in both sexes, while the presence of nicotine countered this effect. Low level of inflammatory cells in the bronchoalveolar lavage fluid of animals in all groups, indicating minimal inflammatory inflammation. Slightly higher activity of alkaline phosphatase, and lower concentrations of metabolic markers were noticed mainly in serum from nicotine-exposed groups. Organ weight changes including higher liver and adrenal weights, and lower thymus weights were observed following nicotine exposure. There were few histopathological findings among which minimal laryngeal squamous metaplasia due to PG/VG exposure, with partial reversibility following a 42-day recovery period. Microscopic findings in adrenal glands and thymus were considered secondary due to stress. In summary, nicotine and PG/VG exposure-related findings were seen without synergistic effects caused by the flavors.

2806 Characterization of E-Cigarette Aerosols in Two Different Exposure Regimens for a Nose-Only Rodent Inhalation System

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Inhalation studies require reliable exposure generation and characterization regardless of the type of product being tested. In this study, an established rodent nose-only inhalation exposure setup was adapted to generate aerosols from a cig-like e-vapor product and conventional (3RF) cigarettes using a rotary cigarette smoking machine (CSM). For the e-vapor product, aerosols were generated in two ways. The first was using the CORESTA CR81 puffing regime (30-sec puff interval, 3-sec puff duration, and a puff volume of 55 mL) for 180 puffs/cartridge. The second was using a modified regime with a longer puff duration (CRM81, except for 5-sec puff duration) and a puff count of 120 to 130 puff/cartridge. The 3RF-cigarette smoke aerosols were generated using smoking regimens 30-sec puff duration and 1500 µg/L nicotine, and the e-vapor TPM concentration met the target concentration within 6% for each exposure cohort with or without active feedback control. The MMAD was initially slightly higher for the modified regime compared to the CRM81 regime, however following modifications to the exposure generation system the particle size was comparable (~1.1 um) for both e-vapor exposure regimes. Ratios of e-vapor aerosol constituents (nicotine, PG, and glycerol) were similar and did not change from the CSM to nose ports. For the 3RF CSM connected to two exposure carousels, TPM was within 1% of target (550 µg/L), the MMAD was at ~0.7 µm and the CO/TPM ratio was ~1.2. This work provides the basis to establish repeated rodent nose-only inhalation exposure regimes.
ogy for the assessment of whole aerosols. Cigarette smoke from TPM test matrices was deemed positive under almost all test conditions in all assays. For NRU, Ames, MLA, Bhas 42 and IVMN assays, responses were observed at 60µg/mL, 240µg/plate, 60µg/mL, 50µg/mL and 30µg/mL, respectively. In contrast, THP TPM failed to elicit a response in each of the assays up to 240µg/mL. Cigarette smoke was also deemed positive in the Ames assay at doses up to 50µg/cm³. THP aerosols were negative at doses exceeded 25µg/cm³. Given a weight of evidence approach, these data demonstrate that THP test matrices are negative at doses equivalent and exceeding those of cigarette smoke where positive responses are observed, suggesting THPs may offer significant reduced risk potential compared to cigarette smoking.

2808 Chronic Toxicity and Carcinogenicity of 2,3-Butanedione (Diacetyl) Vapors in Rodents

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2,3-Butanedione (BD, diacetyl) is a common ingredient in artificial butter flavor formulations used in many food and beverage products. While consumption of the amounts of BD present in food is generally considered to be safe, occupational exposure to BD vapors has been associated with obstructive bronchiolitis (OB), an irreversible fibroproliferative disorder of the small airways. Acute and repeated short-term exposure to BD has been shown to cause significant respiratory tract toxicity and OB in rodents; however, the effects of chronic BD inhalation are unknown and were investigated in this study. Wistar Han rats and B6CF1/N mice (50/sex/species/concentration) were exposed to 0, 12.5, 25, 50, and 100 ppm BD 6 h/day, 5/d/wk for 105 wk. Survival and body weights of male and female rats and mice exposed to 50 ppm were significantly less than controls. BD exposure caused a spectrum of nonneoplastic lesions of the nose, larynx, trachea, lung, and eye in male and female rats and mice, primarily in the 25 and 50 ppm groups. The most severe lesions were observed in the nasal cavity and included respiratory epithelial hyperplasia, squamous metaplasia, and necrosis; olfactory epithelial atrophy, respiratory metaplasia, and necrosis. Although OB was not observed, significant fibrosis was present in the nose of rats and mice exposed to 25 and 50 ppm BD, and in the trachea of both rats and mice and in the lung of rats exposed to 50 ppm BD. Rare squamous cell carcinomas of the nasal mucosa occurred in 3 male and 3 female rats exposed to 50 ppm. A squamous cell papilloma was also present in the nasal cavity of 1 male rat exposed to 50 ppm. No squamous cell carcinomas or papillomas of the nose occurred in control rats. In mice, rare adenocarcinomas of the nasal cavity occurred in 25 ppm females. No nasal neoplasms were observed in exposed male mice or control mice. BD is mutagenic and can form adducts with 2-deoxyguanosine. The sustained cytotoxicity and cell proliferation in the nasal cavity caused by chronic BD exposure may have predisposed animals to nasal carcinogenesis.

2809 Comparison of E-Vapor Carrier Mixtures to Air Control and Cigarette Exposure Using 90-Day Inhalation Studies in Rats

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Propylene glycol (PG), glycerin and water are used as the carrier in e-vapor product formulations. OECD 413 compliant (BALF analysis) 90-day nose-only inhalation studies were performed where Sprague Dawley rats were exposed (160 min/day, 7 days/wk) to aerosols generated from three different carrier mixtures of PG, glycerin and water. Biological responses were compared to the current filtered air (Control). In addition, histopathological findings in respiratory tract were also compared to literature values from Sprague Dawley rats exposed to mainstream (2RF4) cigarette smoke. To acknowledge procedural differences in histopathology evaluations among studies, for each change noted, a “histopathology response” was determined by multiplying the severity of each change by its incidence. The most severe histopathology response from each change, from either sex, was added together to obtain an overall histopathology response for all changes noted in the nose, larynx and lung. The e-vapor and 2RF4 groups were then compared at “daily presented dose (DPD)” (exposure time multiplied by concentration (mg/L)). Compared to the control groups, the e-vapor carrier groups showed no differences in body mass, clinical chemistry, hematology, organ weights or urinalysis, and histopathological findings (incidence and severity). In the bronchoalveolar lavage (BAL) parameters measured after 13 weeks of exposure, e-vapor groups showed a slight but non-statistically significant increase in LDIH in males but not females. In histopathology response, the e-vapor carrier groups displayed a flat dose-response in the nasal and laryngeal findings similar to the air control. At comparable DPD, the average nasal and laryngeal response to cigarette smoke was 3 and 13 times greater, respectively, than the average response from the e-vapor group. The lung histopathology response for the e-vapor carrier group showed increasing scores with increasing DPD; however, it required over 3 times higher DPD to obtain the same response as cigarette smoke. In conclusion, there were no significant differences in biological endpoints between e-vapor carrier and filtered air control groups. In contrast, e-vapor carrier exposures showed substantially reduced histopathological responses in all regions of the respiratory tract compared to mainstream cigarette smoke exposure.

2810 Lung Inflammation and Emphysema in A/J Mice in Response to Chronic Exposure to Aerosol from a Candidate-Modified Risk Tobacco Product and Mainstream Cigarette Smoke

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Chronic exposure to cigarette smoke is the leading cause of chronic obstructive pulmonary disease and lung cancer. The A/J mouse model was used to evaluate lung inflammation, emphysema and the underlying molecular changes upon life-time exposure to cigarette smoke (CS) from the 3RF4 reference cigarette or to aerosol from the Tobacco Heating System (THS) 2.2, a candidate modified risk tobacco product (MRTP) at three concentrations. A/J mice were exposed for 6 hours per day for 5 days per week for up to 18 months. Quantification of pulmonary inflammation, assessment of matrix metalloproteinase (MMP) activity in bronchoalveolar lavage fluid (BALF), lung function tests, lung morphometric assessments by stereological approach, histopathological evaluation, targeted proteomic analysis of BALF, as well as transcriptome and proteome analysis of the lungs were performed at selected interim and terminal dissections. Exposure to CS resulted in pulmonary inflammation, increased MMP activity, altered lung function, enlargement of distal airspaces in the lungs and molecular changes, all indicative of emphysema. Only minimal effects on the lungs were observed following THS2.2 aerosol exposure that were independent of THS2.2 aerosol concentration and duration. Biological analysis identified perturbed molecular processes that are consistent with CS exposure of the lungs, but only minor perturbations of cell stress responses were noted following THS2.2 aerosol exposure. Biological network analysis identified perturbed molecular processes that are consistent with CS exposure, but only minor perturbations of cell stress responses were noted following THS2.2 aerosol exposure. In summary, lung inflammation, emphysematous changes and molecular perturbations were significantly lower in mice exposed to aerosol from THS2.2 as compared to CS exposure.

2811 A Six Month Systems Toxicology Inhalation/Cessation Study in ApoE−/− Mice to Investigate Cardiovascular and Respiratory Exposure Effects of Two Candidate Modified Risk Tobacco Products Compared with Conventional Cigarettes

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Cigarette smoking causes adverse health effects that may occur shortly after smoking initiation and lead to the development of cardiovascular disease (CVD) and chronic obstructive pulmonary disease (COPD) as well as cancers. To reduce the risk for smokers to develop smoking-related diseases, modified risk tobacco products (MRTPs) are being developed. Engaging a systems toxicology approach, combining physiological, histological and omics endpoints, the effects of a 6-month exposure to cigarette smoke (CS) or to aerosols from two candidate MRTPs termed CHTP1.2 and THS2.2, were investigated in ApoE−/− mice. In addition, the impact of cessation or switching to CHTP 1.2 aerosol exposure after 3 months of CS exposure was evaluated. Our results demonstrated that exposure to CS at a concentration of 28.0 µg nicotine/L cause adverse effects on the lungs including increased
lungs, lung inflammation, aortic plaque formation and a dysregulation of the heart transcriptome. In contrast, exposure to either THS2.2 or CHTP1.2 aerosol at matched nicotine concentrations did not induce lung inflammation or enhance plaque development. Cessation or switching to CHTP1.2 aerosol exposure reversed lung inflammatory responses and halted progression of aortic plaques. Biological pathways, including “Drug metabolism-cytochrome P450”, “Focal Adhesion”, “ECM-receptor interactions” and “Tryptophan metabolism” were significantly impacted by CS exposure in heart tissue, but not by exposure to CHTP 1.2 or THS2.2 aerosols. Both, cessation and switching to CHTP1.2 aerosol reduced these perturbations to levels similar to those in sham animals. In conclusion, in this ApoE-/− mouse study, exposure to THS2.2 and CHTP1.2 aerosol had minimal adverse respiratory and cardiovascular effects. In addition, cessation or switching to CHTP1.2 aerosol exposure delayed the progression of CS-induced atherosclerotic and lung emphysematous changes.

2812 Investigating the Effects of Nicotine Using a Human Multi-Organ-Chip Approach

Currently, analyzing the effects of substances and the interaction of a lung and liver co-culture in vitro is unfeasible. However, TissUse’s Multi-Organ-Chip (MOC) platform provides preclinical insight on a systemic level using human tissue. It enables the direct prediction of effects of chemicals and their metabolism on real-life models. In order to be able to elucidate the toxicity of inhaled compounds, we adjusted the MOC design for the optimal co-cultivation of a human liver equivalent and the MucoAir™ lung model. Tissue integrity and function were evaluated using metabolic analysis, measurement of trans-epithelial electrical resistance and cilia beat frequency, immunohistochemistry, gene expression analysis and albumin secretion. Viability and homeostasis could be demonstrated for 14-day lung/liver co-cultures. Initial tests with nicotine, applied either apically to the lung model or systemically, indicated active compound metabolism and the utility of the current MOC set-up for future research into the biological effects of inhaled compounds on the homeostatic co-culture.

2813 Pathway Analyses of Global Metabolomic Profiles Identify Enrichment of Caffeine, Energy, and Arginine Metabolism in Smokers, But Not Moist Snuff Consumers
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Existing US epidemiological data demonstrate that consumption of smokeless tobacco, particularly moist snuff, is less harmful than cigarette smoking. However, the molecular and biochemical changes due to moist snuff consumption relative to smoking remain incompletely understood. Based on metabolomic profiling data of saliva, plasma and urine from moist snuff consumers (MSC), cigarette smokers (SMK), and non-tobacco consumers (NTC), we reported that smokers exhibit elevated oxidative stress and inflammation relative to MSC and NTC. In this study, we investigated the effects of tobacco consumption on additional metabolic pathways using pathway-based analysis tools. To this end, metabolic pathway enrichment analysis, and topology analysis were performed through pair-wise comparison of global metabolomic profiles of SMK, MSC and NTC. The analyses identified >8 significantly perturbed metabolic pathways in SMK compared to NTC and MSC in all three matrices. Among these differentially enriched pathways, caffeine metabolism, energy metabolism, and arginine metabolism were mostly observed. In comparison, much fewer enriched metabolic pathways were identified in MSC compared to NTC (5 in plasma, none in urine and saliva). This is consistent with our transcriptomics profiling results that show no significant differences in peripheral blood mononuclear cell gene expression between MSC and NTC. These findings, taken together with our previous biochemical, metabolomic and transcriptomic analysis results, provide a better understanding of the relative changes in healthy tobacco consumers, and demonstrate that chronic cigarette smoking, relative to the use of smokeless tobacco, results in more pronounced biological changes, which could culminate in smoking-related diseases.

2814 Distinct Gene Expression Changes in Peripheral Blood Mononuclear Cells Exposed to Combustible Versus Non-Combustible Tobacco Product Preparations
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Cigarette smoking exerts diverse physiological effects including immune suppression. Existing US epidemiological data show that consumption of smokeless tobacco products, such as moist snuff, is less harmful relative to cigarette smoking. In efforts to understand the molecular changes due to consumption of different tobacco product classes, we have shown recently that smokers exhibit distinct peripheral blood mononuclear cells (PBMCs) gene expression patterns relative to moist snuff users and non-tobacco consumers (NTCs). To better characterize the biological effects exerted from the use of different tobacco products, a genome-wide gene expression study, using a PBMC in vitro model, was performed. We investigated global gene expression changes in PBMCs exposed to aqueous extracts of cigarette smoke (whole smoke conditioned media [WSCM]) and moist snuff extract (moist smoke tobacco extract [STE]) prepared from reference tobacco products (3R4F and 2S3, respectively). PBMCs were isolated from whole blood of four healthy NTC donors and exposed under different doses of WSCM or one dose of STE. Global gene expression profiling was performed using Affymetrix HTA 2.0 arrays. Our results showed that 5,421 genes (2,809 upregulated and 2,612 downregulated) were dose-dependently changed by WSCM (pFDR < 0.001). Some gene expression changes detected in the in vitro system were also observed in clinical studies. For example, PER2 and SLAMF7 were suppressed by WSCM while CCR2 and HHEX genes were upregulated by WSCM commonly in both in vitro and clinical studies. WSCM exposures, but not STE, uniquely affected genes involved in immune cell development and inflammatory response. Ingenuity Pathway Analysis identified upstream regulators, such as TNF, IL18, and NFκB to be likely responsible for the observed gene expression changes and that these cascading signals were generally suppressed by WSCM, but not STE. Collectively, these findings suggested that combustible and non-combustible tobacco products produce distinct biological effects which could explain the observed chronic immune suppression in smokers.

2815 Heat Shock Proteins Are Key Regulators of Cigarette Smoke-Induced Cardiac Hypertrophy
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Smoking is related to the risk of cardiovascular events. The present study was designed to identify proteins in the left ventricular tissues with altered expression in spontaneously hypertensive rats (SHR) after exposure to cigarette smoke and to find possible molecular targets associated with the pathogenesis or progression of cigarette smoke-induced cardiac hypertrophy. SHR and control, Wistar Kyoto rats (WKY) were exposed to cigarette smoke at low (2 puffs/min for 30 min) or high dose (2 puffs/min for 120 min). Using the two-dimensional fluorescence difference gel electrophoresis (2D-DIGE) combined with MALDI-TOF/TOF tandem mass spectrometry, we compared differences in the expression of proteins in the whole left ventricles after exposure to cigarette smoke for 8 weeks. The left ventricular/body weights were significantly increased by cigarette smoking at high dose in both SHR (3.37 × 10⁻³ vs 3.98 × 10⁻³) and WKY (2.21 × 10⁻³ vs 2.51 × 10⁻³) (p-values < 0.01). Cigarette smoke developed prominently concentric hypertrophy and fibrosis of the heart. Proteomic analysis identified 32 protein spots with significant alterations, including 16 up-regulated and 16 down-regulated proteins in the left ventricles of SHR exposed to cigarette smoke compared with non-exposed SHR. Of the total alterations, heart shock protein 70 kDa and heat shock protein beta-6 showed significant up-regulation in the left ventricles of SHR exposed to cigarette smoke. The study also confirmed that cigarette smoke induced the cardiac hypertrophy. The results suggest that heart shock proteins are key regulators of the cigarette smoke-induced cardiac hypertrophy.
Research has shown that the electronic nicotine delivery system (E-cigarette/vape; ENDS) contains banned toxic chemicals that cause cytotoxicity, oxidative stress, and inflammatory response and induce gene expression signatures similar to tobacco smoke (TS). Our aims were to investigate whether or not ENDS can cause vascular endothelial dysfunction (VED) similar to that induced by traditional cigarettes and to characterize the cellular and molecular mechanisms of such effects. Male C57/BL6 mice were exposed for 32 weeks to vaporized nicotine (VN), TS (4 hrs/day, 5 days/week), or for 6 weeks of infused nicotine (1.5 mg/kg). A smoke/vape generating system (Cairo University Laboratory Inc., Cairo, Egypt) was used to generate TS and VN. The system was designed, tested, and certified by a licensed engineer to deliver TS (35-ml puff of 2-seconds duration once every 30 seconds) and VN (55-ml puff of 4-seconds duration once every minute). 3R4F reference research cigarettes and locally available e-liquid containing 2.4% nicotine were used to generate TS and VN, respectively. VED was evident by observing impairment of acetylcholine (Ach)-induced endothelium-dependent relaxation, with a shift to the right and downward in the Ach-concentration response curve in thoracic aortic segments of test groups. Aorta of test groups, compared to controls, showed increased reactive oxygen species (ROS) generation, increase oxidative damage and elevation of protein carbonylation. Tetrahydrobiopterin (BH4) level and endothelial nitric oxide synthase (eNOS) expression and phosphorylation were decreased in the aorta homogenate of test animals, compared to controls. Furthermore, all groups of exposed animals showed variations in blood pressure and blood pressure compared to controls. Thus, ENDS, and infused nicotine induced VED and hypertension, with TS showing the highest effects, through the generation of ROS that depleted BH4 and impaired eNOS activation. Overall, our study shows that ENDS causes vascular endothelial function similar to that induced by TS, and may trigger vascular disease through similar mechanisms.
Toluene diisocyanate (TDI) is a highly reactive compound used in the production of polyurethane foams and other industrial products. Human exposure to TDI occurs mainly through inhalation of vapors in occupational settings where TDI is produced or used, but dermal exposure to TDI is also possible during some production or use operations.

In a recently published epidemiological study, Pinkerton et al. (2016) reported increased lung cancer risk in workers with potential dermal exposure to TDI. To evaluate the hypothesis that dermal, but not inhalation, exposure to TDI may be associated with an increased risk of respiratory cancers in humans, we critically reviewed and integrated the evidence from relevant epidemiological, toxicological, and toxicokinetic studies of TDI. We evaluated the quality of the studies we identified, then determined how these studies’ strengths and limitations could impact the interpretation of their results. We then applied the Bradford Hill considerations for causal inference to integrate the evidence from these studies to address whether TDI is likely to be carcinogenic via the dermal exposure route. In addition, we considered the potential for confounding and bias in epidemiological studies, as this potential can affect the ability to make causal inferences based on these studies’ results.

In our analysis, we found that the reported associations between TDI exposure and respiratory cancers in epidemiological studies of polyurethane foam workers do not support TDI being a causal factor for those cancers, because there are other explanations that are more likely than causation, such as confounding by smoking and low socioeconomic status. Experimental animal and genotoxicity studies indicate that the carcinogenic potential of TDI depends on its conversion to toluene diisocyanate (TDI). TDI is not known to induce tumors in rodents. TDI has no evidence of TDA being systemically available after dermal exposure to TDI. Also, systemic uptake of TDI is very low after dermal exposure, and any absorbed TDI is more likely to react with biomolecules on or below the skin surface than to form TDA. Even if some TDA formation occurs after dermal exposure to TDI, TDA does not induce respiratory tumors in experimental animals after either dermal or oral exposure. Based on our analysis, we conclude that the available evidence indicates that dermal TDI exposure does not cause respiratory cancers in humans.

**2820 Evaluation of Respiratory Cancer Risk from Dermal Exposure to Toluene Diisocyanate**


**2821 Development of Exposure Limits for Polyalphaolefin Fluids**


**2822 Acute and Chronic Non-Cancer Inhalation Toxicity Factors for C7-C10 Alkanes**

J. Lee, and T. Bredfeldt.

**2823 Derivation of a Parenteral PDE Value for Zirconium Pursuant to the ICH Q3D Elemental Impurities Guideline**


Elemental impurities may arise from various sources and their levels in drug products must be controlled within acceptable limits. The International Conference on Harmonisation (ICH) has derived permitted daily exposure (PDE) values for some elemental impurities, but not for zirconium (Zr). The current work was performed to derive a PDE value for Zr pursuant to the methodology outlined in the ICH Q3D elemental impurities guideline. This involved identifying the most suitable point of departure and applying modifying factors to account for species extrapolation (F1), individual variability (F2), study duration (F3), severity of toxicity (F4), and effect level (F5). Following identification and review of publicly available nonclinical information for various Zr salts, the PDE value for Zr was based on the results of a combined repeat-dose oral toxicity/reproductive and development toxicity screening study in Sprague-Dawley rats that was GLP-compliant and conducted per OECD guidelines. In this study, no toxicologically relevant systemic or reproductive/developmental effects were observed in parenteral animals or offspring at Zr acetate dose levels that provided Zr up to 530 mg/kg/day; thus, this was considered to be the NOAEL. Applying a cumulative modifying factor of 500 (F1 = 5, F2 = 10, F3 = 10, F4 = 5, F5 = 1) to the NOAEL resulted in an oral PDE value of 1.06 mg/kg/day. Given the reported low oral bioavailability of zirconium compounds (<1%), a modifying factor of 100 to extrapolate from oral to parenteral exposure was applied (per ICH Q3D). A final 10-fold factor was applied to reduce potential systemic Zr exposure to a level within reported background circulating levels in humans, resulting in a parenteral PDE value of 0.0606 mg/kg/day (equivalent to 0.053 mg/day, or 53 µg/day, based on a 50 kg body weight). With regard to local effects, the results of various studies indicate that Zr compounds are neither skin irritants nor sensitizers. The major outcome of this work is the establishment of a parenteral PDE value of 53 µg/day for Zr. This PDE value can be used to assess risks associated with exposure to Zr as an impurity in parenteral drug products.
the revised standard, and 2) to quantify dermal loading and hand-to-mouth transfer efficiency of selected FRs from these products. Five manufacturers of baby products containing PUF, one bath product, three changing pads, and one sleep positioner, were purchased in 2015 in both California and Colorado (n=10) and analyzed for the presence of FRs. Tris (1-chloro-2-propyl) phosphate (TCP) was found in all ten products tested (203 to 43,000 μg/g). Tris (2-chloro-1,3-diphenyl) phosphate (TDCPP) was found in only a single product (63 μg/g). Two products that contained the highest levels of TCPP, the bath product and one of the changing pads, were each handled by three participants for 15 s, and a wipe sample of one hand was collected to determine dermal transfer. After accounting for the average of TCPP found in the lab blank and other lab materials (range <0.010 μg/L – 0.237 μg/L), the amount of TCPP loaded onto the hands of the participants was below detection.

Additionally, we evaluated hand-to-mouth transfer from the three participants handling the bath product; after handling, the participants presented three fingers from their second hand into saliva. We concluded that the transfer of TCPP from hands to saliva was below detectable levels. It is hypothesized that the covering foam components reduced transfer to hands. These data show that FRs continue to be added to baby products; however, the transfer of FRs onto hands and exposures as a result of hand-to-mouth transfer from these products is expected to be negligible for TCPP. Further research is needed to estimate the role of other routes of exposure, such as inhalation and dermal absorption.

**2827 Evaluation of Interspecies Differences in Susceptibility to Thyroid Perturbation**

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The potential effect of goitrogenic chemicals on thyroid homeostasis has become a significant concern in the field of regulatory risk assessment. In particular, the developing brain can be affected by changes in maternal thyroid hormones during pregnancy. Much of the experimental work evaluating the thyroid effects of goitrogenic chemicals has been conducted in rats, both as a matter of convention and to allow comparison to the existing scientific literature. Yet rats (or rodents more broadly) are stated to be particularly sensitive to disruption of thyroid homeostasis due to a number of physiological factors these animals do not share with humans (e.g., shorter thyroid hormone half-life, lower colloid stores). While the basic elements of thyroid homeostasis are conserved across species, there appear to be important species differences in sensitivity, which can in turn impact the use of data obtained in rats to derive human safe levels of exposure. We investigated published studies of eight different thyroid active compounds (methyl iodide, ethylene thiocyanate, perchlorate, nitrate, resorcinol, propyl uracil, and sulfamethazine), which produce their adverse effects by interfering with different aspects of thyroid homeostasis. The thyroid disrupting effect of each of these chemicals has been studied in rats as well as at least one other species (e.g., mice, rabbits, dogs, and/or monkeys) which allows for comparison of cross-species sensitivity. Among the studies we reviewed, the same doses that produced effects in the rat often either produced no effects at all or effects of much lower severity. For example, five-week exposures to propyl uracil (a thyroid perturbing inhibitor) at 30 mg/kg produced classic goitrogenic effects (decreased triiodothyronine [T3] and thyroxine [T4], increased thyroid stimulating hormone [TSH]) in rats but no effects in mice at the same dose and similar time frame. The exceptional sensitivity of the rat thyroid to perturbation by exogenous chemicals suggests that caution is warranted in the use of such data for quantitative risk assessment. We also proposed that future studies of potential thyroid hormone effects consider use of alternative animal models in addition to, or in lieu of, rats.
ture database was searched through November 2016. To be included, a study had to assess exposure to environmental chemicals prior to two months of age or 14 days in humans or rodents, respectively. The search returned 18,218 studies that were screened for relevance. Forty human studies, 50 animal studies and 41 reviews were identified as relevant. Human study designs included case-control (n=30), cohort (n=9) and ecological (n=1). The most frequently reported outcomes were: mercury (n=13), air pollution (n=12) and particulate matter (n=11). Rodent studies were considered relevant if they included at least one measurement of deficits in reciprocal social communication and social interaction or repetitive stereotyped behaviors. Twenty five environmental chemical exposures were investigated in developing rodent data. Of these, chlorpyrifos (n=9), mercury (n=5) and lead (n=3) were the most frequently reported. Seven reviews (17%) were systematic-reviews or meta-analyses, but only five assessed the quality (risks of bias) of the primary studies and all were limited to human studies. Pesticides, mercury and air pollution were the most reviewed environmental expo-
sures. This scoping review characterizes and summarizes the primary research and reviews that have been conducted on early life exposure to environmental chemicals and autism. Whereas most research effort to-date has been dedicated to assessing mercury and components of air pollution, other environmental chemicals have not been adequately studied. Validated animal models could improve the efficiency by which environmental chemicals are investigated and provide a way to prioritize chemicals for evaluation in humans.

2829 The Risk Assessment on Human Health Based on Environmental Concentration of Medicinal Chemicals for Human Use in Urban Rivers in Japan

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Chemicals used as the active ingredients in medicines are discharged into the aquatic environment. Although the amount of each chemical might be small present, it has been feared affect derived by the chemical and physiological properties to the organism. However, we still know little about what kind of impact on wildlife in the environment and on human health. In view of the background, we have measured the actual environmental concentrations of 31 kinds of active ingredients in urban rivers in Japan, once per season in 2015-2016. The active ingredients exceeded 100ng/L at maximum detected concentrations were sulpiride, crotamiton, acetoaminofen, clarylcyromycin, olmesartan, lorzepam, valsartan, DEP, bezafibrate, epinastine, irbesartan, ketoprofen, losartan and candesartan. Among target ingredients, the detection concentration of active ingredient in medicines for the lifestyle-related diseases tended to be higher. There was a tendency higher concentrations were detected in winter during four seasons while the flow rate of the river decreased. It must be con-
sidered that five kinds of active ingredients used for antihypertensive agents were detected in high concentrations exceed 100ng/L. These ingredients might be discharged in the same amount every day for long term and are thought to effect on wildlife in the environment and on human health. The similar mechanism due to their similar structure. Evaluating on human health effect, if these five ingredients coexist, the total value of the ratio of the maximum detected concentration to the minimum daily dose became 0.0038, which makes it difficult to keep a three-digit margin. Regarding the case of lorzepam, its value was 0.004 with only one ingredient. It is necessary to grasp the daily actual concentration, and it may be necessary to consider the correspondence. In the future, it would need to be evaluated in consideration the combined action of multiple active ingredients based on the mechanism of action. This research is supported by the Research on Regulatory Science of Pharmaceuticals and Medical Devices from Japan Agency for Medical Research and development,AMED.

2831 Derivation of an Oral Reference Dose for Trimethyl Silanol

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Trimethyl silanol is an organosilicon compound used as the precursor to a variety of silicone materials, including polymeric/oligomeric fluids, elastomers and resins. Trimethyl silanol is identified as a volatile extractible from drinking water using US EPA Method 524.2. This poster describes an oral reference dose (RfD) for trimethyl silanol to assess health risks following oral exposure. Trimethyl silanol is predicted to partition into the lipid bilayer of membranes and cross into the blood brain barrier to cause toxicity. In vivo genotoxicity studies, there is no evidence of mutagenicity, but there is mixed evidence of clastogenicity. In vivo chromosomal aberration assays in rats are negative for clastogenicity. Transient narcotic effects are reported in repeated dose experiments in rodents at oral doses as low as 150 mg/kg/day, and inhalation concentra-
tions of 2.2 mg/L/6hr in an OECD Guideline 412 study. At higher oral doses in rat studies, adverse effects include growth retardation (≥350 mg/kg/day) and hepatotoxicity (750 mg/kg/day), including increased liver weight and proliferation of the bile ducts are reported. A prenatal developmental toxicity study in rats reported reduction in fetal weight, delayed ossification and increased incidence of cartilaginous variations at a maternal oral dose of 450 mg/kg/day, in the presence of maternal toxicity. A NOAEL of 50 mg/kg/day is assigned for this study, based clinical signs of toxicity among dams, including uncoordinated movement and decreased activity, and decreased body weight gain among dams at trimethyl silanol doses at and above 150 mg/kg/day. A NOAEL of 50 mg/kg/day was selected as the point of departure for derivation of trimethyl silanol’s RfD. Using the BMDL scaling approach, a human equivalent dose (NOAELHED) of 13 mg/kg/day was derived from the 50 mg/kg/day NOAEL. Using total uncertainty factors (UF) of 1,000 (3x and 10x for inter- and intra-species extrapolation, respectively, 3x for subchronic to chronic extrapolation, and 10x for database deficiency, as no chronic toxicity, carcinogenicity, or two-generation reproductive toxicity studies are available), a 0.013 mg/kg/day oral RfD was calculated as the quotient of the NOAELHED and combined UF. This oral RfD can be used to derive a chronic drinking water action level for trimethyl silanol.

2830 Oral Risk Assessment and Acceptable Drinking Water Levels for the Organophosphate Flame Retardant Tris (1-Chloro-2-Propyl) Phosphate (TCP) (TCP)

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General population exposure to tris (1-chloro-2-propyl) phosphate (TCP) may occur from the widespread use of this flame retardant in rigid and flexible polyurethane materials, some of which have drinking water contact applications. The purpose of the present assessment was to incorporate toxicity data from both published and unpublished studies in addition to recent NTP data in order to derive an oral RfD from which to establish drinking water criteria. Absent any human health effect data, hazard identification relied on laboratory animal data. Identified treatment-related effects following subchronic oral exposure to TCP in rats and mice included weight loss, lymphocyte depletion as well as hepatic, renal and thyroid effects. In an OECD guideline-compliant two-generation reproduction study in Wistar rats, a significant decrease in uterine weight was reported in all treated groups of F1 female rats and high-dose F1 females as well as increased incidences of runts in all treatment groups. Two developmental studies in rats reported minimal treatment-related adverse effects. The weight of evidence indicates the potential for mutagenic activity in vitro. No in vivo mutagenicity data were identified while in vivo assessments of clastogenicity were either negative or reported effects at high-doses likely exceeding the MTD. Epidemiological or chronic animal data are not available since results from recently conducted NTP bioassays have not yet been released and as such, there is Inadequate Information to Assess Carcinogenic Potential of TCP. Recognizing that a read-across of carcinogenicity based on structurally similar compounds is confounded by differences in metabolism, target organs, severity of effects and potency. The lowest benchmark dose (BMDL010) of 16 mg/kg-day was selected as a weight-of-evidence point-of-departure for the oral RfD, based on the human equivalent BMDL010 of 16 mg/kg/day for the increment in mean relative uterine weight and human equivalent BMDL010 of 16 mg/kg/day for the litter-based incidence of runts at PND 21. Using a total uncertainty factor of 300x (3x interspecies, 10x intraspecies, 3x subchronic, 3x database), an oral RfD of 0.05 mg/kg/day was determined for TCP. When considering RfD or MRL values established for other organophosphate flame retardants, the present RfD value is comparable. The resulting Total Allowable Concentration in drinking water was 0.4 mg/L.
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Kathon CG is a commonly used cosmetic grade preservative that contains active ingredients methylchloroisothiazolione (MCI) and methylisothiazolione (MI), which have been identified to have a high allergen potential. The objective of this study was to perform an evaluation of daily exposure to Kathon CG via application of various personal care products. We estimated the dermal exposure to MCI and MI following daily application of rinse-off (body wash and shampoo) and leave-on (body lotion and face cream) personal care products. We calculated an estimated daily dermal exposure using the amount of product applied, a retention factor, the MI/MCI concentration in the product, and the surface area of the body where the product was applied. We assumed that the products contained the maximum recommended concentration of Kathon CG: 0.1% by weight in rinse-off products and 0.05% by weight in leave-on products. The active ingredients MCI and MI compose 1.15% and 0.35% of Kathon CG, respectively. We used the 50th and 95th percentile amount of product applied per day among adult women from consumer use practice data, and based on dermal modeling data, we conservatively assumed a maximum skin adherence of 10 mg/cm². Additionally, per consumer data, we assumed one application of shampoo, body wash, and body lotion per day, and two applications of face cream. We applied a retention factor of 0.01 in rinse-off products and 1.0 in leave-in products. We compared estimated daily dermal exposures (µg/cm²) to reported MCI and MI no expected sensitization induction levels (NELSILs) of 0.83 µg/cm² and 15 µg/cm², respectively. The estimated product-specific daily dermal exposures did not exceed the NELSILs in either the 50th or 95th percentile exposure scenarios. Under the 95th percentile exposure scenario, the highest daily dermal exposure was from face cream (6.7x10⁻² µg/cm² [MCI] and 2.1x10⁻² µg/cm² [MI]), while the lowest daily dermal exposure was from body wash (1.9x10⁻³ µg/cm² [MCI] and 5.9x10⁻³ µg/cm² [MI]). This evaluation provides evidence that use of the products under the examined exposure scenarios does not result in an exposure above the MI and MCI NELSILs, and that use of different products may drive an individual's exposure to MI and MCI.

2833 Assessment of Risks to Home Health Care Workers from Exposure to Nebulizer-Administered Pharmaceuticals during Patient Care
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Home health care workers (HHCWs) are one of the fastest-growing workforces in the US public sector. The home healthcare workplace presents numerous challenges for occupational risk assessment because the agents are highly variable environments and engineering controls for environmental risks, such as ventilation, are often absent. The use of inhaled medications administered via nebulizer is very common in home healthcare. Nebulizers used in healthcare are not self-contained, and medications administered via nebulizer are used in a use simulation. The objective of this work is to assess risks of HHCW exposure to common nebulizer-administered drugs when they administer care in the home. Based on consultation with subject matter experts in home healthcare, we assessed the exposure and health risks of three medications commonly encountered in home healthcare for assessment. These were albuterol, budenoside, and ipratropium. Acceptable daily exposure (ADE) limit values were derived for the inhalation route based on clinical safety/efficacy and non-human toxicity data for each drug with consideration of short-term vs chronic dose-response and local vs systemic effects. Occupational exposure scenarios were determined based on expert consultation and inhalation dosimetry relevant to these was modeled using the Multiple Path Particle Dosimetry model and data characterizing nebulizer aerosols from the HHCW use simulation. Deposition of nebulizer aerosols was increased at tidal volumes and breathing rates representing moderately heavy work as compared to resting levels. This increased deposition in the head region, however, deposition to the tracheobronchial and pulmonary regions was slightly reduced in the heavy work scenario. Mass deposition rate increased sharply as a function of airborne concentration as the distance from the nebulizer output closed to 6’. Variation of body posture did not significantly impact the deposited dose of nebulized medication. Together, these data provide industrial hygienists with risk information applicable to the most common use scenarios of nebulized medicine in the home healthcare workplace.

2834 Investigation of Multiple Modes-Of-Action of Octylphenol and Nonylphenol and Their Relevance to Human Health Risk Assessment
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Nonylphenol (NP) and 4-tert-octylphenol (OP) are data-rich compounds that have a range of adverse effects, some linked to estrogenicity, but all having demonstrable thresholds. Based on the effects seen in rat multigenerational in vivo guideline tests, the weak estrogenic activity of NP and OP does not predict the entire suite of observed adverse effects in biological systems. In the case of OP, the Points of Departure effect (decreased body weight and body weight gain) at the Lowest Observed Adverse Effect Level (LOAEL) is not due to an estrogenic MOA. For NP, estrogenic activity (accelerated vaginal opening in offspring) as well as non-reproductive systemic toxicity (kidney effects and decreased sperm motility) are found at the same concentration. The LOAELs for each of these effects were below combustible cigarette yields. These findings indicate that NP and OP have multiple modes of action (MOAs) that influence the toxic responses in biological systems. In a battery of high throughput in vitro assays, (i.e., ToxCast®), NP and OP both interacted with multiple biological pathway targets below their lower cytotoxicity limits, including estrogen receptor, pregnane-X receptor/xenobiotic metabolism, vitamin D receptor, mitochondrial toxicity, cell cycle targets, and cytokines. Moreover, in vitro and in vivo mechanistic studies also demonstrate presence of multiple MOAs such as endocrine activity, oxidative stress, calcium signaling perturbations, cellular toxicity and inflammation/immune responses that can contribute to observed atypical effects. Overall, the evidence demonstrates that multiple biological pathways are perturbed by NP and OP within the same concentration range as estrogenic effects. Thus, multiple MOAs need to be considered when defining the Points of Departure for these compounds, and their weak estrogenic activity may not be the most sensitive effect for use in hazard and risk assessments. Concentrations of NP and OP detected in human biomonitoring studies are orders of magnitude below Points of Departure for human health. The NP and OP case studies illustrate the need to incorporate the concept of potential MOAs and their potential to contribute to observed effects, in the context of expected human exposure in characterizing potential risk to humans. Sponsored by ALKYLPHENOLS & ETHOXYLATED RESEARCH COUNCIL.

2835 Electronic Nicotine Delivery Systems (ENDS) Open Devices and Market Fruit Liquid Chemical Risk Assessment
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Open electronic nicotine delivery systems (ENDS) are refillable devices and have grown in popularity within recent years. 2017 Market analysis indicates fruit flavors are part of a top selling category of liquid formulations. Identification of interactions between liquid formulations and orally-inhaled devices may mitigate potential unexpected consumer exposures. In this study, nineteen popular fruit flavored e-liquid mixtures and their open ENDS generated aerosols were screened for thirty compounds. Aerosolization used a machine puffing regime of 55 mL puff volume, 30 second interpuff and 3.3 second puff duration (0.3 seconds for push button actuation). Liquid and aerosol measurements include propylene glycol, glycerol, flavor ingredients, carbonyls, and metals. Flavor ingredients detected with higher frequency included diacetyl, acetoic acid, acetic acid, acetyl propionyl, benzoaldehyde, butyric acid, and propionic acid. Although considered generally recognized as safe (GRAS) by recognized bodies for oral consumption, these ingredients have been identified as possible inhalation hazards. ENDS aerosol levels of these flavor ingredients underwent a risk assessment with results indicating lower levels than the lowest observed adverse effect, and lower than estimated non-cancer hazards from relevant regulatory-derived thresholds. Lead, chromium, and nickel were not present in liquids but detected in aerosols suspecting the device as the source of leachates. The majority of formaldehyde yields generated from ENDS were below combustible cigarette yields. These findings indicate that the presence of carbonyls and metals were dependent on liquid formulation interaction with the device. Generally, the lower yields of select analytes from ENDS, compared with combustible cigarettes, supports migration of current adult smokers to ENDS would be a benefit to public health.
### 2836 An Inhalation Risk Assessment of Indium and Indium Compounds for Derivation of an Occupational Exposure Limit

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Indium is an important metal used in many modern-day technologies such as flat panel displays, solar panels, and other microelectronic devices. Over the last decade, the industrial use of indium has grown appreciably due to increased demands for consumer and commercial electronics. The increased industrial use of indium has also lead to a greater number of potential occupational exposures to indium. Case reports have emerged describing a progressive and often irreversible lung disease in a small number of workers primarily employed in indium production and reclamation facilities. OSHA has not yet established an occupational exposure limit (OEL) for indium. Both NIOSH and ACGIH have evaluated OELs for Indium compounds, but these recommendations were made more than 20 years ago. The purpose of this evaluation was to comprehensively review the current toxicological information pertaining to indium to derive an updated OEL for indium and indium compounds. Based on the current data, three critical health endpoints were identified: alveolar proteinosis (animal and human), bronchiolo-alveolar hyperplasia (animal), and lung tumors (animal). The available data suggest that cellular infiltration such as accumulation of alveolar macrophages, chronic inflammation, and remodeling (e.g., proteinosis, fibrosis, hyperplasia) are precursor events to neoplasia. For this analysis, two different approaches were used to derive an OEL. Depending on the dose-response curve for each end-point, a LOAEL approach (alveolar proteinosis) or bench mark dose approach (hyperplasia, lung tumors) was used to derive a point-of-departure (POD) from chronic inhalation studies conducted in rats exposed to indium oxide. Each POD was evaluated based on a weight-of-evidence consideration of toxicokinetics, toxicodynamics, and other uncertainties. Overall our analysis supports the use of animal data for the derivation of an OEL for respirable indium of 0.03 µg/m³.

### 2837 Dose-Response Effects of Dietary Methionine Intake on Radiation-Induced Toxicity: An Experimental and Computational Study

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Methionine (Met) is an essential amino acid needed for a variety of processes in living organisms. Ionizing radiation (IR) depletes tissue Met concentrations and leads to reduced DNA methylation and glutathione synthesis. Hence, Met supplemented diet (MSD) was proposed to improve the response to total body irradiation (TBI). To test this hypothesis, we exposed mice to 3 or 8.5 Gy of TBI while feeding either Met adequate diet (MAD) or MSD for 3.5 or 6 days (tissue harvest) or up to 30 days (survival). Strikingly, our studies indicated that MSD, compared to MAD, potentiated acute IR toxicity, resulting in earlier lethality at sub-lethal dose of 8.5 Gy. Early lethality post TBI was also observed after exposure to 3 Gy, a dose generally not associated with mortality. Hind-limb protection during exposure to 8.5 Gy of TBI resulted in 50% mortality by day 9 post-IR in mice on MSD, while 90% of mice fed MAD survived 30 days. These findings suggest the prevalence of gastrointestinal syndrome in MSD-induced acute radiation toxicity. MSD also led to substantial alterations in one-carbon metabolism in proximal jejunum and disbalance between the jejunal and plasma Met concentrations. Thus, we sought to quantify the Met whole-body kinetics and the combinatorial dose-response relationship using computational analysis. A PBPK model is currently being developed to describe systemic and tissue distribution of Met across various doses. Metabolism, the primary clearance pathway for Met, was included in the model in liver, kidney, and small intestine compartments using saturable enzyme kinetics. Oral doses of Met for MAD (650 mg/kg) were simulated and compared with experimental data for model calibration and verification purposes. The first-generation PBPK model predicted the measured systemic exposure of Met and the tissue-specific distribution at MAD. We are currently developing the dose-response model taking into account the PK alterations due to MSD. The findings of the PBPK model calibrated in vivo in mice will be extrapolated to humans with the ultimate goal of assessing the risk of co-exposure to Met and IR and developing appropriate clinical mitigation strategies for adverse effects in cancer patients.

### 2838 A Critical Analysis of the Toxicological Mode-of-Action of Automotive Brake Dust

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The role of inhaled chrysotile-containing brake dust as a causative agent in the development of mesothelioma has been a source of debate with some epidemiological studies reporting a significant association, while others report no association. To address the plausibility of an association between brake dust containing chrysotile and mesothelioma, a critical analysis of the available in vitro and in vivo evidence was conducted to identify plausible mechanisms of action (MOA) and their implication for causality and understanding of dose-response. Using literature from multiple sources, including the National Library of Medicine’s PubMed database, the Scopus literature search engine and TOXNET, and MOA frameworks developed by different organizations such as the International Programme on Chemical Safety (IPCS), we identified two possible MOAs for further consideration: 1) frustrated phagocytosis resulting in an inflammatory response; and 2) direct physical interaction with chromosomes. It was found that the literature better supported frustrated phagocytosis leading to an inflammatory response and that this MOA involves the following sequence of events: 1) deposition of fibers deep into the lung; 2) production of reactive oxygen species (ROS) by macrophages unable to engulf the fibers; 3) inflammatory response and exacerbated ROS production, possibly potentiated by fiber-associated iron; 4) DNA damage subsequent to persistent ROS generation; and 5) dysregulation of cell proliferation, including cells that contain damaged DNA, by growth factors related to the inflammatory response. Fiber type, length, and biopersistence substantially influence this MOA, with studies showing longer and more durable fibers to be more likely to trigger an adverse physiological response. We considered this MOA in the context of the short fiber length, limited biopersistence in animal studies, and limited biological reactivity of chrysotile-containing brake dust, as well as the inconsistent human epidemiological evidence. It was concluded that the evidence supports a threshold dose-response relationship and that there are exposure concentrations of chrysotile-containing brake dust below which no adverse health effects would be expected to occur in humans.

### 2839 Safety in Mice of a Nanovaccine against Nicotine Addiction

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The Zhang laboratory has developed a series of biodegradable lipid-polymeric hybrid nanoparticles as potential vaccines against nicotine addiction. Safety of one vaccine was examined in groups of male, CD-1 mice given a one-time, subcutaneous dose that included 0.356 mg/mL of carrier protein (keyhole limpet hemocyanin (KLH)) in PBS. Doses were administered to provide 0 (vehicle control, Group 1), 25 (low dose, Group 2) or 125 µg (high dose, Group 3) of the KLH carrier protein. A baseline neurobehavioral assessment was performed 3 days prior to dosing, and then again at 1, 2, 4, 7, 14, 28 and 56 days post-dosing (n=10/dose group). Each assessment consisted of clinical observations scored as either normal/abnormal or yes/no (observational category is present/absent) along with a recording of body weight. Evaluation of behavioral endpoints indicated that the groups did not differ for any of the categorical endpoints, which included home cage, open field and reflex assessments. Also, after adjusting for baseline weight, the groups did not differ for all follow-up time points. Cohorts of animals (n=3/dose group/time-point) were sacrificed on post-dose days 1 and 7 to collect tissues for clinical pathology and histopathology. Review of clinical pathological findings did not reveal obvious abnormalities. Histopathological analysis of multiple mouse organs only revealed minimal to moderate focal acute inflammation of the deep dermis and adjacent subcutis of the nanovaccine injection site in 3/3 mice on post-exposure day 1. At day 7 a more chronic appearance of the injection site inflammation was noted, with lymphocytes and macrophages seen in the deep dermis, panniculus muscle and adjacent subcutis. With no detectable lesions outside of the site of injection after nanovaccine administration, results do not tentatively support its safety. Funded by U01DA036850 to C. Zhang.
Alzheimer’s Disease (AD) is a neurodegenerative disease with the majority of occurrences being sporadic in nature. In this sporadic form, environmental factors such as air pollution are believed to contribute to the pathological development of the disease. Of particular concern is exposure to the ultrafine (UFP, <0.1 μm in aerodynamic diameter) fraction of particulate matter air pollution. UFPs can deposit in all regions of the respiratory tract, evade macrophage mediated clearance, undergo secondary translocation, and induce inflammation. We hypothesize that exposure of a triple transgenic AD mouse model (3xTgAD) to concentrated UFPs can accelerate AD progression and affect performance in tests of cognitive function as compared to filtered air (FA) exposed mice. UFPs from ambient air were concentrated using the Harvard Ultrafine Concentrated Ambient Particle System (HUCAPS). Cohorts of male mice were exposed at 2.5-3 months of age for 2 weeks (4 hours/day, 4 days/week) to FA or HUCAPS. Starting one-month following exposure, a battery of behavioral tests was performed to assess spatial learning and memory, recognition memory, and locomotor function. We previously showed that the overall rate of spatial learning was diminished in the radial arm maze test following HUCAPS exposure, independent of genotype. Further analysis using linear mixed modeling shows that these deficits resulted from decreased recognition memory in all regions due to HUCAPS exposure while working memory remained unaffected. No effects were found on long-term recognition memory (novel object recognition test), but the initial session showed lower exploratory activity in non-transgenic (NTg) mice and HUCAPS exposure diminished exploratory activity in both NTg and 3xTgAD mice. Locomotor testing showed that basal locomotor activity for NTg mice was lower than in the 3xTgAD mice and HUCAPS exposure diminished exploratory activity specifically for 3xTgAD mice. Overall these results suggest that HUCAPS exposure prior to onset of AD pathology can diminish the rate of learning by inhibiting reference memory while having no effect on working or long-term recognition memory and that this effect is not dependent on genotypic background. Funding Sources: NIH R01ES020332, T32ES007026, P30ES001247.

Traffic-Generated Air Pollution-Exposure Mediated Expression of Factors Associated with Onset and/or Progression of Multiple Sclerosis in a Female Mouse Model

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Multiple Sclerosis (M.S.) is disorder of the central nervous system (CNS) associated with inflammation, aberrant immune signaling, and demyelination, which has a higher prevalence in females. Over the past several years, epidemiological studies have reported strong associations between air pollution exposure and occurrence of M.S.; however, the mechanisms involved have not yet been fully elucidated. Thus, we examined the sequence of events leading to a mixture of pulmonary and engine emissions (MVE) on the brains of female Apolipoprotein (Apo) E-/- mice, both ovary-intact (OV+) and ovariectomized (OV-). Mice were exposed to either filtered air (FA, controls) or mixed gasoline and diesel vehicle emissions (MVE: 200 PM, μg/m³) for 6hr/d, 7 d/wk, for 30d. We then analyzed MVE-exposed mediated alterations in myelination, CD4+ and CD8+ T cells, and expression of reactive oxygen species (ROS) via immunohistochemistry and DHE staining, respectively. Alterations in interleukin (IL)-1β, tumor necrosis factor (TNF)-α, estrogen receptor (ER) α, estrogen receptor (ER) β, and progestrone receptors A/B (PRo A/B) transcript were analyzed via real-time RT-qPCR. MVE-exposure mediated significant alterations in myelination across multiple regions in the cerebrum (decreased by as much as 50%), which was associated with a 4-fold increase in CD4+ and 5-fold increase in CD8+ positive staining. We also observed a 2-fold increase of ROS in the CNS, as determined by DHE, which appeared to be related to MVE-exposure vs. ovary status. MVE-exposure induced expression of PRoA/B (18-1, 5-fold) and TNF-β (1, 2-fold) mRNA in the cerebrum that was strongly associated with ERα expression, which was interestingly observed to be reduced in expression with MVE-exposure. Modification of PRoA/B expression in the CNS was not significant. In conclusion, these findings suggest that exposure to inhaled MVE may mediate changes in ER expression, associated with increased CD4+/CD8+ infiltration into the CNS, regional demyelination, and induction of inflammatory mediators IL-1β, TNF-α, and ROS in female mice +/- ovaries. Research funded by NIEHS R00 ES0126586 and UNT RIG Grant GA9306 to A.K.L.

Assessment of Study Quality (Risk of Bias) in Understanding the Relationship between Congenital Heart Defects (CHDs) and Exposures to Trichloroethylene (TCE)

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In the US EPA IRIS assessment of TCE, the Agency selected CHDs obtained in a rat drinking water study as a sensitive endpoint for development of both the RFD and RIF. This decision has been controversial due to notable study limitations and the inconsistency of the findings relative to the rest of the TCE literature. Due to “concerns raised about study quality,” the TCE-CHD literature was recently re-evaluated in what was termed a “systematic evaluation” (Makris et al., 2016). While this re-evaluation has the appearance of a “systematic review” (SR), it lacks several critical elements of an SR, most notably an assessment of risk of bias (RoB) - a measure of confidence in whether the design and conduct of a study compromised the credibility of the link between exposure and outcome. Herein, we evaluated RoB for the TCE-CHD human and animal literature and integrated such using the NTP OHAT approach. To account for critical study design features of animal studies, some of the relevant RoB domains in the NTP OHAT RoB tool were refined and expanded. The single animal study reporting TCE-CHD effects was found to have high RoB for multiple domains, including selection bias and performance bias. Most of the remaining animal studies in the evidence base demonstrated low RoB for these and other domains. RoB was present for all studies for study group concealment and blinding as these elements were generally not reported by study authors. The human studies tended towards high RoB in the domains of detection bias (e.g., deficiencies in exposure characterization) and confounding bias; one human study was insufficient for RoB analysis (ecological study). Integration of RoB results with other factors (e.g., inconsistency, lack of dose-response, low magnitude) supports a moderate to high level of confidence that TCE is not associated with CHDs. Results of this evidence-based approach suggest that the single CHD-positive study is unreliable for use in developing toxicity factors based on: high RoB, inconsistency with animal studies of lower RoB, and a limited human dataset that does not demonstrate causality. These results demonstrate the importance of assessing study quality (recommended by NAS and a required component of TSCA assessments) and highlight the need to re-evaluate the current toxicity values for TCE using an evidence-based approach that integrates data quality.
Ten Factors for Considering the Mode-of-Action of Cr(VI)-Induced Intestinal Tumors in Rodents

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The determination of whether a chemical induces a specific cancer through a mutagenic or non-mutagenic mode of action (MOA) plays an important role in whether linear or nonlinear low-dose extrapolation is used to derive safety criteria. Currently, no formal framework exists for determining whether environmental chemicals act through a mutagenic or non-mutagenic MOA. As a result, most MOA determinations are made on an ad hoc basis. Eastmond (Mutat Res 751 (2012)) recently conducted a systematic investigation of MOA determinations by U.S. and international regulatory agencies and organizations, and identified 10 key factors that influence MOA determinations. These factors include: i) nature of tumors, ii) mutations in tumors, iii) chemical properties, iv) toxicokinetics, v) structural similarity to other carcinogens, vi) understanding relevant genotoxicity assays, vii) in vivo genotoxicity, viii) origins of observed genotoxicity, ix) data quality and reproducibility, and x) evidence for alternative MOAs. These 10 factors were used to assess the likelihood of a mutagenic or non-mutagenic MOA for gastrointestinal tumors induced by oral exposure to hexavalent chromium (Cr(VI)) in drinking water. Comprehensive assessments were conducted on in vivo genotoxicity, toxicokinetics, and MOA. To our knowledge, this is the first demonstration of using the 10 factors identified by Eastmond for making MOA determinations. Based on these analyses, we conclude that the MOA for Cr(VI) induced gastrointestinal tumors is non-mutagenic. Comparisons between Cr(VI) and intestinal carcinogens previously determined to have non-genotoxic MOAs revealed similar data sets, thus further supporting the MOA conclusion for Cr(VI). As such, threshold risk assessment approaches for oral Cr(VI) exposure are appropriate. The analyses also indicate that an adverse outcome pathway (AOP) for intestinal carcinogenesis mediated by chronic villus wounding and regenerative hyperplasia would benefit future risk assessment efforts of intestinal carcinogens.

Toxicity and Recovery in the Duodenum of B6C3F1 Mice following Treatment with Intestinal Carcinogens; Captan, Folpet, and Hexavalent Chromium: Evidence for an Adverse Outcome Pathway

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High concentrations of hexavalent chromium [Cr(VI)], captan, and folpet induce duodenal tumors in mice. Many regulatory agencies have concluded that captan and folpet induce duodenal tumors by non-genotoxic threshold mechanisms, despite some evidence for genotoxicity in vitro. Recent studies on the effects of Cr(VI) in the small intestine support that the mode of action (MOA) for Cr(VI)-induced tumors involves non-genotoxic threshold mechanisms. Evidence is mounting that there may be a generic adverse outcome pathway (AOP) for intestinal tumors mediated by chronic villus wounding and regenerative crypt hyperplasia. Using standardized tissue collection procedures and diagnostic criteria, we compared the duodenal histopathology in B6C3F1 mice following exposure to captan, folpet, and Cr(VI) to determine whether they share similar histopathological characteristics. B6C3F1 mice (n=20 per group) were exposed to 180 ppm Cr(VI) in drinking water, 12000 ppm captan in feed, or 16000 ppm folpet in feed for 28 days. After 28 days of exposure, examination of H&E stained transverse sections revealed villus enterocyte hypertrophy and mild crypt epithelial hyperplasia in all exposed groups. A subset of mice allergic to recover after 38 days of duodenal samples were generally indistinguishable from those of unexposed mice. The changes in the villi and lack of observable damage to the crypt compartment suggests that toxicity was mediated in the villi, which is consistent with earlier studies on all three agents. In addition to H&E staining, immunohistochemical (IHC) staining for Ki-67, a well-known marker of cell proliferation, was similar among treated animals. These findings indicate that structurally diverse agents can induce similar (and reversible) phenotypic changes in the duodenum. These intestinal carcinogens likely converge on common pathways involving irritation and wounding of the villi leading to crypt regenerative hyperplasia that, under protracted high-dose exposure scenarios, increase the risk of spontaneous mutation and tumorigenesis. These findings set the stage for the development of an AOP for intestinal cancer.

Use of Clinical Data to Inform Risk Assessments of Food Additives: A Case Study of Sodium Benzoate

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Sodium benzoate and its potassium salt are common preservatives in foods and beverages and have an acceptable daily intake (ADI) of 0-5 mg/kg body weight (bw) based on a no observed adverse effect level (NOAEL) of 500 mg/kg bw/day in rodents and a total uncertainty factor (UF) of 100. We recently derived a chemical-specific adjustment factor (CSAF) of 2 for benzoic acid and its salts for the pharmacokinetic component of the interspecies UF based on pharmacokinetic data in rodents and humans, which would change the total UF to 50 and increase the ADI to 0-10 mg/kg bw. Sodium benzoate is also used clinically as a therapeutic agent to treat urea cycle disorders, but risk assessments of benzoic acid and its salts by various regulatory and scientific bodies have not considered clinical data. Here, we evaluate clinical studies of sodium benzoate to inform the basis of a risk assessment of benzoic acid and its salts. Through a thorough review of clinical studies, we identified 29 published clinical studies of sodium benzoate, including 13 case reports, 11 case series, and 5 clinical trials involving administered doses of sodium benzoate greater than the current ADI. These studies involved patients across all life stages, including infants. Doses up to 500 mg/kg bw/day (100-times the current ADI) were administered and well tolerated in patients, with no treatment-related adverse effects. Most of these clinical studies were observational in nature, however, and none were designed to systematically evaluate long-term safety of sodium benzoate. Toxicity, such as vomiting and irritation, occurred only after a single or accidental overdose at around 800 mg/kg bw-day and ceased once exposure was discontinued. The highest therapeutic dose of 500 mg/kg bw-day for sodium benzoate in humans is comparable to the NOAEL of 500 mg/kg bw-day for benzoic acid in rodents, which supports that the UF for interspecies differences in pharmacodynamics can be reduced or possibly eliminated. Also, the doses of sodium benzoate in children with urea cycle defects and in adults with impaired liver functions (250-500 mg/kg bw-day and 71-143 mg/kg bw-day, respectively) are comparable and are at least one order of magnitude higher than the current ADI, and
also suggest that the UF for human variability in pharmacodynamics may be reduced. In conclusion, though limited, the clinical evidence reduces the uncertainties in the ADI derivation and demonstrates that clinical data can inform risk assessments of food and beverage ingredients.

### 2848 A Class-Based Evaluation of 2,5-Dimethyl-2,5-Hexanediol in Drinking Water

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2,5-Dimethyl-2,5-hexanediol is an unregulated semi-volatile chemical identified as a drinking water contaminant using US EPA Method 525.2. This poster uses a class-based risk assessment to establish a corresponding drinking water action level for 2,5-dimethyl-2,5-hexanediol according to the requirements of NSF/ANSI Standard 61: Drinking Water System Components - Health Effects. This class-based, read-across risk assessment approach operates on the hypothesis that structurally similar chemicals have similar mechanism of action. Structurally similar chemicals identified ChemIDplus included propylene glycol mono-t-butyl ether, which is part of a chemical class that is cleared to a Total Allowable Concentration (TAC) in water of 0.4 mg/L. To determine whether or not 2,5-dimethyl-2,5-hexanediol could be included as part of this class-based consistency, ToxMatch was employed to calculate a Tanimoto coefficient to assess the similarity of 2,5-dimethyl-2,5-hexanediol and propylene glycol mono-t-butyl ether. A Tanimoto coefficient of 0.6 has been suggested as a cut-off in the evaluation of structurally similar chemicals. ToxMatch predicted a Tanimoto coefficient of 0.333, indicating that 2,5-dimethyl-2,5-hexanediol is not structurally similar to propylene glycol mono-t-butyl ether. 2,2,4-Trimethyl-1,3-pentanediol, a plasticizer known as TXIB, is cleared to a TAC and SPAC of 0.4 and 0.04 mg/L along with its metabolites. The metabolism of TXIB following oral exposure produces a diol, 2,2,4-trimethyl-1,3-pentanediol (TXMP). Comparison of 2,5-dimethyl-2,5-hexanediol to TXMP yields a Tanimoto coefficient of 0.786, indicating these chemicals are structurally similar enough to justify including 2,5-dimethyl-2,5-hexanediol in TXIB's risk assessment. Inclusion of Tanimoto coefficients as part of drinking water risk assessments will help to ensure that properly structured surrogates are selected as part of this risk assessment process.

### 2849 Evaluation of Potential Health Risks Associated with the Ingestion of Asbestos


The inhalation of asbestos, depending on the fiber type and dose, is associated with the development of mesothelioma and other asbestos-related diseases. However, little is known about the risk and development of asbestos-related disease associated with the ingestion of asbestos, which can occur through multiple exposure pathways. There is evidence of release of asbestos fibers from asbestos-cement pipes used in water distribution systems that potentially contaminate drinking water. Data from animal and human studies were analyzed using a weight-of-evidence approach to make conclusions regarding the potential risk of disease associated with asbestos ingestion. Human and animal studies that evaluated exposure due to ingestion were identified by peer-reviewed literature searches. Disease endpoints of interest included all gastrointestinal-related cancers. Regarding animal studies, our analysis identified 23 studies that examined multiple organ-specific cancer pathways after ingestion of various asbestos fiber types; most studies exposed animals to asbestos-containing food. These studies employed a variety of doses in multiple animal species. Inconsistent dose-response protocols made it difficult to compare doses between studies that used different protocols (e.g. 0-360 mg/week versus a 1-10% diet). Regardless of this challenge, the studies reported that the asbestos fibers, irrespective of fiber type and dose, failed to produce any definitive gastrointestinal carcinogenic effect. Our analysis also identified 10 studies that examined the influence of ingestion of asbestos-contaminated water, for concentrations from 1 to 71,350 million fibers per liter (MFL), on the incidence of cancer in humans for different sources of asbestos and cohorts from world-wide. A majority of the epidemiology studies reported statistically significant increases in multiple organ-specific cancer endpoints for concentrations much higher than the EPA’s maximum concentration level of 7 MFL. However, these findings are inconclusive due to several critical study limitations including flawed study design, small sample size, selection bias, lack of individual exposure history, lack of adequate latency, and the inability to account for confounders including occupation history, diet, and smoking history. Based on our analysis, there is no evidence of a relationship between ingestion of asbestos and an increased incidence of gastrointestinal tract cancers.

### 2850 Use of the ATSDR Intermediate MRL for Soluble Uranium Compounds in lieu of an Outdated IRIS Reference Value (RfD) for Soluble Uranium Salts in the Regional Screening Levels (RSLs) for Chemical Contaminants at Superfund Sites

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Human health toxicity values in the RSLs are selected based on a recommended hierarchy of sources, namely 1) U.S. EPA’s Integrated Risk Information System (IRIS), 2) Provisional Peer-Reviewed Toxicity Values (PPRTVs), and 3) other (“Tier 3”) sources (e.g. ATSDR, CalEPA). However, the PPRTVs provide flexibility to encourage risk assessors to select toxicity values according to the “best science” available (up-to-date data that are relevant, publicly available, and peer-reviewed). The purpose of this study was to determine if the ATSDR intermediate MRL for soluble uranium compounds was more suitable for use in the RSLs than the IRIS RfD. The chronic RfD (last revised by IRIS in 1989) is 0.003 mg/kg-day based on moderate nephrotoxicity in a 30-day study in rabbits (Maynard and Hodge 1949). Several limitations to this study have been noted (including few animals per group, limited endpoints evaluated, and the absence of raw datasets); IRIS identified (in 2002) new studies which were likely to change the RfD value, but did not produce an updated assessment (Gilman et al. 1998). A subsequent analysis of the uranium dataset by the ATSDR program (in 2013) resulted in 15X lower intermediate MRL of 0.0002 mg/kg-day based on histopathological kidney effects in a contemporary 91-day study in rats (Gilman et al. 1998). Based on these data, the Superfund Technical Support Center (STSC) responded to a request from EPA Region 10 to evaluate the use of the intermediate duration MRL (rather than the chronic RfD) for the RSLs. STSC supported the use of the intermediate MRL because the new data were considered reliable, and the methods used to derive the toxicity values were similar (excepting small differences with respect to dosimetries adjustments and the adjustment of kidney endpoints). ATSDR concluded that continued exposure is not likely to induce more severe effects since the critical effect (regeneration of the tubular epithelium) occurred at low doses. Therefore, the intermediate MRL is expected to be protective for chronic exposure durations (365 days or more), OSRT suggested that regional risk assessors consider whether additional uncertainty is warranted on a case-by-case basis.

### 2851 Derivation of a Human Estimate of Toxicity for the Ultra-Potent Opioid Carfentanil: Reducing Animal Usage

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Carfentanil is an ultra-potent opioid of public health and chemical weapons defense concern. Exposure to carfentanil has been seen in both illicit drug use and in the resolution of a hostage situation in Russia in 2002. While opioid pharmacology and toxicology are well researched topics, carfentanil remains unstudied relative to its clinically used counterparts like fentanyl. Similarly to fentanyl, carfentanil elicits toxicity from central nervous system depression, largely in regions of the brain involved in spontaneous respiratory rhythmogenesis. By depressing the respiratory centers of the brain, respiratory failure occurs, and can lead to death. Carfentanil is of concern because it is reportedly 100 times more potent than its prototype, fentanyl, in analgesic assays in rodents. Carfentanil has very little human relevant data to indicate its toxicity in man for use in public health or chemical defense risk assessments. The present study was designed to test the hypothesis that carfentanil physiologically based pharmacokinetic (PBPK) modeling could accurately reflect observed PK in vivo in a surrogate animal model, and be translated to a human equivalent predication of toxicity. Studies were carried out to assess opioid receptor subtype specificity, potency, and efficacy. Carfentanil metabolism was studied in both rabbit and human liver microsomes to assess its intrinsic clearance. A metabolite identification study was undertaken to identify metabolites that could contribute to prolonged exposure or toxicity, and to generate a library of metabolites to be used in a forensic setting to identify carfentanil as a culprit agent in overdose or mass casualty exposures. Additionally, two key physicochemical properties of carfentanil were quantified in both rabbit and human blood: plasma protein binding and red blood cell - plasma partitioning. These properties have important roles in PBPK modeling, and can be used in a forensic setting to indicate where carfentanil can be found. Finally, the rabbit in vitro derived properties were incorporated into the PBPK model to accurately model the in vivo PK seen in the very
limited rabbit exposures. This model was then translated to a human PBPK model and an equivalent toxic dose was optimized. This predicted human toxic dose (0.34 μg/kg) was calculated to be very close to a post hoc human LD50 value published in a study by researchers at the U.S. DEA (0.29 μg/kg).

2852 Acrobiphylloine: A PDE Case Study
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After the revisions of Chapters 3 and 5 of the EU GMP Guide in 2015, more attention has been given to the cross contamination topic. At the same time, a guideline of the EMA was published introducing the concept of Permitted Daily Exposure (PDE) values. The approach for setting PDE limits is the same outlined in ICH Q3c guideline on residual solvents and in ICH Q3d guideline on elemental impurities. The background for that was the request of the pharmaceutical industry to be able to decide itself on a risk-based approach whether a product can be produced in a multipurpose facility or not. Acrobiphylloine (ACE) is an interesting case because there are no pre-clinical data available on ACE in public literature. Nevertheless, ACE is obtained by targeted salification of ambroxol (AMB) and theophylline-7-acetic acid (TAA). Therefore, the PDE assessment has been performed on its two components: TAA and AMB. The Point of Departures (POD) for AMB and TAA are a 52-week study in dogs and a reproductive toxicity study in mice, respectively. Regarding selection of the AFs, interspecies and intraspecies variability, reference study duration and bioavailability adjustment have to be considered. The peculiarity of ACE case study is the possibility to readjust PDE values according to the stochiometric ratio of TAA and AMB in ACE. The resulting oral PDE for ACE is equal to 3.7 mg/day. The daily therapeutic dose is used as alternative approach to assess PDE value for ACE. Regarding selection of the AFs, interspecies variability, duration of therapy, adverse effects, therapeutic dose and bioavailability adjustment have to be considered. The results of PDE for ACE is equal to 4 mg/kg/day. The results from the two different methodologies above exposed suggests that the assessment performed on the two components of ACE should be considered in order to evaluate similar contingencies. Moreover, the approach based on the daily therapeutic dose is more expeditious than the approach based on the components of the reference API.

2853 Derivation of an Oral Reference Dose (RFD) for Di 2-Ethylhexyl Cyclohexan-1,4-Dicarboxylate (DEHC): An Alternative to Phthalate Plasticizers
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Di 2-ethylhexyl cyclohexane-1,4-dicarboxylate (DEHC, CAS 84731-70-4) is an ester of polyaromatic acid assessed in a wide variety of ecotoxicological and mammalian toxicity assays as a substitute for phthalate ester-type plasticizers. Laboratory animal data indicate that DEHC has a low acute oral and dermal toxicity, is non-irritating to the skin and eyes, and is a non-sensitizer. In an OECD 422 combined repeat dose toxicity study with a reproductive/developmental toxicity screening test with Sprague-Dawley rats, minimal effects were observed on the liver, spleen, and thyroid. In a subsequent 90-day subchronic study in Sprague-Dawley rats, no toxicological effects were noted. Therefore, DEHC was neither mutagenic nor clastogenic in in vitro studies with or without metabolic activation. Results from the OECD 422 study suggest that DEHC is neither teratogenic or a reproductive toxicant. Although the dataset lacks chronic and multigenerational reproduction studies, the current evidence from screening studies indicates low potential for genetic toxicity or reproductive effects. Where data gaps exist for DEHC, a read-across approach was used to assess the toxicological endpoints of interest. Di-ethylhexyl terephthalate (DEHT, CAS 6422-86-2) and 1,2-cyclohexane dicarboxylic acid, diisomonyl ester (DINCH, CAS 4744919-59-0) have higher tiered studies to provide the data lacking for standard setting. DEHT and DINCH were chosen as the closest read-across source substances due to similar anticipated physical/chemical properties, metabolism and available safety profiles, one a terephthalate and the other an ortho-cyclohexane diacid. The current study emphasizes the challenges of evaluating the safety of novel compounds that lack certain empirical data and the proper selection of analogs for read-across. An oral reference dose (RFD) for DEHC was calculated using the human equivalent NOAEL from the OECD 422 study in Sprague-Dawley rats. A total uncertainty factor of 100 was comprised of interspecies (3x), intraspecies (3x), subchronic to chronic (1x), LOAEL to NOAEL (10x), subchronic to chronic (1x) and database uncertainty (3x) factors, resulting in an RFD of 0.3 mg/kg/day.

2854 The Evaluation of Snus Toxic Effects on Human Vascular Endothelial Cell
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Snus is popular in Europe and America. Although Snus is considered to be healthier than cigarettes, it is recently reported that Snus may have adverse effects on cardiovascular system. In this study, we evaluated the toxic effects of Snus solution on human vascular endothelial cell (HUVEC). DMSO and aqueous solutions of Snus were produced, then HUVEC was exposed to these two solutions with certain concentration gradient for 3h and 24h. Next, HUVEC cytotoxicity was examined by neutral red and LDH. The results showed mild toxic effects on the cell survival for both solutions. In addition, Snus aqueous solution could promote cell proliferation under low concentration. After that, the cell apoptosis was tested by Annexin V/FACS after 3h exposure and TUNEL assay after 24h exposure, but in either case, no effects were observed for both solutions. We then tested the mRNA expressions of four apoptosis associated genes by qPCR. The expression level changed differentially for different solutions treatment. In the case of 3h treatment, both solutions did not affect BAD, BAX and TP53 expressions, but inhibited BCL-2 expression. For 24h, Snus aqueous solution increased BAD and TP53 expressions, but reduced of BCL-2 expression, while Snus DMSO solution increased the expression of BAX, BAD, TP53. We also checked the IL-6 and IL-8 expression by ELISA to examine whether the inflammation was affected after 24h exposure. We observed that Snus aqueous solution could promote IL-8 secretion, and Snus DMSO solution only inhibited IL-6 secretion. In conclusion, Snus solutions showed slight toxicity for HUVEC based on the evaluations of survival, apoptosis and inflammation effects in vitro.

2855 Genotoxicity and Carcinogenicity Risk Assessment of Prucalopride
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Prucalopride (PRU) is a highly selective 5-hydroxytryptamine type-4 (5-HT4) receptor agonist used to treat chronic idiopathic constipation. During development, follow-up mechanistic studies were conducted to de-risk and understand the positive Ames test (positive in the TA100 strain with absence of or reduction in revertant frequency) that was observed in the Aroclor-induced genotoxicity assay of PRU. The acute oral toxicity was examined in a 14-day gavage study in rats and mice, and the NOAEL was 3500mg/kg/day. The NOAEL for the 28-day repeated dose toxicity was 1200mg/kg/day for rats and 500mg/kg/day for mice. The NOAEL for the 2-year rodent bioassays (neoplasia of the liver in rats and tumors in mice) was 150mg/kg/day for rats and 100mg/kg/day for mice. In these studies, the absence of any genotoxicity or evidence for tumorigenicity was considered to be positive. Additional mechanistic studies in mice and rats revealed the potential for PRU to biomarker animal data, but no studies showed positive results. Following the mechanistic studies, follow-up genotoxicity studies were performed to further characterize the genotoxic hazard, including a mouse lymphoma assay, an in vitro human lymphocyte assay, a UDS assay in primary rat hepatocytes, an in vivo UDS-test, an in vivo gene mutation assay with liver tissue from a Big Blue transgenic rodent model, and a transgenic mouse model. In the transgenic mouse model, no increases in liver neoplasms were observed in mice injected with PRU. However, in the UDS-test, PRU increased the expression of BAX, BAD, TP53. We also checked the IL-6 and IL-8 expression by ELISA to examine whether the inflammation was affected after 24h exposure. We observed that Snus aqueous solution could promote IL-8 secretion, and Snus DMSO solution only inhibited IL-6 secretion. In conclusion, Snus solutions showed slight toxicity for HUVEC based on the evaluations of survival, apoptosis and inflammation effects in vitro.

2856 In Vitro Oxidative Stress Responses of Different Arsenic Species

Arsenic (As) is a known carcinogen. Previous studies have demonstrated that As would induce oxidative stress in the in vivo tests, which could be an important part to explain the mechanism on causing cancers. However, the environmental and occupational exposure levels of As will make a great difference according to the chemical species. In this study...
the cytotoxicity and oxidative stress responses of four As species, i.e. arsenite (As(III)), arsenate (As(V)), monomethylated arsenic (MMA) and Dimethyl arsenic (DMA), were tested to analyze oxidative stress caused by these chemical species. GBW standard reagents of the four arsenic species were used for the tests and were investigated for the cytotoxic response and oxidative stress response (intracellular reactive oxygen species, ROS and extracellular reactive oxygen species, SOD) on A549 cells after 4-hour exposure for ROS and SOD and 4-hour and 24-hour exposure for cytotoxicity at the same molar mass of As. Results showed that As(III) had exhibited cytotoxicity greatly (i.e. less than 70% viable cells vs. negative control) at the range from 60-100 µmol/L after the 24-hour exposure, whereas the other 3 species did not show significant cytotoxicity in both 4-hour and 24-hour exposures. Under the 4-hour exposure condition, ROS fluorescence intensity increased to 140.3% and 177.6% for As(III); 117.1% and 108.0% for MMA; 109.3% and 120.0% for DMA, respectively at the concentrations of 20 µmol/L and 100 µmol/L As, when compared with the negative control. Extracellular SOD generated under the condition of 100 µmol/L As and 4-hour exposure was 3.28-fold, 1.93-fold, 1.97-fold and 0.96-fold for As(III), As (V), MMA and DMA, respectively, of negative control. In general, all the As species have induced clear oxidative responses of A549 cells, although the cytotoxic results of As(V), MMA and DMA did not show significant differences at low doses. The study on oxidative stress caused by arsenic species might plot a clear picture to identify the different toxic characters. These results could be important to further reveal the mechanisms of As toxicity and help assess the hazards of environmental As exposure.

2857 Toxicological Challenges for Indigenous People in Colombia

In Colombia indigenous people are minority groups settled in specific areas legally constituted and protected by the constitution, granting them their territories and natural resources. However, they are experiencing different toxicological vulnerabilities due to several anthropogenic activities, mostly mining operations, with few regulatory mechanisms to guarantee their ethnic and cultural diversity. The aim of this work was to identify negative impacts of external activities on environmental degradation and the effect on human health in indigenous people of Colombia. To review the subject, text mining was carried out in several databases. The results showed that the most common activities carried out in indigenous territory are coal and gold mining; mining promotes air and water pollution, together with deforestation, loss of biodiversity and soil for cultivation and incorporation of metals to the trophic network. Exposure to toxics has been measured at the individual level, especially mercury, found at high concentrations in indigenous living in several river basins of the Colombian Amazon. In the department of Guajira, open-pit coal mining has been linked to respiratory diseases and displacement-related physiological effects. It has to be underlined, that in many native communities where mining activities are carried out, there is no public health data related to their environmental or toxicological impacts, and health information is based just on perception complaints. The lack of data and the existence of many mining exploration applications and title requests in protected areas makes it clear, that it is necessary to take urgent measures for the preservation of these indigenous territories to warrant their survival, and in the long run, our own as well. It is also important their participation in the decision-making processes regarding their territory, making use of the ethnic minority prior, free and informed consultation for any type of project in their zones, aiming to reduce mining vulnerability and intervention in their ancestral territories.

2858 Incorporating ToxCast Data into Naphthalene Human Health Risk Assessment
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Chronic inhalation of naphthalene vapor causes nasal olfactory epithelial tumors in rats and benign lung adenomas in mice. Assessment of the available human data does not establish an association between naphthalene and increased respiratory cancer risk. Therefore, cancer risk assessment of naphthalene in humans depends entirely on experimental evidence derived from rodents. The United States Environmental Protection Agency’s (US EPA) Toxicity Forecaster (ToxCast) Database contains 882 in vitro assays for naphthalene, with more than 750 assays conducted with human cells. All of the available naphthalene ToxCast assays were reviewed and used for the following analyses: 1) a physiologically-based pharmacokinetic (PBPK) model for naphthalene (Campbell et al. 2014), the naphthalene inhalation concentrations corresponding to relevant activity concentrations for all active naphthalene assays were determined, and compared to the naphthalene human equivalent concentration (HEC) derived in our recent naphthalene paper (Bailey et al., 2015); 2) Target endpoints for active assays in the context of proposed modes of action for naphthalene were evaluated; and 3) ToxCast assays were reviewed to determine which assays might be used for the positive based on proposed modes of action and carcinogenic endpoints identified for naphthalene. Although there are numerous and recognized limitations and uncertainties within the naphthalene ToxCast data, the results from our analyses of these data are consistent with and provide additional support for the conclusions described in our 2015 analysis of naphthalene.

2859 Prenatal Exposure to Bisphenol A and Hyperactivity in Children: A Systematic Review and Meta-Analysis
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Attention-deficit hyperactivity disorder (ADHD) has increased in prevalence in the past decade. Studies attempting to identify a specific genetic component have not been able to account for much of the heritability of ADHD, indicating there may be gene-environment interactions underlying the disorder, including exposure to environmental chemicals. Based on several studies, we chose to examine bisphenol A (BPA) as a possible contributor to ADHD in humans. BPA is a widespread environmental chemical that has been shown to disrupt neurodevelopment in rodents and humans. Using the Office of Health Assessment and Translation (OHAT) systematic review framework, we designed a protocol to determine the association between early life exposure to BPA and hyperactivity, a key diagnostic criterion of ADHD. Searches of PubMed, Web of Science, and Toxline were completed for all literature to January 1, 2017. All rodent and human studies measuring exposure to BPA and hyperactivity were included. Studies were evaluated using the OHAT risk of bias tool. A review of the literature identified 29 rodent and 3 human studies. The effects in humans were assessed qualitatively including difference between the sexes. For rodents exposed to 20 µg/kg/day BPA, we estimated the size and direction of the effect in a random effects meta-analytical model. A random effects meta-analysis showed significantly increased hyperactivity in male rodents. In humans, early BPA exposure was associated with hyperactivity in boys and girls. The systematic review, meta-analysis, and the hazard identification conclusion will be presented, as well as suggestions for future research and risk assessment. This study indicates that BPA may contribute to hyperactivity in children, and precautions should be taken to avoid exposures, especially in pregnant women.

2860 Risk Assessment of Polycyclic Aromatic Hydrocarbons in Some Herbal Remedies in Enugu, Nigeria

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental pollutants that contaminate various biological media. Some PAHs are known carcinogens with broad range of toxicity to human health. Benzo[a]pyrene (BaP), is the most toxic PAH characterized toxicologically. This study was set to determine the risks posed by PAHs in some herbal remedies in Enugu. Two hundred and thirty samples were analyzed for the presence of the United State Environmental Protection Agency (US EPA) 16 priority PAHs using gas chromatography-mass spectroscopy/flame ionization detection (GC-MS/FID) (Clarus 500 Series). Determinations of PAH concentration, total toxicity equivalency, total carcinogenicity, carcinogenic index and carcinogenic risk were assessed. Results showed that 11 out of the 16 priority PAHs by US EPA, 3 out of the 8 carcinogenic PAHs by IARC and 3 of the 4 European Union PAHs (EU-PAHs) were present in all the herbal remedies studied. Benzo(a)pyrene was not detected in any of the samples. The total PAH concentrations, total toxicity equivalency and total carcinogenicity of the herbal remedies were normalized to the cancer potency equivalent factor of BaP (BaP = 1), and were found to be higher than the WHO target limit of BaP-TEQ of 1 ng/m³. The percentage carcinogenicity of the 3 carcinogenic PAHs found in the samples ranged from 88.98% to 98.09%, indicating that the herbal remedies have the potency to induce cancer. The sources of PAH contamination as determined by the diagnostic ratios indicated pyrolytic and petrogenic sources, and amounts of contamination in the herbal remedies. The carcinogenic risk assessment showed the
The current oral MRLs for DEHP are based on reduced fertility at ≥140 mg/kg/day in intermediate-duration studies and testicular at ≥25 mg/kg/day in chronic-duration studies. Numerous studies published since the last toxicological profile update indicate that reproductive effects can occur following exposure to much lower doses. Furthermore, while reproductive effects are among the most well-studied endpoints associated with DEHP exposure in experimental animals, recent studies show that DEHP exposure may induce other effects at lower doses. Following acute oral exposure to DEHP in animals, effects observed at low doses include: 1) altered glucose homeostasis in rat offspring following maternal exposure to ≥1 mg/kg/day during gestation; 2) abnormal Leydig cell clustering in fetal rat testes following maternal exposure to ≥10 mg/kg/day during gestation; and 3) Leydig cell toxicity (proliferation, reduced testosterone production) in male rats exposed to ≥10 mg/kg/day for 11-14 days. Recent intermediate-duration studies in animals have shown the following low-dose effects: 1) enhanced immune responses in sensitized mice exposed to ≥0.03 mg/kg/day for 28-52 days; and 2) various developmental effects, including decreased offspring body weight, male and female reproductive effects, impaired renal function and altered glucose homeostasis in rodents following in utero and/or early postnatal exposure to doses as low as 0.04 mg/kg/ day. Human epidemiological studies published since the last profile update have also suggested potential associations between DEHP exposure and endocrine, immunological, reproductive, and developmental effects. Taken together, the updated information suggests that it may be appropriate to derive a new acute oral MRL and revise the existing intermediate oral MRL for DEHP. In addition, this information also suggests that derivation of a chronic oral MRL may no longer be supported, because recent acute and intermediate-duration studies identify adverse effects at doses much lower than those tested in available chronic studies.

**2862 Identifying Systematic Review Topics through Scoping Reviews of PAHs and Melamine**

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Systematic reviews are increasingly being used to answer important questions in toxicology and environmental health, including for the purpose of risk assessment. Surveying the available literature through “scoping reviews” is a valuable first step in determining the feasibility and scope of subsequent systematic reviews. Scoping reviews aid in the formulation of research questions and development of protocols, including the selection of populations, exposures, comparators, and outcomes. Scoping reviews do not assess study quality or direction of effects, they are useful for identifying research gaps and recommending specific avenues for future research. We recently published two reviews that demonstrate the role of scoping. For both, the methods included a priori protocols, comprehensive literature searches, independent screening for study inclusion, and systematic categorization and summarization of relevant studies. Data extraction included the number and age of subjects, the models used, exposure routes and duration, doses administered or concentrations measured, and outcomes assessed. The review of polycyclic aromatic hydrocarbons and female reproduction identified 75 relevant studies. The top three studied chemicals were benzo[a]pyrene (42 studies), naphthalene (14), and phenanthrene (10). Two endpoints were deemed the most appropriate for systematic review: fertility (36 studies), and pregnancy/fetal viability (15 studies). These endpoints have ample research for systematic review, as well as an appropriate level of evidence. The design of the scoping reviews demonstrated the feasibility of completing systematic reviews of these endpoints.

**2863 Prostaglandin Analog: PDE Assessment and Relationship with the Therapeutic Dose**

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After the revisions of Chapters 3 and 5 of the EU GMP Guide in 2015, more attention has been given to the topic cross contamination. At the same time, a guideline of the EMA was published introducing the concept of Permitted Daily Exposure (PDE) values. The approach for setting PDE limits is the same outlined in ICH Q3c consensus guideline on residual solvents and in ICH Q3D consensus guideline on elemental impurities. The background for that was the request of the pharmaceutical industry to be able to decide itself on a risk-based approach whether a product can be produced in a multipurpose facility, or not. Bimatoprost (amide prodrug of 17-phenyl-PGF2α), Tafluprost (difluoroprostaglandin derivative of PGF2α), Travoprost (isopropyl ester prodrug of PGF2α), and Unoprostone (free acid analog of PGF2α) were selected as examples for clinical use to lower patients’ intraocular pressure (IOP). Recently, PGAs were approved as a first-line treatment for glaucoma. Though PGAs are intended for ocular administration, it may occur, particularly in chemical-pharmaceutical companies, that PGAs contaminate molecules intended for other routes of administration. Our assessment focuses on the intravenous route. By definition, when a medication is administered intravenously, its bioavailability is 100%. A common approach for the selection of the Point of Departure (POD) and Adjustment Factors (AFs) can be applied to the prostaglandin analogs group. The result of the above suggests that the substance has a POD of PDE. The purpose of this review is to demonstrate a well-established safety profile at therapeutic dose and comparable preclinical data. Pre-clinical studies conducted during the organogenesis period were judged to be the most relevant POD. Regarding selection of the AFs, interspecies and intraspecies variability, NOAEL and bioavailability adjustment have to be considered. The assessment results highlight a consistent relationship between the therapeutic dose of PGAs and the PDE value irrespective of the therapeutic route of administration. Moreover, a group assessment methodology focused on APIs with the lowest therapeutic doses is considered a possible appropriate and resource efficient solution for companies, particularly those in the chemical-pharmaceutical industry, with a large portfolio of APIs.

**2864 Toxicological Evaluation of a Sulphonated Nanocellulose from Khaya senegalensis Seeds in Rats**

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Nanocellulose is currently gaining attention due to its unique properties. This attention includes its application as building blocks for developing novel functional materials, plant drug and also in drug delivery systems. However, its safety remains largely untested or less understood. Thus, sulphonated nanocellulose (KSS) was prepared from cellulose (KSC) isolated from Khaya senegalensis seed (KS). KS, KSC and KSS were characterized using Fourier transformed infrared (FTIR), X-ray diffraction (XRD), thermogravimetric analysis (TG), particle size distribution (PSD), zeta potential and scanning electron microscopy (SEM). The impact of KSS on selected rat markers of oxidative stress, inflammation and apoptosis in Wistar rats was also investigated. Thus, male rats were randomly assigned to four groups of five animals each and were treated with KSS (0, 50, 75 and 100 mg/kg BW) respectively, for 14 days. Thereafter, bio-markers of renal oxidative damage, inflammation and immunohistochemical expressions of iNOS and COX-2 in the kidney of the rats were evaluated. The results revealed that the crystallinity of KSS was 70.40%, monomodal with a mean size of 0.0149 μm and a flaky surface with few agglomerations. KSS had no effect on markers of kidney function and oxidative damage, although there was a generalized hypotension after 14 days of exposure. Lastly, KSS enhanced the antioxidant status and immunohistochemical expressions of iNOS and COX-2 in the kidney of the rats. While the biomedical applications of KSS may appear plausible, our data suggest that it could induce renal toxicity via the combined impacts of electrolyte imbalance and inflammation.
The neonicotinoid insecticide nitenpyram has excellent efficiency against pests and low toxicity for mammals. However, increasing studies have shown that neonicotinoids have adverse effects on non-target organisms. It is not known whether nitenpyram exposure negatively affects human health. Genotoxic potential of nitenpyram on human bone marrow mesenchymal stem cells (HBMSCs) was evaluated. Cytotoxicity was analyzed using the Water-soluble formazan method (WST-8) in a 48-h assay. For following exposure studies, non-cytotoxic concentrations of nitenpyram (50-250 μg/ml) were selected. Nitenpyram (>125 μg/ml) significantly induced formation of MNs and the counts of MNs was concentration-dependent. According to the results of Annexin-FITC assay, no apoptotic bodies were observed, thus eliminating the interference of apoptotic bodies on MNs counting. Nitenpyram (>125 μg/ml) led to the formation of oxidative stress in HBMSCs, as evidenced by an increase in reactive oxygen species (ROS) content, the decrease of total antioxidant capacity (T-AOC) and changes in the activity of superoxide dismutase (SOD). Furthermore, the expression levels of key antioxidants (e.g. bromine and chlorine) were similar, with differences depending on acute dose-response data. The toxicidrome-based approach allows for applicability across a range of chemicals, as the results of a given analysis can be applied to chemicals with similar acute clinical signs and symptoms. Risk assessors and planners may use this approach to estimate the long-term health effects following acute chemical exposures using more robust data available.
Acetaminophen (APAP) is one of the most commonly used as an over-the-counter analgesic/antipyretic drug given to children around the world. It is known to cause high dose related hepatotoxicity in children and adults. APAP metabolism involves cytochrome 450 (CYP) enzymes and several Phase II conjugation reactions, including glutathione (GSH). The vulnerability to hepatotoxicity is driven by the balance between metabolic activation and detoxification and may be altered in early-life stages. This study evaluates how APAP is a primary hepatotoxic drug to N-acetyl-p-benzoquinone imine which conjugates by direct interaction with GSH and excreted in urine via glucuronide, sulfate and GSH enzymatic pathways. At high acute dose, detoxification rapidly depletes GSH allowing excess benzoquinone imine to bind critical proteins in the liver leading to hepatocyte injury. The primary objective of this study is focused on conducting a comprehensive systematic literature search of ontogeny of enzymes (CYP2E1, UGT, SULT, GST) involved in metabolism in humans and experimental animals and integration of hepatotoxicity data for early life-stages and adults. The data analysis suggests that the causes hepatotoxicity profile shifts in early-life. The human metabolic profile was obtained from the liver bank pediatric pharmacokinetic data for APAP. However, the major determinant of early-life vulnerability appears to be lower expression of CYP2E1, resulting in less oxidation of APAP to toxic metabolites in children compared to adults. There is little indication from metabolic studies that supports the contention that there is a window of vulnerability to APAP liver toxicity in early-life. It would be useful to explore these findings further in physiologically based toxicokinetic/toxicodynamic models incorporating life-stage differences in APAP metabolism enzymes including their polymorphisms and nutritional status (e.g., malnutrition) data. One might employ enzyme ontogeny database for example from the URL (https://www.dropbox.com/s/uzizi5q3jdjov0gm/Enzyme_Ontology_Master_11_14_2013_mdb?dl=0) together with the physiological parameters database (https://cpub.epa.gov/ncea/cfm/recorddisplay.cfm?deid=202847) including model development software (https://megens.useconnect.co.uk). Disclaimer: The authors declare there exist no real or perceived conflict of interest.
feine, did not induce lipid accumulation at treatment concentrations up to 100 μM. Rat and human steatosis assays were run with concentrations of AMP that encompass the rat in vivo LOEL. In preliminary data, 48 hour treatment with AMP induced modest neutral lipid accumulation at 316 μM in rat vs. 1000 μM in human MPCPs, without significant effect on viability. After 96 hours, lipid accumulation was only detected at concentrations that caused statistically significant loss of viability. We are currently refining evaluation of the time- and dose-dependence of steatotic response and will perform reverse dosimetry using the human PBPK model.

Multiple regulatory bodies (US EPA, ECHA, Health Canada) are currently tasked with prioritizing chemicals for data collection and risk assessments. These prioritization efforts are driven by the many chemicals in commerce, or in the environment for which detailed risk assessments have not been performed. Ideally, the incompletely assessed chemicals posing the highest risk would be given the highest priority to go into a time-consuming and expensive high-level risk assessment process. In order to assist these efforts, we have developed a web-based application that enables a rapid, flexible and transparent prioritization process. The tool includes multiple data streams related to human and ecological hazard, exposure, and physico-chemical properties (persistence and bioaccumulation). For human hazard, the data streams include quantitative points of departure (PODs) that are compiled from multiple sources such as EPA ToxRefDB, ECHA, COSMOS; estimated PODs from high-throughput in vitro screening assays and computational models; and qualitative measurements and predictions of specific endpoints (e.g., genotoxicity, endocrine activity). For ecological hazard, quantitative PODs are taken from the EPA EXPO databases. Exposure information includes production volume, quantitative predictions using the EPA (EPA) ExpoCast and SHEDS models, biomonitoring data, and qualitative information such as media occurrence, use profiles and likelihood of consumer and childhood exposures. The tool allows users to select a chemical list, as well as to specify input data types, weights, and overall prioritization scoring schemes. The use of the tool is illustrated by prioritizing chemicals related to TSCA and the Safer Choice Ingredient List. The TSCA list has more “High” priority chemicals than does SCIL, as expected from their use profiles. Additionally, we show that there are significant data gaps for many chemicals in both of these lists, which may need to be filled using additional experiments or computations. The underpinning data streams for this application are already available in the EPA CompTox Chemistry Dashboard and have been repurposed to deliver this application. This is in keeping with our overarching software development methodology of providing multiple “building blocks” in the form of databases, web services and visualization tools to inform web services and visualization components to deliver fit-for-purpose applications to the relevant audiences. This abstract does not necessarily represent US EPA policy.

Human health risk assessment relies on comprehensive toxicity studies, mostly in animals, for identifying potential adverse health effects and corresponding effect levels from chemical exposures. However, the majority of chemicals currently in commerce lack the data necessary for traditional hazard identification and dose-response analysis, preventing regulatory agencies from establishing chemical-specific reference values and clean-up levels at contaminated sites. Previously, we introduced a read-across framework to address data gaps for target chemicals with limited in vivo toxicity information. The methodology relied on the integration of evidence for evaluation of structural, metabolic and toxicity-like analogues in order to select the single best analogue as the basis for quantitative read-across to target chemicals. This approach has been implemented to support screening-level risk assessment of data-poor chemicals of interest to the U.S. Environmental Protection Agency (EPA) Superfund program. Case study examples are presented herein, outlining lessons learned from the practical application of techniques in chemical grouping and read-across to quantitative risk assessment. Furthermore, a revised framework is proposed, incorporating lessons learned coupled with scientific and technological advances in the field of read-across and predictive toxicology. The new framework includes important considerations for fit-for-purpose, target chemical profiling, analogue identification, and evaluation and incorporation of data derived from new approach methods. Altogether, this work emphasizes the translational nexus between computational and expert-driven risk assessment with the potential to inform a broad landscape of regulatory decision-making. The views expressed in this abstract are those of the authors and do not necessarily reflect the views and policies of the US EPA.

Systematic reviews involve the identification and synthesis of information from all available evidence related to a review question. Conducting 10-fold increases in eosinophils and neutrophils). Histopathologic evaluations, propose a framework to minimize relevant data losses and maximize efficiency.

Systematic reviews are expensive and time-consuming, especially in terms of data extraction, in which subject matter experts must review full text documents to extract specific data elements. While natural language processing-based information retrieval technologies are now widely used to increase the efficiency of the literature screening and prioritization step, the use of information extraction algorithms in the data extraction step is still nascent. In this research, we used published evidence tables from EPA’s IRIS assessments, together with the corresponding full text articles, to test the accuracy of a combination of information extraction algorithms on a number of structured data elements. Specifically, we used a combination of conditional random field algorithms, supervised machine learning based on support vector machines, Naive Bayes, and maximum entropy classifiers, and hand-crafted rules based on parsing sentences into their grammatical elements, to extract structured data elements including species, strain, route of exposure, dose regimen, and chemical. Extracted entities included sentences, in which the sentence containing the information relevant to the data element is extracted, and concepts, in which the specific concept is fully extracted. By comparing the machine extracted results to the expert extracted results in the published evidence tables for the same set of studies, we found extraction accuracy rates ranging from F1-scores of 28% to 89%. We quantitatively assess the effect of training data size and present the comparative performance of the various algorithms tested individually and in a pipeline using the F1, recall, and precision metrics. Finally, we estimate potential time savings from the use of automated data extraction algorithms and, based on our presented empirical findings, propose a framework to minimize relevant data losses and maximize efficiency.

Currently, there is no fully accepted approach to identify chemicals as respiratory sensitizers. This work was conducted in vivo approach to identify low molecular weight respiratory sensitizers and differentiate them from dermal sensitizers and irritants. BN rats were administered 2 equipotent dermal applications of a dermal (2,4-dinitrochlorobenzene; DNBC or hexyl cinnamaldehyde; HCA) or respiratory (trimellitic anhydride; TMA) sensitizer or a highly reactive aldehyde (orthophthalaldehyde; OPA) in methylenetric ketone (Mek, vehicle) to induce systemic sensitization. Two weeks later, treated and control (MEK only) rats were challenged with the same substances via inhalation of TMA induced pulmonary inflammation in sensitized rats, IgE levels increased relative to baseline in rats sensitized with TMA and control (MEK only) rats were challenged with the same substances via inhalation for 0 (air only), 15, 30 or 60 min (n=4/dose) to characterize the C x dose response. Measured endpoints included functional respiratory parameters, serum IgE, airway hyperreactivity (AHR), pulmonary inflammation, and nasal/lung histopathology. In addition, the right apical lung lobe was analyzed for targeted gene (Th1/Th2 immunological pathways, airways, inflammation) expression changes. Serum levels increased relative to baseline in rats sensitized with TMA and OPA but not DNBC or HCA. Bronchoalveolar lavage (BAL) indicated only inhalation of TMA induced pulmonary inflammation in sensitized rats, compared to controls. At peak response, exposure to TMA decreased BAL total protein (4-fold), DLH (3-fold), and total cells 5-fold (approximately) and induced significant increases in NKC. Microarray analysis revealed no changes in pulmonary inflammation in any treatment group when compared to controls. OPA-exposed rats exhibited...
dose-dependent nasal lesions indicative of exposure to a direct-acting highly reactive vapor. Functional respiratory parameters and AHR were not discerning endpoints in this short-term assay. Candidate gene biomarkers contribute to the ability to identify and distinguish potential respiratory sensitizers. This integrated in vivo approach enabled identification and differentiation of respiratory sensitizers, dermal sensitizers and irritants. The relevance in the mouse study and characterization of the immune, cellular and inflammatory responses underscore the need to examine multiple endpoints and take a WOE approach as the effects of epithelial irritation and chemical reactivity can impact regional dosimetry and interpretation of results.

**2877 Missing Toxicokinetics Data Offers Opportunities to Improve an Oral Cancer Slope Factor for Isoprene**

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General population exposure to isoprene occurs via inhalation with background environmental exposures of 0.5 ppb. Oral exposure may occur when isoprene is used as a monomer in rubber articles intended to contact food or drinking water. The NTP considers isoprene as "reasonably anticipated to be a human carcinogen" and IARC classified isoprene as possibly carcinogenic to humans (Group 2B) based solely on inhalation exposure data given the lack of chronic oral data. Although an oral slope factor for isoprene has not been established, no oral slope factor has been proposed. In the current analysis, an oral cancer slope factor is derived based on the chronic inhalation studies in rats and mice and considers toxicokinetic and metabolic data after inhalation in rats, mice, and humans. These studies suggest interspecies differences in metabolism contribute to varying sensitivities to isoprene toxicity among humans, rats, and mice. Benchmark dose modeling of adverse effects from the chronic rodent studies identified the combined incidence of mammary gland adenomas and carcinomas in rats as the most sensitive endpoint and thus critical effect. Humans are anticipated to be less sensitive than rodents due to more closely resemble rats than mice with respect to sensitivity to isoprene exposure. However, species-specific areas under the curve (AUC) values in humans, rats or mice are not available for the toxic moiety (or the parent compound) to derive a dosimetric adjustment factor for inhalation-to-oral extrapolation. Without these data, the resulting oral cancer slope factor of 7.7 x 10^{-3} (mg/kg-day)^{-1} considers chemical- and species-specific blood:air partition coefficients, but is unable to account for metabolic rate differences that likely result in much lower AUC in humans compared to rats or mice. The missing toxicokinetics data could inform a physiologically-based pharmacokinetic model that offers opportunities to improve an oral cancer slope factor for isoprene.

**2878 Using Machine-Learning and SWIFT-Active Screener to Reduce the Expense of Evidence-Based Toxicology**


Evidence-based toxicology employs the rigorous and transparent methodologies of Systematic Review (SR) in order to synthesize and reach consensus about targeted questions related to the health effects of environmentally important chemicals. A critical and time-consuming step in this process is screening the available literature to select relevant studies for further analysis. We have recently evaluated our collaborative, web-based screening tool, SWIFT-Active Screener, in order to assess its ability to reduce the overall screening burden associated with creating and maintaining a systematic review. By employing a machine learning methodology called "Active Learning", and through a novel statistic, we have developed an automated, supervised machine-learning method that can automatically classify the relevance of studies screened. Active Screener can significantly reduce the overall screening burden compared to traditional approaches. Previously, we have demonstrated the theoretical advantage of using Active Learning to prioritize rank documents by simulating the review process using a large set of more than 100,000 titles and abstracts already screened by human screeners during 20 different systematic reviews. Compared to traditional screening, this method resulted in an average 54% reduction in screening burden, while still achieving 95% recall or higher; when tested on a subset of the 13 studies containing >1,000 articles, the reduction in screening burden improved to 71%. Here, we extend that work by evaluating the performance of our SWIFT-Active Screener application on several recent systematic reviews conducted using our software by organizations such as NIEHS, EPA, USDA, TEDX and EBT. Results from these "real world" data sets are similar to results previously observed in simulation. For example, in the largest of the projects evaluated, 20,883 references were dual-screened using Active Screener, with 99% recall achieved after screening only 42% of the total collection. Given that each abstract takes, on average, 1 minute to screen per screener, the resulting savings in effort for this review alone was approximately 400 hours. More than 4,000 systematic reviews are performed each year in the fields of environmental health and evidence-based medicine, with each review requiring, on average, between six months to one year of effort to complete; for this reason, we anticipate that machine learning screening tools such as Active Screener can help to greatly reduce the total cost of evidence-based toxicology.

**2879 Accelerating the Pace of Chemical Risk Assessment Workshop: Advancing Use of New Alternative Methods for Regulatory Support**

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The tides of acceptance of new alternative methods (NAMs) are changing. Numerous scientific papers have been published recently that explore the boundaries of data applicability and propose approaches that combine new and conventional methods. The modernization of the Toxic Substances Control Act, the implementation of REACH, the next phase of the Canadian Chemical Management Plan, and many international chemical management policies and laws have escalated the demand for sharing of data and knowledge across the regulatory landscape. This interest and regulation is fueling the momentum to examine how NAMs might transform regulatory evaluation of chemicals and pragmatically evaluate barriers to acceptance. These barriers include potential limitations of existing technologies, differing regulatory needs for decision making, and lack of understanding in applying NAMs. In order to better understand what is needed for the acceptance of the use of NAMs for chemical risk assessment, recent workshops were convened comprising key international regulatory agencies to discuss progress in applying the new tools to prioritization, screening, and application to quantitative risk assessment (QRAs) of different levels of complexity. Most progress has been made in screening and prioritization, but ultimately to modernize quantitative risk assessment, there is a need to demonstrate how the data and tools can be incorporated into future QRAs. Scientific and regulatory needs for the quantitative application of NAMs to QRAs were identified, and example case studies were undertaken as intergovernmental collaborations to address these needs. Case study topics include use of NAMs for exposure evaluation, assessing data poor chemicals, or specific chemical classes, including per- and poly-fluorinated substances. Results of these case studies will be presented and the role of the NAM to address the chemical management or risk assessment challenge will be discussed. These efforts are an important step in increasing the confidence in use and acceptance of NAMs in regulatory chemical risk assessment. Disclaimer: The views expressed are those of the authors and do not necessarily represent the policies of the US EPA, the European Chemicals Agency, or Health Canada.
set to identify relevant studies, which saves the effort of finding seeds within the dataset to be classified. Recall in the chemical-agnostic simulations was between 83 and 100% and we eliminated the need to manually screen approximately 65-80% of studies; however, variance between actual and predicted recall was greater than the random seed method owing to the external and non-random origin of agnostic seed data. The mutual recall for agnostic simulations was significantly improved using supervised machine learning on the discards from the supervised clustering stage to reach recall of 90% while still eliminating upwards of 40% of studies from screening. These simulations demonstrate that supervised clustering is a transparent and reliable method for reducing manual screening while ensuring literature searches are comprehensive. Further, a chemical-agnostic seed set was shown to be effective in identifying relevant studies in search results with very low precision.

2881 Comparing Toxicological Tipping Points from High-Content Imaging to Rat Subchronic Hepatotoxicity Doses


A major challenge to using in vitro high-throughput screening (HTS) data in risk assessment is the identification of toxicological “tipping points” between adaptation and adversity. Toxicological tipping points represent a systems threshold, or critical point, beyond which biological pathways invoke permanent perturbations that eventually lead to adverse effects. Previously, we have proposed a formal approach to utilize time-course high-content imaging (HCI) data to identify tipping points in vitro. Here, we analyzed toxicological tipping points for chemicals in rat primary hepatocytes and used quantitative in vitro to in vivo extrapolation (QIVIVE) to compare them with rat subchronic lowest observed adverse effect levels (LOAELs). First, we selected 88 chemicals from ToxRefDB that produced subchronic effects in rats. Next, we treated rat primary hepatocytes with 10 concentrations (0.2 to 100µM) of these 88 chemicals. We used HCl to measure endoplasmic reticulum stress, mitochondrial function, lysosomal mass, steatosis, apoptosis, DNA texture, nuclear size and cell number at 6 time points (1, 3, 6, 24, 48 and 72h). After processing and normalizing the data to calculate cell-state trajectories produced by each chemical treatment, we examined the occurrence of tipping points. For chemicals that produced tipping points, critical concentrations were estimated and extrapolated to oral equivalent doses using (q)IVIVE, and were compared rat subchronic LOAELs for liver effects. Overall, we found 47/88 chemicals produced tipping points while the remaining 41/88 chemicals showed transient perturbations followed by system recovery. For 20/47 chemicals the oral equivalent doses corresponding to tipping points were 5-100 times lower than LOAELs observed in rat subchronic. Our results show the utility of in vitro tipping points as a sensitive estimate of a systems threshold between adaptation and adversity that is supported by in vivo data. Toxicological tipping points offer a novel approach for estimating their point of departure and evaluating chemical safety using HTS data. This abstract does not reflect US EPA policy.

2882 Building toward an Environmental Health Sciences Ontology


Environmental Health Science (EHS) information is increasingly complex and voluminous. Coordination in how this information is collected and shared across organizations has been an ongoing topic for government agencies, academics, and professional scientific societies. Consistent capture, transmission, and analysis of these data for comprehensive risk assessment, scientific, and regulatory applications will depend upon standardization and integration of the data across all of these organizations. Simultaneously, systematic review is increasingly used across many organizations to assist in gathering, screening, reviewing and extracting data collections for specific purposes. While data collection methods have been identified for a systematic review process are a valuable asset that is often not recognized as such. Proper organization and curation of these data collections will further increase their value not only for the initial purpose but also for future applications, yet to be defined. The proof-of-concept pilot project described in this presentation focuses on human health risk assessment to develop a data coordination blueprint that is flexible and sustainable, based in large part on the definition of needs established in a 2014 EHS Language Workshop (http://dx.doi.org/10.1289/ehp.1510438) and several prior SOT sessions. The intent of the project is to move from simple constructs (glossaries) to more harmonized, integrated and interoperable taxonomies and domain-specific ontologies. First, an inventory was developed of work already in progress across EPA (e.g., EPA’s Science Vocabulary) and other federal agencies (e.g., the Standard for Exchange of Nonclinical Data [SEND] Protocol developed by FDA). The second phase develops a strategy to leverage commonalities and bridge inconsistencies in moving toward a holistic, sustainable and consistent approach to gathering, extracting, curating, and disseminating EHS data. The initial results of this work will be presented. The views expressed in this abstract are those of the authors and do not necessarily reflect the views or policies of the US EPA.

2883 The Many Ways for Keratinocytes to Influence Dendritic Cells and Their Response to Chemicals

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Small chemicals can induce skin sensitization upon dermal exposure, which may result in tolerance or allergic sensitization. Here, activation of dendritic cells (DC) is crucial. However, keratinocytes provide additional capacities for xenobiotic metabolism and can release inflammatory mediators, modulating the DC response to chemicals. We addressed the question how keratinocytes influence DC and their response to stimuli, and studied changes at early time points in a coculture of THP-1 cells as surrogates for DC with HaCaT keratinocytes. Focussing on phase I xenobiotic metabolism revealed an increased cytochrome P450 1 (CYP1) enzyme activity in THP-1 cells at 48h for HaCaT cells at all time points that even increased at 72h. Synthetic enzyme activity at 48h. In contrast to that, but as expected, THP-1 cells were found to not contribute to CYP1-dependent metabolism. Coculturing THP-1 with HaCaT also added further glutathione and other targets for protein-reactive chemicals as well as phase II enzymes. Exposure in vitro to 10 µM 4-nitrobiphenyl diminished the rapid depletion of glutathione in THP-1 cells, underlining the enhanced capacity for conjugation of xenobiotics. Upregulation of DC activation markers such as CD86 and CD54 occurs in response to inflammatory molecules. While keratinocytes including HaCaT can release a plethora of mediators such as interleukin-1α and tumor necrosis factor (TNF)-α, THP-1 cells lack significant competence, and were reported to not upregulate CD86 in response to TNF-α. We found that in the presence of keratinocytes, exposure to cytokines such as TNF-α upregulated both CD86 and CD54 on THP-1 cells. Enhanced responsiveness was also observed after exposure to chemicals in coculture, enabling sensitive upregulation of CD54 after exposure to a set of sensitizers. In sum, keratinocytes contribute to the DC response to chemicals and can enhance their responsiveness, indicating that both cell types are needed for a balanced assessment of sensitization potential and potency of chemicals.

2884 The Correlation between Liver Tumor Incidence and Short-Term Liver Weight Change in Rodents

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Chemically induced liver neoplasms are serious adverse effects and require expensive long-term animal carcinogenesis studies to detect. Therefore, it is invaluable to identify some early indicators of liver tumor occurrences to prioritize research effort and allocate resources. In this study, we focus on investigating the correlation between short-term liver weight change and liver neoplasm incidence using published National Toxicology Program (NTP) data. We screened all 593 published NTP technical reports (TRs) and identified compounds investigated in 174 TRs that have at least one species/sex combination with positive clear-evidence liver tumor. Then, we collected the data of absolute and/or relative liver weight at early stages reported in these TRs. If no liver weight data were reported in the same TR report, we attempted to find data from short-term toxicity studies documented in NTP’s TOX reports. Finally, we found 78 compounds with both long-term cancer data and short-term liver weight data available for data analysis. We first employed the benchmark dose (BMD) method to analyze the dichotomous (long-term) and continuous (short-term) dose-response data and to estimate a biologically meaningful BMD for each dataset. Then we calculated the correlation coefficient for those corresponding BMDs estimated from adequately fitted model to determine the correlation between these two sets of BMDs. Multiple dose-response models were applied to take model uncertainty into account. Preliminary results show that the correlation coefficients can range from -0.26 to 0.86, with a mean of 0.53. In addition, more than 43% of correlation is greater than 0.6, which indicates a fairly strong association. Generally, although considerable uncertainties still exist, there is a relatively high agreement between the BMD estimates from the liver tumor data and short-term liver weight data.
Recent determinations by regulatory agencies are focused on safe doses ((e.g., reference concentrations (RfCs) or reference doses (RfDs)) for developmental and reproductive toxicity (DART) endpoints (i.e., RfD, DT or RfC, DT). However, there seems to be an inconsistent approach being used for calculating these safe doses. We identified and examined seven specific issues based on recent regulatory activities for TCE, PFOA, PFOS, and paracetamol. Based on our review, we determined that the following are pertinent to development of a DART safe dose: (1) Use of effects from acute and short-term exposures. These effects should be used for long-term risk management decisions when they represent the chemical’s critical effect, but with some caveats. (2) Use of appropriate dose metrics. The dose metric metric, for example, accumulation of damage or tissue concentration of the chemical may be better characterized by either the area under the curve (AUC) or the peak concentration (Cmax), depending, as appropriate, on a broad view of the pharmacological and toxicological exposure of the chemical and an embryological foundation. (3) Incorporation in the risk assessment of the severity of DART endpoints in relation to the toxicity evident in other studies. DeRosa et al. (1985) ranking scheme provides a useful tool for incorporating severity of effects into the risk assessment process. (4) Consideration of maternal toxicity when deriving RfD, DT or RfC, DT. Estimation of a DART safe dose may be appropriate, if it is determined that the developmental toxicity is due to the chemical itself and not from maternal toxicity evoked by the chemical. (5) Appropriateness of adjusting exposure durations in DART studies for continuous exposure. Such duration adjustments depend on whether the observed developmental toxicity is due to Cmax or AUC. Development of a DART safe dose when the population exposed does not include pregnant women or children. While it is not necessary to develop RfD, DT or RfC, DT when the population exposed does not include pregnant women or children, exceptions were identified that call for such safe doses to be considered. (6) Incorporation of critical information into the risk DART assessment process. Such integration is appropriate, but on a case-by-case basis. Other issues relevant to DART are identified and recommendations are made to further assist in deriving appropriate DART safe doses

2886 Toxological Risk Assessment for Respiratory Medical Devices: Comparison of Methodology ISO 10993 Vs. ISO 18562

Respiratory medical devices come into contact with patients at a particularly vulnerable point and time. As a result, the exposure assessment for these devices is an important aspect of evaluating the safety of these products, and needs to be particularly concerned with potential releases of trace chemicals from the device materials, with special attention given to airborne releases of volatile organic compound (VOCs). ISO 10993 is the traditional standard for the evaluation of biocompatibility of medical devices and provides a framework for the safety evaluation. However, in addition to not sufficiently address the exposure through the airway, ISO 18562, on the other hand, focuses on of exposure via inhalation, while ISO 10993 does not sufficiently address the exposure through the air pathway. Therefore, we systematically examined acute median lethal dose (LD50), and reproductive/developmental no-observed-adverse-effect level (NOAEL) and lowest-observed-adverse-effect level (LOAEL) values for rat, mouse and rabbit models (oral) from the Cleaning Product Ingredient Safety Initiative database. Probabilistic distributions were subsequently constructed using data from several study durations for all available ingredients in all purpose cleaners. Based on data availability, product type-specific and chemical category-specific CTDs were also generated and compared. For each CTD, Cmax and AUC and their 95% confidence intervals were calculated using the log-normal model. To test whether the common default uncertainty factor (UF) approach (e.g., 10) in mammalian health risk assessment provides sufficient protection, we also derived UFs for acute (LD50) and chronic (reproductive/developmental NOAELs (ACRs) and reproductive/developmental LOAELs/NOAELs) via different models. In general, our novel analysis appears particularly useful for hazard and risk practitioners when identifying TTS for ingredients in all purpose cleaning products and other chemical classes. This approach can also support developmental data (e.g., maternal) for chemicals, such as cleaning product ingredients (e.g., read across) and screening-level health risk assessments when limited or no empirical toxicity information exists for specific chemicals.

2887 Probabilistic Health Hazard Assessment of Cleaning Product Ingredients in All-Purpose Cleaners
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Though numerous chemical ingredients are used in cleaning products, empirical mammalian toxicology information is often limited for many substances. Such limited data inherently presents challenges to environmental health practitioners performing hazard and risk assessments. Probabilistic hazard assessment using chemical toxicity distributions (CTDs) is alternative approach for assessments of chemicals when toxicological information is lacking. The CTD concept allows for derivation of toxicological thresholds of concern (TTCs) to predict adverse effect thresholds for mammalian species, including humans. Unfortunately, health hazard assessment of cleaning product ingredients in different use categories has not been well studied. However, all purpose cleaners are used routinely for household and industrial applications, resulting in residential and industrial exposures. Therefore, we systematically examined and analyzed acute median lethal dose (LD50), and reproductive/developmental no-observed-adverse-effect level (NOAEL) and lowest-observed-adverse-effect level (LOAEL) values for rat, mouse and rabbit models (oral) from the Cleaning Product Ingredient Safety Initiative database. Probabilistic distributions were subsequently constructed using data from several study durations for all available ingredients in all purpose cleaners. Based on data availability, product type-specific and chemical category-specific CTDs were also generated and compared. For each CTD, Cmax and AUC and their 95% confidence intervals were calculated using the log-normal model. To test whether the common default uncertainty factor (UF) approach (e.g., 10) in mammalian health risk assessment provides sufficient protection, we also derived UFs for acute (LD50) and chronic (reproductive/developmental NOAELs (ACRs) and reproductive/developmental LOAELs/NOAELs) via different models. In general, our novel analysis appears particularly useful for hazard and risk practitioners when identifying TTS for ingredients in all purpose cleaning products and other chemical classes. This approach can also support developmental data (e.g., maternal) for chemicals, such as cleaning product ingredients (e.g., read across) and screening-level health risk assessments when limited or no empirical toxicity information exists for specific chemicals.

2888 Application of Quantitative Approaches to Assess Uncertainties in the Development of Toxicity Values: A Case Study Involving the Reference Dose (RfD) for 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD)
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Recent efforts to improve risk assessments include recommendations from The National Academy of Sciences (NAS) to incorporate quantitative uncertainty analysis to better inform risk decisions. However, there are relatively few examples where such has been applied in development of toxicity values. Using the RfD for TCDD as an example, we investigated the impact of uncertainty related to selection of critical studies, points of departure (PODs) from key datasets, and application of uncertainty factors (UFs) on the RfD. The current TCDD RfD is based on equivalent PODs (20 pg/kg/day) for sperm concentration in males and neonatal TSH screening levels from two human studies, with a 30x UF applied to arrive at an RfD=0.7 pg/kg/day. Significant limitations in internal validity (e.g., high risk of bias in key domains including exposure and outcome assessment) of each of the critical studies were identified but could not be evaluated quantitatively (though the potential impact is described qualitatively). With respect to identification of a POD, multiple aspects of uncertainty were assessed quantitatively: addition of TEQ, kinetic model uncertainties (estimation of daily intake associated with serum concentration in children), clinically relevant thresholds (e.g., TSH screening levels of 10 µU/ml) and designations of such as no- or low-effect levels, and dose-response information for neonatal TSH and risk of congenital hypothyroidism from multiple study populations was combined using Bayesian statistics to develop PODs for specified risk levels. Due to lack of statistical significance and/or clinical significance, alternative UFs were also applied (e.g., elimination of 10x for LOAEL to NOAEL). This assessment, including consideration of study quality, dose-response, magnitude, and clinical relevance, supports RfD values ranging from <0.7 to >70 pg/kg/day. This range of plausible RfD values informs risk management decisions and highlights key uncertainties, as well as demonstrates the utility of evidence-based approaches in deriving health-based guidance values.
Reducing the number of laboratory animals and refining experimental procedures to enhance animal welfare are fundamental questions to be considered in connection with toxicology testing. However, it is not obvious how the number of animals and the degree of distress should be weighed against each other, and there is a lack of guidance on prioritizing between the 3Rs when they are in conflict. In other words, would it be better to use fewer animals in order to reduce the distress of the few animals that experience more distress? Here, we first explored the use of cardinal ethical weights for clinical signs and symptoms in rodents by conducting trade-off interviews with members of Swedish Animal Ethics Committees in order to derive such weights for nine typical clinical signs of toxicity (i.e., the number of non-responding animals with an equal weight as one responding animal). The mild examples of signs, such as 30 minutes of decreased motor activity or 5% weight loss during one week, had median ethical weight between 2 and 4 whereas more severe examples of the signs such as 4 hours of decreased motor activity or 10% weight loss during one week had median ethical weights between 5 and 20. However, some interviewees assigned considerably higher ethical weights, as indicated by 75th percentiles for the ethical weights of 15 and 220, respectively. A similar range of ethical weights was then used to examine designs with four dose groups, varying the total number of animals, keeping the ethically weighted dose level constant between the 3 present chemicals. Two sets of quantal dose-response data (with and without background incidence) were generated by Monte-Carlo simulation. The informative value of the simulated data was estimated in terms of accuracy of benchmark dose (BMD) estimates. Our simulations suggest that if animal distress is given an ethical weight, it is preferable, in terms of BMD quality, to use high dose placements but increase the total number of animals. In conclusion, BMD data of higher quality may be obtained without higher ethical cost as long as the top dose shows a clear response rate, as compared to the background incidence. Conversely, a similar BMD quality as with a standard design may be possible to obtain at a lower ethical cost.

We have recently developed the subacute reference doses (saRfDs) of the 19 drinking water quality standards (DWSQs) in Japan) for emerging short-term exposure period. The DWSQs are required to monitor as legally-binding standards by water suppliers in Japan, because most of the DWSQs chemicals are often detected on the level of over 10% of its limit. For each DWSQ, the “Complementary Chemicals”. Additionally, about 50 chemicals are listed as the “Items for Further Study”, because of provisional risk assessment. The exposure levels of these chemicals are usually low, but are still highly concerned in public health. As the next step, we simulated 16 chemicals and two sets of the “Complementary Items” and “Items for Further Study”, and evaluated the toxicological profile of the chemicals for short-term exposure. After estimating the saRfDs, we calculated the health advisory subacute exposure levels of these chemicals in drinking water as one of the risk management tools. In order to estimate the saRfD, in principle, the NOAEL of a one to three-month rodent oral study was used as a Point of Departure (PbD), and a total uncertainty factors of 100 was generally applied to the PbD. The saRfD of about half of 16 chemicals were calculated according in this manner. As for four genotoxic chemicals, 10 times the Virtual Safe Level was defined as a saRfD. However, saRfDs of phthalate esters were evaluated as same as TDIs, because the most sensitive toxicological endpoints of developmental toxicity are independent from the exposure periods. Finally, most of the health advisory values of concentrations in drinking water for sub-acute exposure were determined by the monitoring values for lifetime exposure. We are going to make further evaluations for rest of the listed chemicals. This study was supported by a Health and Labour Sciences Research Grant (H28-Kenki-Ippan-005) from the Ministry of Health, Labour and Welfare, Japan.
We have described a tiered approach that moves from lower tiers focused on rapid decision making and prioritization to Tier 0–1 to higher tiers (Tier 2) that incorporate biologically relevant in vitro models to provide necessary dose-response information for making risk-based chemical safety decisions. Here, we used estrogenic chemicals in a case study for this tiered approach. Using published QSAR models for estrogen receptor (ER) binding (Collaborative Estrogen Receptor Activity Prediction Project; CERAPP) and high-throughput assays for parent chemical activity (ToxCast), 45,000 chemicals can be screened quickly for parent chemical activity and prioritized for further testing in a Tier 2 biologically relevant fit-for-purpose assay. To comprehensively evaluate chemical safety for prioritized chemicals, we used QSAR models of metabolism coupled with the estimates of ER binding to predict compounds likely to be bioactivated by metabolism and to develop a prioritization scheme based on parent and metabolite risk. Potential metabolite structures and bioavailability of 1,677 parent chemicals and related metabolites from the ToxCast library as well as an additional list of 593 purchasable metabolites. We are currently testing the top ranked parent and metabolite compounds using phenotypic cell based assays for the breast and uterus to determine a point of departure for parent and metabolite activity. We will then use IVIVE to determine a region of safety for estrogenic compounds that accounts for both activity and exposure to the parent chemicals and their metabolites.

**2895** Assessment of Risk Associated with Drug Treatment Coupled with Procedural Intervention for Non-Traumatic Neurological Events

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Cerebrovascular disease is a leading cause of death in the United States. Acute neurological events are a result of cerebrovascular disease. Acute neurological events comprise a large array of conditions that include acute ischemic attacks (strokes), transient ischemic attacks, hemorrhages, and un-ruptured aneurysms. Stroke is the leading cause of long-term disability. We estimated the associations between treatment methods and discharge rates and the prevalence of non-traumatic cerebrovascular events using the database from Florida’s Healthcare Administration (FLAHCMA). Drug therapy, commonly tissue plasminogen activator (t-PA), has been the cornerstone of treatment for stroke patients. The advent of new technologies (mechanical thrombectomy) has allowed surgeons to use intraluminal devices to repair damaged vessels and alleviate symptoms resulting from these events. The focus of this study is to assess the risks associated with the treatment based on discharge outcomes of visits to three hospital departments. Discharge outcomes are considered unfavorable when a patient is discharged into hospice care or dies. Patient outcomes for stroke patients regardless of treatment type were determined. Morbidity is common in stroke survivors, intermediate care that requires skilled medical staff is required after hospital discharge. A cross-sectional analysis of FLAHCMA databases explored the risk between treatment method and discharge rates using logistic regression. The step of length for each treatment was compared using the F-test. We identified 2142 cases of treatment with drug and 375 cases of treatment with mechanical thrombectomy (MT) in 2013. There were 41,033 cases of stroke, 45.8% of those who underwent treatment were discharged home while 7.8% died or entered hospice care. Stroke mortality for MT patients was 105 deaths per 100,000 while drug treatment patients was 251 deaths per 100,000. Stroke patients treated with MT were 3 times (OR = 0.32; 95% CI: 0.25-0.40) less likely to die or enter hospice care than drug treated stroke patients. Stroke patients treated with MT were 5 times (OR = 0.20; 95% CI: 0.13-0.29) less likely to require intermediate care than drug treated stroke patients. Although, stroke patients received drug therapy as the primary treatment approximately 6 times more often than MT, the risk of an unfavorable outcome was greater among drug treated patients.
were collected from 5 animals of each species and assayed in 5 replicates as soon as possible after collection (Time 0). Hematopathology analysis was performed using an Advia® 2120 and samples stored at ambient temperature were run at multiple times within the first 12 hours after Time 0, and, following storage at 2-8°C, at 24, 48, and 72 hours after Time 0. Coagulation and clinical chemistry analyses were performed using a Stago STA Coagu巣 and Olympus AU 5800, respectively. Samples for coagulation and clinical chemistry were analyzed at multiple time points following storage at ambient temperature, refrigeration at 2-8°C, and up to 84 days at -80°C with 3 freeze/thaw cycles. Additionally, coagulation samples were analyzed after storage on wet ice. Values from all analyses were compared to Time 0 to calculate the percent difference. Samples were considered to be stable when the mean percent difference was less than or equal to the established Testing Facility acceptance criteria, which was 25% for hematopathology parameters, 20% for coagulation parameters, and between 5% and 30% for clinical chemistry parameters. For hematopathology parameters at low physiologic levels, the absolute difference between the mean Time 0 value and the mean obtained value was evaluated. All clinical pathology parameters at all time points and storage conditions evaluated were stable for rats and NHP. Mean platelet volume in canines was only stable up to 12 hours at ambient temperature. Although changes in clinical pathology parameters were minimal, timely processing and analysis of samples is recommended to ensure integrity of the data and to avoid misinterpretation due to artifactual changes.

2898 Bolstering the Existing Database Supporting the Non-Cancer Threshold of Toxicological Concern Values with Toxicity Data for Fragrance-Related Compounds

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The concept of threshold of toxicological concern (TTC) supports safety assessment of exposure to low levels of chemicals when toxicity data is limited. The TTC concept proposes that de minimis exposure values can be established for chemicals, including those of unknown toxicity, based on comparison with known toxicity of a wide range of chemicals. RIFM delivers safety assessments for fragrance materials that result in safe products for the consumer. The TTC safety assessment is based on exposure information on fragrance materials from oral, inhalation and dermal routes. A major goal of the TTC safety assessment program is to invest in alternative methods to animal testing for use in assessment of fragrance chemicals. The TTC concept provides a pragmatic approach for safety evaluation of fragrance materials of known chemical structure in the absence of chemical-specific toxicity data. The RIFM database provides the largest available inventory of fragrance and flavor materials classifying more than 6000 materials of which there are over 2600 discrepancy fragrance materials. The TTC database was used to identify TCT chemicals with at least a single dose, reproducibility or developmental toxicity studies. This database was refined to identify over 540 chemicals (TCT-TTC dataset) that are currently used in the fragrance industry to develop fragrance related non-cancer thresholds (i.e., NOAELs). These fragrance chemicals were individually assigned a Cramer Class via expert judgment and a threshold concentration derived from the organic functional group profiler from OECD toolbox v3.4. The chemical space comparisons were made with the Munro-TTC dataset. The toxicity studies associated with each chemical were then carefully evaluated to identify a NOAEL/NOAEL associated with associated chemical specific toxicity information. This information was used to generate a TTC-TTC dataset that is referenced with several toxicity databases, including some that provide robust safety/risk assessments (FEMA, EPA, OECD, EFSFA, SCCS, JECFA, CPDB among others). The ultimate goal is to enhance the existing TTC database with risk values for fragrance chemicals, similar to the work done on Carcinogenicity chemicals (COSMOS: Yang et al., 2017). This work will provide further support for the use of TTC as a tool to conduct safety assessments for fragrance chemicals.

2899 Toxic Load Exponents for Select Priority Chemicals on the ERPG List


Estimates of health outcomes resulting from inhalation exposures to hazardous chemicals (HCs) are needed for emergency response and preparedness. For short-term exposures, the National Academy of Sciences (NAS) recommends that estimates be derived from the toxic load equation, C = n * T * L. It states that both the exposure concentration, C, and duration, T, contribute to a toxic load, TL, that causes an equal level of injury at different levels of a steady-state concentration and duration. The empirical parameter n, the toxic load exponent (TLE), is chemical-specific. For each HC and health effect, a TLE can be extracted from appropriate in vivo toxicological studies. In the present work, TLEs for select HCs reviewed by the American Industrial Hygiene Association (AIHA) were derived. These HCs have publicly available health guidance values (HGVs) for only one duration but sufficient information for bivariate probit modeling to derive TLE estimates that make extrapolation to other durations possible. TLEs and their 95% confidence intervals were derived using animal mortality data from AIHA-cited studies for the following chemicals: benzyl chloride, 1.55 (0.29-2.81); hexachlorobutadiene, 1.16 (0.57-1.75); hexafluoracetone, 0.79 (0.52-1.05); hydrogen peroxide, 0.77 (0.69-0.85); 2-isocyanoethyl methacrylate, 0.97 (0.84-1.11); and 1-octene, 2.21 (1.46-2.96). Cobalt hydrocarbonyl, dimethyl disulfide, n-butyl acetate, tetraethoxysilane, and toluene disocyanate were also considered for probit modeling; however, data presented in the AIHA-cited documents were insufficient for derivation of a statistically significant model for these HCs. These chemical-specific TLEs (aka n-values) can be considered for extrapolation of HGVs available for the aforementioned HCs to other short-term durations instead of general-use default n-values recommended by NAS in the absence of empirical TLEs. Disclosure: The findings and conclusions in this presentation have not been formally disseminated by CDC/ATSDR and should not be construed to represent any agency determination or policy.

2900 The Use of Standard Toxicology Data to Substantiate the Safety of Feed Additives

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A core component to regulatory approval of any new feed additive is that it is safe and suitable for the intended feed use. The typical process to establish safety of a feed additive is based on studies in the specific species of interest; however, target species studies remain a significant impediment to the approval of new feed additives given the time and resources invested to conduct these studies. The extent that toxicity data from common laboratory species may be useful in extrapolating to safety in target species has been considered. The purpose of the current work was to evaluate whether standard toxicology studies are a good alternative to target species studies for establishing a safe use level for feed additives and identify any methodological limitations. The initial proof-of-concept study was conducted using examples of simple chemical compounds (potassium diformate, propionic acid, fumaric acid and formic acid) that have been approved as feed additives in the EU based on target species studies. Relevant toxicology data were systematically identified for these compounds within the best available and appropriate uncertainty factors and species-specific risk factors were applied to determine whether there were any differences in the safe use concentrations on the basis of laboratory animal data relative to that determined from target animal studies. In all additives examined, while the safe use level derived from laboratory species data were within the levels of use that are currently approved, the observed trend was that levels were at least an order of magnitude below the presently established use level derived on the basis of target species studies. The safe use concentration derived from standard toxicology studies uses safety factors which give an adequate safety margin for target species and may be a useful tool in the preliminary assessment of feed additives. The differences between laboratory and target animal species is likely related to practical limitations regarding feed additive levels which can be included in laboratory species diets. In practice, these could limit the utility of certain feed additives in target species and as such target species studies will continue to be the animal model of choice despite the improved margin of safety afforded by the derived safe use concentration. Further investigations should be conducted to determine whether this process can be extended to more complex feed additives.

2901 Decision Tree Approach for Cosmetic Ingredient Safety Evaluation with Botanical Biofunctionalss: Case Study with Baobab Seed Extract Rich in Plant Small RNAs

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Today, there is a growing consumer demand for cosmetics containing sustainable botanical ingredients, with a wide range of biofunctional activities that contribute to skin wellness. However, there is a dearth of information on approaches in addressing the consumer safety of these ingredients. To address the concerns with assessing botanical ingredients in personal care products, Ashland has developed a decision tree approach to regulatory approval of any new feed additive is that it is safe and suitable for the intended feed use. The typical process to establish safety of a feed additive is based on studies in the specific species of interest; however, target species studies remain a significant impediment to the approval of new feed additives given the time and resources invested to conduct these studies. The extent that toxicity data from common laboratory species may be useful in extrapolating to safety in target species has been considered. The purpose of the current work was to evaluate whether standard toxicology studies are a good alternative to target species studies for establishing a safe use level for feed additives and identify any methodological limitations. The initial proof-of-concept study was conducted using examples of simple chemical compounds (potassium diformate, propionic acid, fumaric acid and formic acid) that have been approved as feed additives in the EU based on target species studies. Relevant toxicology data were systematically identified for these compounds within the best available and appropriate uncertainty factors and species-specific risk factors were applied to determine whether there were any differences in the safe use concentrations on the basis of laboratory animal data relative to that determined from target animal studies. In all additives examined, while the safe use level derived from laboratory species data were within the levels of use that are currently approved, the observed trend was that levels were at least an order of magnitude below the presently established use level derived on the basis of target species studies. The safe use concentration derived from standard toxicology studies uses safety factors which give an adequate safety margin for target species and may be a useful tool in the preliminary assessment of feed additives. The differences between laboratory and target animal species is likely related to practical limitations regarding feed additive levels which can be included in laboratory species diets. In practice, these could limit the utility of certain feed additives in target species and as such target species studies will continue to be the animal model of choice despite the improved margin of safety afforded by the derived safe use concentration. Further investigations should be conducted to determine whether this process can be extended to more complex feed additives.

2902 Decision Tree Approach for Cosmetic Ingredient Safety Evaluation with Botanical Biofunctionalss: Case Study with Baobab Seed Extract Rich in Plant Small RNAs

S. Kim1, E. Oger1, E. Bauza2, K. Gondran2, K. Cucumel2, C. Choi3, and A. Schatz2. 1Ashland LLC, Lincoln, CA; 2Ashland Global Skin Research Center, Sophia-Antipolis, France; and 3Ashland LLC, Bridgewater, NJ.

Today, there is a growing consumer demand for cosmetics containing sustainable botanical ingredients, with a wide range of biofunctional activities that contribute to skin wellness. However, there is a dearth of information on approaches in addressing the consumer safety of these ingredients. To address the concerns with assessing botanical ingredients in personal care products, Ashland has developed a decision tree...
approach to conduct a safety evaluation and used a Baobab seed extract (CASRN 91745-12-9) as a case study. The decision tree involves the following aspects: (1) botanical source identification and biological activities assessment, (2) intended use application and usage concentration assessment, (3) literature-based demonstrated safety or toxicological review, (4) potential safety concern identification, analysis, and mitigation, and (5) confirmatory safety testing. A Baobab seed extract from *Adansonia digitata* species was obtained by valorizing oil by-product to retain the richness of cold pressing seeds and particularly to preserve small RNAs. Rich in small RNAs, this Baobab seed extract has been shown to have positive effects on microRNA maturation machinery dysregulation that may limit the appearance of skin aging and the presence of enzymes associated with skin hydration level, based on in vitro assays with fibroblast cells. Baobab seed extracts are not known to contain chemicals of toxicological concern. In *in vitro* dermal/eye irritation studies, the Baobab seed extract was not irritating (Reconstituted Human Epidermis, 24 hour contact). The oral dermal and Epithelium of Hen’s Eye Test-Chorioallantoic Membrane, Neutral Red Release Uptake Assays). It was also non-mutagenic (Ames assay) and non-phototoxic (3T3 Neutral Red Uptake Phototoxicity assay). In clinical studies, the Baobab seed extract was not irritating (48-Hr Human Patch Testing, N = 10) or a sensitizer (Human Repeat Insult Patch Testing, N >200). Our scientific and systematic decision tree approach, as exemplified with this Baobab seed extract, provides a sufficient margin for consumer safety while minimizing the need for extensive physicochemical and toxicological characterization of botanical biofunctional ingredients intended to be used in cosmetics.

**2902 An Approach for Evaluating the Skin Sensitization of Cosmetic Ingredients Using Non-Animal Safety Assessment Methods**


Development of non-animal safety evaluation method for cosmetic ingredients is essential from the viewpoint of animal welfare and to meet the 7th amendment of the European cosmetics directive. Recently, many non-animal skin sensitization evaluation models are developed based on *in vitro* assays and *in silico* approaches (quantitative structure-activity relationship (QSAR) and Read-across). In this study, we developed a skin sensitization evaluation system based on the *in vitro* or *in silico* quantitative risk assessment models and read-across approach. Using an artificial neural network (ANN) based on several *in vitro* assays (h-CLAT, DPRA and Keratinosens™) and *in silico* QSAR approach (using MOPAC2002) to predict stimulation index of 3 (EC3) of the local lymph node assay (LLNA) followed by Read-across approach. *In vitro* or *in silico* artificial neural network (ANN) models predicting the EC3 value of the local lymph node assay (LLNA) were constructed with a dataset (a training dataset) of 134 and 206 general compounds respectively. Read-across approach was composited by structure, chemical structure, physical properties and toxicological concerns (ex. protein binding, structural alert, Cramer classification) using OECD QSAR Toolbox and DEREK. The correlation coefficient of *in vitro* and *in silico* ANN prediction models were 0.89 (RRM error = 0.51) and 0.73 (RRM error = 0.64), respectively. If predicted EC3 value of each chemical is decided by adopting a lower value of both models. The differences between actual and predicted EC3 values were less than 10 times in approximately 80% of the chemicals. To avoid misjudgment of strong and extremely sensitive, chemicals which evaluated strong or extremely sensitive by read-across approach are imposed safety margin to the predicted EC3 value. We concluded that this integrated evaluation system was useful for evaluating cosmetic ingredients in terms of risk assessment of skin sensitization. Reference: Hirata et al., SOT, PS 2735, 2017.

**2903 Derivation of Maximum Allowable Dose Levels for Bisphenol A**

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In 2015, California’s Office of Environmental Health Hazard Assessment (OEHHA) added Bisphenol A (BPA) to the State’s Proposition 65 list of chemicals “known to cause reproductive toxicity.” OEHHA based its decision on the conclusion of its Developmental and Reproductive Toxicant Identification Committee (DART-IC) that BPA has been shown to cause female reproductive toxicity. A critical factor in determining compliance with Proposition 65 is evaluating whether the reasonably anticipated use of a product could result in an exposure above a Maximum Allowable Dose Level (MADL). However, OEHHA has not derived oral BPA MADL. Thus, we independently derived an oral BPA MADL, based on many factors, including study selection and metabolism considerations. More specifically, the oral BPA MADL is based on Delclos et al. (2014), a 90-day subchronic study that evaluated the effects of BPA exposure on rat dam and their offspring, which was identified as the most appropriate study among the many peer-reviewed studies available. It is of sufficient quality, has the lowest no observed effect level (NOEL), and results in the most conservative oral BPA MADL of 157 mg/day. This oral BPA MADL is generally supported by other studies, including those that are considered by DART-IC in its evaluation of BPA toxicity. Also, the oral MADL for BPA provides a similar margin of safety as OOHHAA’s dermal MADL and other BPA regulatory guidelines.

**2904 Integration of 2D and 3D In Vitro Cytotoxicity and In Silico Models for Predictive Toxicology**

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The development of new crop protection products (i.e. pesticides) is a complex process that requires large expenditures in time and cost. Embracing high throughput predictive models for assessment of potential hazards of a database early in the development phase can aid in analog selection with the ultimate goal of generating studies with a more favorable human health profile. We assessed the predictive utility of two cytotoxicity endpoints by comparing *in vitro* IC50 values to reported rat 90-day *in vivo* NOAEL and LOAELs. Human hepatocellular carcinoma HepG2 cells (20 μM) were exposed to a set of 23 pesticides for 24 hours at 6 different concentrations, and cytotoxicity was assessed by calculating IC50 values for mitochondrial membrane potential (MMP) and adenosine triphosphate (ATP) content. Using a linear mixed-effects model accounting for a grouping structure of pesticidal use (fungicide, insecticide or herbicide), as random effect increased prediction accuracy comparing to a simple regression model, *2D in vitro* cytotoxicity and *in vivo* NOAEL and LOAELs were correlated (e.g. the predictive relationship of *in vitro* 2D MMP IC50 to *in vivo* LOAELs was r = 0.407). To determine if *3D* cell culture conditions increased predictivity, 21-day HepG2 3D spheroids were exposed to the same set of pesticides for 24 hours for assessment of ATP content. The predictive relationship for *in vitro* ATP IC50 and *in vivo* LOAEL was strengthened with 3D compared to 2D cultures (r = 0.528 and r = 0.348, respectively) but was similar for predicting the NOAEL. Given that molecules can have varying absorption and bioavailability profiles, which can impact *in vivo* points of departure but are accounted for in *in silico* approaches, we in-house developed a GastroPlus to estimate the plasma Cmax of a molecule at the 90-day LOAEL applied dose and compared these Cmax values to *in vitro* cytotoxicity IC50 values. Utilizing the Cmax at the LOAEL increased the predictive relationship of *2D* ATP IC50 (r = 0.54) and *2D* MMP IC50 (r = 0.534) compared to NOAEL based upon *in vitro* IC50 (r = 0.448 and 0.348, respectively) and *2D* ATP (r = 0.407 (2D MMP)), but remained similar for ATP in 3D spheroid culture (r = 0.522 (3D) and 0.528 (2D)). These data demonstrate that harnessing in *vitro* cytotoxicity and *in silico* BPBK models can predict in *in vivo* points of departure, which can aid in new molecule prioritization decisions.

**2905 An Interdisciplinary Approach to Setting Science-Based Occupational Exposure Levels (OELs)**

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OELs are generally established to assure adequate protection of workers in the workplace. Typically, such values are expressed as an 8-hr time weighted average (TWA), Short-term Exposure Limit (STEL) or a ceiling value. While there are numerous OEL setting bodies, there is no common approach. At ExxonMobil, we extensively make use of the AGICH TLVs and the OARS WEELs. In cases where an exposure limit is needed and there is no TLV or WEEL, or if the scientific derivation of those limits does not appear to be aligned with current scientific evidence, an internal OEL may be established. ExxonMobil has been setting internal science-based OELs for several decades, and under the formal guidance of a senior science, staffed OEL Committee since the late 1970s. A number of learnings about the process are shared here. OEL-
setting benefits from an interdisciplinary approach. The available health data come from across the fields of epidemiology and/or toxicology, and always exposure. EM OEL teams always include scientists from all of these disciplines. Value is enhanced through development of OEL recommendations by working level scientists, followed by expert review by an interdisciplinary group of senior scientists. In this way, balance is achieved between novel approaches and evolving technology with historical perspective and broad experience. Well-articulated rationale for applying assessment factors improves transparency and consistency across time and between periodic OEL reviews. Integrating information across all evidence streams informs assessment factor selection rationale. Judgment remains critical to address either database insufficiency or unaccounted uncertainty. Consideration should be given to establishing OELs as STEL or ceiling values when the primary or initial occupational hazard is upper respiratory irritation. Special notations, e.g., for skin absorption, sensitizers, cancer potential, etc are informative to Occupational health staff. Regarding notation of potential skin absorption, skin penetration modeling, e.g, with SkinPerm, informs decision making. For complex mixtures, e.g., hydrocarbons, the Reciprocal Calculation Procedure, which is addressed in the TLV booklet, is a useful approach.OELs are not particularly useful without appropriately sensitive methods for measurement; at EM, the analytical methods are updated or developed as needed. Science is dynamic; hence, OEL re-reviews on a regular basis are recommended.

2906 An Exposure-Led Safety Assessment Framework for Systemic Toxicity: Initial Case Studies from Cosmetics Europe

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A battery of toxicological studies was conducted on a proprietary hemp oil (ca. 25% cannabinoids) extracted from the aerial parts of the Cannabis sativa plant. No evidence of genotoxicity was found in a bacterial reverse mutation test (Ames), an in vitro mammalian chromosomal aberration test, and an in vivo mutagenicity/carcinogenicity study. Based on the results of a 14-day repeated dose range finding study, a 90-day repeated dose oral toxicity study was conducted in rats using doses of 100, 360 and 720 mg/kg bw/day, followed by a 28-day recovery period for two satellite groups (control and high-dose). No deaths occurred. Significantly decreased body weight and body weight gain were noted in males in the 360 mg/kg bw/day group and in both sexes in the 720 mg/kg bw/day group. Various significant differences in organ weights compared to controls were found in all groups; however, only elevated liver (360 and 720 mg/kg bw/day for males and females) and adrenal mass weights (720 mg/kg bw/day males and females) were considered to be test article related. There were no histopathological variations in the livers of these animals and related histopathological findings in the adrenals were only noted in the 720 mg/kg bw/day group. In addition, at the end of the recovery period, both male and female high-dose sat-ellite groups’ organ weight results were trending toward normal; thus, the changes appeared reversible. No toxicologically relevant test item related changes were observed in animals administered 100 mg/kg bw/day. The NOAEL for this hemp oil in Hsd. Han: Wistar rats was considered to be 100 mg/kg bw/day for males and 360 mg/kg bw/day for females.

2908 Potential Utility of Juvenile Animal Study Protocols for Infant Food-Contact Safety Assessment

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The US FDA Center for Food Safety and Applied Nutrition (CFSAN), Division of Food Contact Notifications (DFCN) performs infant lifestyle-specific safety assessments for food contact materials intended to contact human milk or infant formula. DFCN reviews developmental and reproductive toxicity (DART) studies conducted in laboratory animals to identify potential hazards for the infant population. DFCN examined the potential utility of the juvenile animal study (JAS) protocol for use in the OFAS infant food contact safety paradigm. We previously shared the study characteristics and common outcomes in our JAS library and factors that enhanced JAS dose selection (Neal-Kluever et al., SOT 2017; Wu et al., SOT 2017). In the present analysis, we evaluated how the JAS compared to the sub-chronic (SC) toxicity protocol as well as other DART studies that measured postnatal toxicity (PN: generational DART protocol and pre- and post-natal toxicity protocol). For each study type, we harvested metrics associated with protocol design, endpoints measured, and dosage prepar-ation (POD), test-article related effects, toxicokinetics, and affected biological systems. Our database had 11 drugs with concordant SC and PN studies for comparison. We observed that 1) the JAS and PN studies performed similarly by providing the lowest LOAEL in 4/11 drugs (36%) and 2) PN studies identified more drugs (48%) compared to PN studies (22%), or similar internal exposure (3/9 drugs, 33%) compared to adult rats, as measured by blood AUC or Cmax; 3) the most common toxicological effects in the JAS were target organ and behavioral in SC studies and target organs and behavioral in PN studies; 4) the most common effects were reproductive effects and body weight, and 4) the PN and SC studies identified potential hazards for developing systems that were largely confirmed in the JAS. The JAS provided unique results in 7/9 drugs (78%) and was more sensitive than the PN and SC studies in 2/9 drugs (67%). The SC and PN studies predicted similar toxicity as the JAS in 7/9 drugs (78%) and 4/9 drugs (44%), respectively. In the DFCN paradigm, our analyses suggest that the PN and SC studies may be helpful to design a targeted JAS in cases with an outstanding question regarding infant safety.
2910 Evaluation of FXR-Active Chemicals Identified from Tox21 Screening

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Nuclear receptors play a key role in physiological functions. Assessing how chemicals interact with this superfamily of proteins can provide mechanistic data that supports the construction of toxicity pathways related to human disease. Farnesoid X receptor alpha (FXRa, NR1H4) is a member of the nuclear receptor superfamily with demonstrated importance in bile acid homeostasis, glucose metabolism, lipid homeostasis, and immune system generation. In this study, we screened two sets of compounds previously identified in Tox21 qHTS in vitro screens as FXRa agonists and antagonists using four experimental approaches. Transactivation studies were conducted to validate potency and efficacy of putative FXRa agonists and antagonists using four experimental approaches. Transactivation studies were conducted to validate potency and efficacy of putative FXRa agonists and antagonists using four experimental approaches. Transactivation studies were conducted to validate potency and efficacy of putative FXRa agonists and antagonists using four experimental approaches. Transactivation studies were conducted to validate potency and efficacy of putative FXRa agonists and antagonists using four experimental approaches. Transactivation studies were conducted to validate potency and efficacy of putative FXRa agonists and antagonists using four experimental approaches.

2911 Chemical Stability of a Cleansing Conditioner Product under High-Heat Conditions Experienced during Consumer Use


Chemical stability is a key component of ensuring that a cosmetic personal care product is safe for consumer use. Current industry guidance for stability testing of products considers whether foreseen transport, storage or handling impacts the integrity of the product. Typical testing conditions include evaluations of thermal stability at temperatures up to 40 °C for several months. However, such testing protocols may not reflect specific stress conditions experienced during the actual use of the product. For example, hair care products may be exposed to high-heat stresses during the styling process, including blow-drying and hair straightening. In this study, a hair cleansing conditioner product was subjected to temperatures of 60 and 185 °C to simulate the use of a hair dryer and flatiron hair straightener, respectively. Matched heated and unheated samples were analyzed via GC-MS HPLC, and FT-IR to capture a widespread profile of the product which included volatile and non-volatile organic and non-organic chemicals. The resulting spectra from matched heated and unheated product samples were analyzed to identify any changes in chemical composition or generation of degradation product(s). Together, no differences in the spectra were observed between the heated and unheated samples. Individual chemicals identified during GC-MS were all known ingredients of the cleansing conditioning changes. In addition, no alteration of the active preservative ingredients, methylisothiazolone and methylchloroisothiazolone, upon heating. Therefore, no changes in chemical composition are expected to occur under high-heat conditions associated with the use of the product. Therefore, the potential health risk of this product would not be impacted by heat modulation during use.

2912 Four-Week Continuous Nicotine Treatment of Immortalized Bronchial Epithelial Cells Does Not Contribute to Tumorigenesis

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Cigarette smoking is one of the major risk factors for the development of lung cancer, but relatively little is known about the effects of nicotine, a major constituent of cigarette smoke, on lung epithelial cells in the context of lung tumorigenesis. In order to investigate whether nicotine elicits differential effects on mechanisms promoting or leading to carcinogenesis, immortalized non-tumorigenic BEAS-2B and tumorigenic (BZR) human bronchial epithelial cells were continuously exposed to low, medium and high concentrations of nicotine (10, 100 and 1000 nM) for 4 weeks. Proliferation, apoptosis and expression of metalloprotease-1, -3 and -9 were evaluated using western blot and qRT-PCR and compared to untreated control cells. In addition, tumorigenicity of the cell lines was assessed using the xCELLigence platform, high-content imaging and Lumexin technology. Gene expression analysis was performed on nicotine-treated and untreated cells collected weekly during microarray experiments with a computational network approach. In summary, nicotine treatment did not increase the proliferation of immortalized BEAS-2B or BZR cells as determined by cell counting. Real-time impedance data indicate a small, but transient pro-proliferative effect on BEAS-2B cells at week 3. Nicotine treatment had no impact on stauroporine-induced apoptosis of BEAS-2B and BZR cells. Furthermore, nicotine treatment did not increase the levels and activity of metalloproteases. Systems toxicological analysis indicated a small, but non-consistent impact when treating BEAS-2B cells with nicotine, while BZR cells seemed to be more responsive to nicotine than BEAS-2B cells. Finally, anchorage-independent growth was not observed in BEAS-2B or BZR cells when treated for four weeks with nicotine. Chronic nicotine treatment of immortalized non-tumorigenic BEAS-2B and tumorigenic BZR cells did not promote cell proliferation, suppress apoptosis or initiate any mechanisms that favor tumorigenesis. Longer exposures might be necessary to elucidate the contribution of nicotine to cancer promotion and progression.

2913 Microsampling Method That Does Not Cause Anemia or Hematopoiesis in Rats


Microsampling improves animal welfare (refinement and reduction) and enables to evaluate the relationship between safety profile and drug exposure in the same animal. Many facilities have reported the relationship between microsampling and anemia, but that between microsampling and hematopoiesis caused by anemia has little been evaluated. We examined anemia- and hematopoiesis-related changes at endpoints of 1, 3, and 7 days after blood sampling by clinical pathology and histopathology. Seven-day oral dosing with microsampling was also conducted to mimic a 1-week screening toxicity study with toxicokinetics. Six-week-old female Crl:CD(SD) rats (5 animals/group) were used, and microsampling was conducted via the jugular vein without anesthesia. Experiment 1: Blood was collected by serial sampling (6 × 0.05 mL, 0.10, and 0.15 mL of blood; approximately 3, 6, and 9% of the circulating blood volume, respectively. Sampling points were 0, 1, 2, 4, 8, 24 h) on Day 1, and the animals were necropsied on Day 2 or Day 4. Untreated control and sham groups (restraint and needle puncture) were also set. Experiment 1: Animals received 10 mL/kg of water orally, once daily for 7 days. Animals underwent serial microsampling (6 × 0.05 mL at 0.5, 1, 2, 4, 8, 24 h on Day 1 and 7 × 0.05 mL on Day 7). Untreated control and sham groups were also set. Experiment 1: The 0.05 mL sampling at 6 points did not affect results in any examination. Anemia and hematopoiesis...
and extramedullary hematopoiesis in the spleen and liver on Day 4. The thymic hemorrhage. Microsampling with 6% and 9% of the circulating blood volume caused anemia and/or hematopoiesis, but 3% did not. Microsampling within 3% of circulating blood volume enabled toxicity to be appropriately evaluated.

**2914 Withdrawn by Author**

**2915 Marijuana-Induced Hepatic and Renal Mitochondrial Lipotoxic and Non-Lipotoxic Dyslipidemia in Female Albino Rats**

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Intake of marijuana has been associated with debilitating outcomes. Various reports have appeared in the literature suggesting a role for mitochondrial dysfunction in the pathophysiology of several diseases. To investigate the effects of marijuana on mitochondrial function, female albino rats (n=36) were exposed orally to marijuana (25 and 50 mg/kg body weight) (extracted with petroleum ether and suspended in olive oil) for 2, 4 and 6 weeks. Control animals (n=18) received the vehicle for the same period. Mitochondria were isolated from the kidney and liver of the animals and analysed for their lipid profile. Both lipotoxicity and non-lipotoxicity characterised the effects of marijuana on renal and hepatic mitochondria. Induction of both cholestero genesis and phospholipidosis in hepatic and renal mitochondria at the highest dose characterised the lipotoxic effects of marijuana at the end of 6 weeks. Comparatively, while cholestero genesis was more pronounced in the kidney, phospholipidosis was more pronounced in the liver. Triglyceride constipation was the hallmark of the highest dose of marijuana exposure at the end of 4 weeks in both renal and hepatic mitochondria. Reduced hepatic and renal mitochondrial triglyceride (2 weeks), as well as a dose-dependent reduction of renal mitochondrial cholesterol (2 weeks), were the hallmarks of non-lipotoxicity of marijuana. These findings suggest that marijuana-induced mitochondrial dysfunction characterised by dyslipidemia might mediate its disease endpoints.

**2916 Challenges in Assessing Health Risk from Exposure to Bisphenol A (BPA) in Consumer Products**

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BPA is regulated differently by various national and international agencies due to discrepancies in the characterization of health risks posed by BPA. For example, current "safe harbor level" estimates for BPA from different sources range from 3 to 290 µg/day, nearly a 100-fold dose range. The recent State of California Proposition 65 listing of BPA was accompanied by a yet-to-dermal route-specific maximum allowable dose level, but not yet a new challenge for assessing health risk for BPA-containing consumer products. This challenge is further compounded by the absence of well-accepted methods for estimating BPA transfer from products to skin or from packaging to foods. This presentation describes our experience in assessing BPA transfer from a variety of consumer products and food for estimating potential BPA dermal and oral exposures. Most of our dermal transfer testing to date is based on either wiping the product with a cotton pad or rolling the product, indicating minimal potential dermal exposure. Similarly, model evaluations of several paper packaging and food combinations, including long-term storage and heated preparation scenarios, produced BPA daily dose estimates from packaged food consumption substantially less than 0.1 µg/day. We will present our understanding of the relationship between BPA transfer and the characteristics of material, BPA content, time of contact, receptor media and environmental variables, and discuss uncertainties in current BPA health risk assessments of consumer products and their effects on evaluating product compliance with health-based criteria.

**2917 Tier-Based Safety Testing of Common Personal Care Products and Their Constituents**

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The Food and Drug Administration (FDA) does not require testing of personal care products or their individual constituents before the products enter the market. Various trade associations such as the Scientific Committee on Consumer Safety and the Personal Care Product Council recommend tier-based assessments to evaluate the safety of personal care products and their individual constituents. The goal of this study is to evaluate the dermal irritation and sensitation potential of several commercially available hair care products using the methodology recommended by personal care product and consumer product trade associations. The first tier of testing utilized the OECD Toolbox to execute an in silico evaluation of the 30 identified ingredients in the hair care products for potential dermal irritation and sensitation potential of each of the identified constituents was evaluated using this tool. Subsequently, two OECD in vitro guideline tests were executed to evaluate the dermal irritation and sensitation potential of the commercially available hair care products. The OECD 439, EpiDerm Skin Irritation Test (SIT), utilizes a reconstructed human epidermis (RHE) to evaluate the irritation potential of a test article. The OECD 442C, Direct Peptide Reactivity Assay (DPRA), utilizes high-performance liquid chromatography (HPLC) to evaluate test article-peptide reactivity to evaluate the sensitation potential of a test article. The results from tier one in silico testing showed that 6 of the 30 ingredients for dermal irritation and sensitation testing included: behentrimonium methosulfate, dicetyldimonium chloride, methylchloroisothiazolinone, methylisothiazolinone, panthenol, and stearamidopropyl dimethylamine. However, tier two in vitro testing showed that all of the commercially available hair care products tested were non-irritants and non-sensitizers. The results of this study demonstrate that although several constituents had structural alerts from in silico testing, subsequent in vitro testing showed that the products of interest were non-irritants and non-sensitizers. Therefore, the presence of potential irritants or sensitizers alone does not determine the safety of personal care products.

**2918 Does Raising E-Liquid pH Increase Saliva Nicotine Absorption?**

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Recent journal articles have suggested that unprotonated nicotine in e-liquids, when vaporized, can partition between the gas-vapor phase (GVP) and the particulate phase (PP) of the aerosol, potentially increasing the addictiveness of use of e-vapor products. However, the research reported to date has used toluene-extracted nicotine from water-diluted e-liquids or PP of the aerosol to estimate the fraction of nicotine that is not protonated. Consequently, a series of experiments were set up containing artificial saliva. Experimental e-liquids were formulated using commercial 50 mg/mL nicotine in propylene glycol and additions of propionic acid to make it acidic and ammonium carbonate to make it basic. The amounts of propionic acid (PA) and ammonium carbonate (AC) added, respectively, were equimolar with the theoretical amount of nicotine (3.08 millimoles per 10 mL).

Two samples of each formulation were prepared. Apparent pH-values (Hanna 1083B microelectrode with IQ150 pH meter) were PA1 6.61, PA2 6.77, AC1 9.28, and AC2 9.29. For comparison, the apparent pH-values for major-brand red 2.4 was 7.03; major-brand green 2.4 was 8.49; and 50 mg/mL nicotine was 9.19. The term apparent pH-value is used here as samples were not diluted with water, and no water was added to the PA and AC formulations. The e-liquids (about 500 mg) were loaded into 2 blank cartomizers and allowed to age at RT for at least 24 hours prior to use. Each cartomizer was affixed to a V2 battery, and the device was inserted into the mouth end of a glassmouth using a latex tubing sleeve to seal the connection. The glassmouth was filled with artificial saliva (10 mL), a HI-1053 pH electrode inserted in the aerosol sampling port and
the device puffed using a 55/3/30 sequence for 50 puffs. The saliva was removed and nicotine content determined by HPLC using a C18 column and UV detection at 259 nm. Although the final pH-values of the saliva ranged from 6.6 to 8.6, the nicotine content of those saliva samples did not change by more than 4%. These findings do not support partitioning of nicotine between the GVP and PP.

2919 The Future of Agrochemical Risk Assessment: Establishing a Platform for Early Estimation of Chronic Dietary Exposure for Agrochemicals

Z. Zhang, P. Geurs, and Z. Yan. Dow AgroSciences, Indianapolis, IN.

Human health assessment of agrochemicals requires a comprehensive battery of toxicological animal studies as well as estimations of human exposure, culminating in risk assessment(s). Among the necessary exposure data, chronic dietary exposures are primarily based on results from extensive residue trials on multiple crops. Given the potentially multi-year study durations and significant number of studies, adequate information for assessments is not usually available until late stages of new agrochemical Research & Development. Thus, it is important to provide a tool that could minimize the residue trials needed to provide exposure estimations to inform safety and enable early decision-making. This study used Clopyralid as a case study to demonstrate a platform for chronic exposure prediction with minimal field residue trials. In the first step, residue predictions were generated using DynamicCROP Model. Available Good Agricultural Practices (GAPs) used for 67 crops were collected. Calculated chemical-specific DT50s from decline trials were used for representative and closely-related crops. Residue predictions were made for different portions of crops based on different application methods, application rates and pre-harvest intervals. The summation of different portions was calculated for each crop. The predicted worst-case scenario (highest value) for each crop was compared with actual data from appropriate field residue trials. Using the default model, the predicted and observed residues were similar for stem, foliar and root crops with correlations of 0.64 and 0.53, 0.32 and 0.28, respectively. However, when a meta-modeling approach was incorporated as a remedy for overcoming model limitations (e.g. underestimation of stem and root), substantially improved predictive performance (R² = 0.79-0.94) for all crop portions was achieved. The second step used the predicted residues from step 1 as inputs for Dietary Exposure Evaluation Model (DEEM) for chronic exposure estimations. The average estimated exposure using predicted residues was within 5% relative to that from measured residues for all subpopulations. In summary, the established platform could significantly improve residue prediction for multiple crops and provide a basis for human exposure-based toxicity testing and early decision making for novel agrochemicals.

Good Agricultural Practices, physicochemical-chemical properties, and crop DT50s from limited field residue trials, residue levels of all crops for a specific agrochemical can be predicted by the modified DynamicCROP model, which can then be used for estimating chronic exposures for different subpopulations. Finally, chronic risk characterization can be performed based on results from step 1 and 2, as well as other relevant toxicological animal studies, such as those from developmental and subchronic studies. Overall, this 3R-focused framework provides a practical tool for conducting chronic risk assessment using data from enhanced subchronic studies and limited residue data to enable agrochemical product development and, potentially, regulatory decision-making.

2920 The Future of Agrochemical Risk Assessment: Framework for Conducting Chronic Risk Assessment with Enhanced Short-Term Toxicological Studies and Significantly Reduced Residue Trial Studies

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Human health assessment of agrochemicals requires a comprehensive battery of toxicological animal studies as well as estimations of human exposure, culminating in risk assessment(s). Within the required animal studies, chronic rat and mouse studies and potentially multi-year residue studies are amongst the most time- and resource-intensive. Given the significant animal use, an increased body of evidence indicating many rodent tumors are not relevant to humans, and advances in computational tools, it is important to consider alternative approaches for conducting chronic human health assessment. A framework has been developed for conducting a chronic risk assessment using data from enhanced short-term toxicological studies and significantly reduced residue trial levels. In this framework, four steps of the risk assessment paradigm were addressed. First, for hazard identification and dose-response assessment, data-driven retrospective analyses that investigated correlations between rat short-term and long-term critical toxicological points of departure (PODs) at the levels of apical effects (for 143 agrochemicals) and transcriptional effects (for 7 phenoxyacetates) were conducted. These results demonstrated that chronic PODs can be reasonably estimated using apical and transcriptome data from subchronic studies by applying an extrapolation factor of 2-10. Second, for exposure assessment, a platform was established by integrating a modified DynamICROP and DEEM models. Using agrochemical-specific computational tools, it is important to consider alternative approaches to early decision making for novel agrochemicals.

2921 The Future of Agrochemical Risk Assessment: Benefits, Challenges, and the Evolution of Integrated Endpoints for the Rat 90-Day Toxicity Study

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The basis of human health assessment of agrochemicals is hazard identification via regulatory guideline toxicity studies. Traditionally, endpoints such as immunotoxicity, genotoxicity, neurotoxicity, toxicokinetics (TK), Mode of Action (MoA) and toxicogenomics (TxG) were evaluated in separate studies. While these endpoints are critical to establish toxicity endpoints and human safety margins, separate endpoints for an adverse effect are animal-, labor-, time- and cost-intensive. There has been considerable effort to adapt testing strategies incorporating 3R principles of animal welfare, and integration of testing endpoints in a single study design. An integrated 90-day study, using 120 rats, reduces total animal use by more than 70% compared to 440 animals used in separate studies. Here we discuss the evolution of integrated testing strategies for rat 28- and 90-day dietary toxicity studies for the herbicides, haloxfen-methyl and florpyptrauxfen benzyl, and the insecticides, sulfoxaflor and a bis-heterocyclic amide. TK data to assess systemic exposure relative to observed toxicity were critical for dose selection and use of KMD approaches resulting in definitive rat and dog studies for florpyptrauxfen benzyl conducted at a high dose of 300 mg/kg/day, as opposed to the limit dose, in the absence of toxicity. Liver MoA and TxG have proven critical for early decision making and prediction of carcinogenic effects for the long-term studies as data were available earlier and were utilized to test sulfoxaflor, haloxfen-methyl, and a bis-heterocyclic amide. Adding the mammalian erythrocyte micronucleus test for the bis-heterocyclic amide and florpyptrauxfen benzyl revealed potential new challenges to address for integrated studies such as dose level, blood or bone marrow, and demonstration of adequate test substance exposure to the target tissue. These innovative testing approaches can provide a comprehensive evaluation of regulatory required toxicity endpoints in a single study using a route of exposure relevant to human health risk assessments. As toxicity testing continues to evolve, the rat 90-day toxicity study should be considered for use as the definitive study for toxicity endpoint evaluation and human health risk assessments, including potentially chronic effects.

2922 The Future of Agrochemical Risk Assessment: Predicting Toxicity Point of Departure with Transcriptome Data

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Human health assessment of agrochemicals requires identification of a toxicity point of departure (POD) from the comprehensive battery of regulatory, guideline toxicity studies conducted when developing a new agrochemical. Currently, the toxicity POD is determined from developmental and subchronic studies, chronic dietary exposures are primarily based on results from enhanced short-term toxicological studies and significantly reduced residue trial levels. In this framework, four steps of the risk assessment paradigm were addressed. First, for hazard identification and dose-response assessment, data-driven retrospective analyses that investigated correlations between rat short-term and long-term critical toxicological points of departure (PODs) at the levels of apical effects (for 143 agrochemicals) and transcriptional effects (for 7 phenoxyacetates) were conducted. These results demonstrated that chronic PODs can be reasonably estimated using apical and transcriptome data from subchronic studies by applying an extrapolation factor of 2-10. Second, for exposure assessment, a platform was established by integrating a modified DynamICROP and DEEM models. Using agrochemical-specific computational tools, it is important to consider alternative approaches to early decision making for novel agrochemicals.

Good Agricultural Practices, physicochemical-chemical properties, and crop DT50s from limited field residue trials, residue levels of all crops for a specific agrochemical can be predicted by the modified DynamicCROP model, which can then be used for estimating chronic exposures for different subpopulations. Finally, chronic risk characterization can be performed based on results from step 1 and 2, as well as other relevant toxicological animal studies, such as those from developmental and subchronic studies. Overall, this 3R-focused framework provides a practical tool for conducting chronic risk assessment using data from enhanced subchronic studies and limited residue data to enable agrochemical product development and, potentially, regulatory decision-making.
agrochemicals. To examine the utility of rat liver microarray data from a subchronic study to predict an apical endpoint POD from a chronic study, data from 7 drugs in the TG-GATES database were examined. The rat liveromic POD was determined from TG-GATES microarray data following 29 days of exposure using BMDExpress. BMDs was used to identify the apical POD from publicly available cancer bioassay data. For 6/7 molecules, the chronic omic-biomarker (O-B) was within 20% of the chronic study apical POD. For acetaminophen, the rat liver omic-based POD was nearly identical to the chronic study apical POD, which was based upon effects in the kidney. These data suggest an omic-based POD derived from subchronic studies approximates a chronic study apical POD. Further, liver omics data may have the potential to be surrogate measures to derive an apical POD in non-liver organs. With additional research and refinement, a transcriptome-based POD from a subchronic study can be considered for determining an apical endpoint POD for both subchronic and chronic study designs.

2923 An Approach to Evaluate Chemical-Induced Biological Response Similarities across Multiple Echinacea purpurea Extracts

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Botanical dietary supplements are complex mixtures that can display significant variability depending on raw plant material and manufacturing processes. *Echinacea purpurea* (EP) is consistently one of the top 10 best-selling supplements in the market. As such, the National Toxicology Program (NTP) is exploring the potential for it to cause toxicity. To probe the extent of variability of representative species, rat liver microarray analysis was performed on 15 samples from the selected EP root extract to other *Echinacea* products available in the marketplace. The NTP developed a case study to assess the chemical-induced biological response similarity of 23 purchased samples, including standard reference materials, to the NTP EP root extract. All samples were extracted with a mixture of ethanol/water and HPLC profiles were generated. The peaks were aligned using standards of known *Echinacea* constituents and evaluated for similarity to the selected EP root extract by hierarchical clustering. By this approach, 9 samples including the standard reference material for EP were highly correlated (Pearson correlation coefficient > 0.9) to the NTP selected EP root extract, while 8 samples, including standard reference materials for other *Echinacea* species had correlation coefficients < 0.5. Next, biological-response similarity of 15 *Echinacea* samples, including the NTP selected EP root extract, were evaluated in organotypic cultures of primary human hepatocytes for liver enzyme induction/receptor activation. Concentration-response data were fit to sigmoidal concentration-response models to determine their respective impacts on target gene expression for major hepatic receptor pathways. Eight out of 15 samples demonstrated marked (10-30-fold) induction of CYP2B6 mRNA consistent with activation of the constitutive androstane receptor (CAR). Interestingly, CYP3A4 mRNA content (along with HMGCS2 and ABCB11) appeared to be suppressed in the majority of samples examined (12 out of 15), and modestly induced in 3 samples consistent with competing dynamics in these complex mixtures. Hierarchical clustering revealed biological-response similarities which were mapped to chemical compositions to characterize lot similarities and identify effector constituents.

2924 Evaluation of Heat-Treated Aqueous Extracts of Hibiscus sabdariffa Calyces for Phytochemicals, Cytotoxicity, Antioxidant, and Anti-Inflammatory Activity

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The extract of *Hibiscus sabdariffa* calyx is widely consumed as beverage and is used in folk medicine to treat diseases. Due to its low cost, the demand for the drink is high and many individuals are involved in its production and sales. The choice of using a water extraction method depends on processor’s preference and safety of product is of great concern. The safety or potential toxicity of regularly consumed medicinal remedies needs to be carefully considered, investigated and validated, more especially when traditional use of plant extracts has been reported to cause deaths due to toxic effects. Hot water extraction of dried and ground *H. sabdariffa* calyx was performed with and without additional heat treatment at 60°C for 0, 10, 20 and 40 min, respectively were prepared. After centrifuged and filtration, clear extract supernatants were used for assays. Cytotoxicity against Vero kidney cell lines was evaluated by MTT, anti-oxidant activity by trolox equivalent antioxidant capacity, total phenolic by gallic acid equivalent and flavonoid by quercetin equivalent. Heat treatment time had no significant effect on parameters investigated in comparison to extracts without additional heat treatment. However with increasing heating time there was a trend of a decrease in the total phenolic content. Extract without additional heat treatment had the highest phenolic content (88.1 ± 0.62 mg/ml) was with heat treatment POD and 40 min (73.5 ± 0.28). Irrespective of the extraction method, all extracts exerted anti-inflammatory activity by the inhibition of 5-lipoxygenase enzyme (58.97 ± 1.17 to 78.77 ± 3.47 μg/ml) although to a moderate extent. This was the first study to demonstrate the anti-inflammatory activity of *H. sabdariffa* extract by inhibition of 5-lipoxygenase activity. Also all extracts were relatively cytotoxic to Vero cells in a dose dependent manner with LD₅₀ ranging from 257.77 ± 1.73 to 280.37 ± 1.52 μg/ml. The aqueous extract of *H. sabdariffa* calyx possesses health benefits but with moderate cytotoxicity. Although there has been no reports of the suspected toxicity of this plant since its long traditional use in folk medicine caution should be exercised in using this extract especially at high concentration and for long periods which could be detrimental to health.

2925 Green Tea Extract and Hepatotoxicity: An Extensive Review by the US Pharmacopeia


Following routine review of literature on hepatotoxicity of green tea extract (GTE), the USP Dietary Supplements Admission Evaluations J3 recommended that USP seek the assistance of toxicologists, to conduct a risk assessment Panel to GTE. The Expert Panel was convened to review toxicity and safety data related to GTE, and to define a label cautionary statement for inclusion in the USP Powdered Decaffeinated Green Tea Extract (PDGTE) monograph. The panel considered three topics relevant to potential hepatotoxicity of GTE: 1) chemistry, manufacturing and control (CMC) pharmacokinetics/pharmaco-dynamics, and clinical/non-clinical data and adverse event reports for risk assessment. A review of information on these topics suggest that constituents, particularly epigallocatechin gallate (EGCG) and other catechins of GTE may be involved in hepatotoxicity, not contaminants, and suggest that fasting increases the absorption of GTE constituents which can lead to hepatotoxic levels in rodents and humans. To evaluate safety data, a comprehensive literature search was performed for clinical and nonclinical studies on GTE and major constituents published since the USP assessment in 2008. All studies were categorized for relevance based on pre-established criteria. We also obtained human case reports, and FDA MedWatch Adverse Event Reports (AERs) related to the use of dietary supplement products containing GTE for the period January 1, 2008 to October 31, 2016. The non-clinical data demonstrated dose-dependent hepatotoxicity in rodents and dogs, with fasted dogs being the most sensitive model (NOAEL of 40 mg/kg EGCG content). In nine clinical studies reviewed, one reported clear evidence of hepatic toxicity in 4 out of 44 participants, and the panel recommended that it be added to the list of potential GTE adverse effects. The GTE monograph currently recommends that USP seek the assistance of toxicologists, to conduct a risk assessment on GTE. An Expert Panel was convened to review toxicology studies and a risk assessment on GTE. An Expert Panel was convened to review toxicology studies and a risk assessment on GTE. An Expert Panel was convened to review toxicology studies and a risk assessment on GTE. An Expert Panel was convened to review toxicology studies and a risk assessment on GTE. An Expert Panel was convened to review toxicology studies and a risk assessment on GTE. An Expert Panel was convened to review toxicology studies and a risk assessment on GTE. An Expert Panel was convened to review toxicology studies and a risk assessment on GTE.

2926 Therapeutic Effects of Astilbin from Smilax glabra Roxb. Targeting Inflammatory Mediators in Rheumatoid Arthritis Disease

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Astilbin, a flavonoid compound, was isolated from the rhizome of *Smilax glabra* Roxb. (with red cross section) grown in Guizhou province, China. We assessed its effect and potential mechanism on attenuation of the inflammatory response in adjuvant-induced arthritic rats. Our results showed that daily oral administration of astilbin at 5.3 mg/kg reduced joint damage in the hind paw of rats with adjuvant arthritis (AA) induced by complete Freund’s adjuvant (CFA), as assessed by radiographic analysis and histopathological evaluation. Accordingly, astilbin exhibited remarkable inhibitory effects on tumor necrosis factor (TNF)-α, -
interleukin (IL)-1β, and IL-6 mRNA expression. Significant decrease of serum cytokine levels of TNF-α, IL-1β, and IL-6 was also observed in astilbin-treated AA rats compared to the vehicle-treated AA rats. The reduced expression of these cytokines was associated with protein activity suppression of three key molecular targets in the pathogenesis of rheumatoid arthritis: inhibitory protein kappa B (IkBκ), nuclear factor kappa B (NF-κB) subunit p65, and toll-like receptor (TLR) adaptor MyD88. Furthermore, the therapeutic effects of astilbin on the inhibition of cytokines production as well as the reduction of inflammatory response in AA rats are close to a commonly used disease-modifying antirheumatic drug, leflunomide (LEF). Collectively, our data suggest the action mechanism of astilbin, as an anti-inflammatory agent, for rheumatoid arthritis treatment, is associated with modulating the production of pro-inflammatory cytokines and inhibiting the expression of key elements in NF-κB signaling pathway mediated by TLR.

**2927** 'H-NMR-Based Chemometric Analysis of Myelostimulatory Activity Exhibited by Echinacea

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The three medicinal species of Echinacea that are widely used are E. purpurea, E. angustifolia, and E. pallida. We previously described myelostimulatory activity of aerial parts of Echinacea. In present study, we evaluated myelostimulation by 8 different accessions of Echinacea spp. We employed chemometrics to identify regions of 'H-NMR spectra associated with activity. We also performed concurrent studies to monitor toxicity of accessions. We obtained aerial parts of accessions E. purpurea PI-631307, PI-63668, PI-649040, E. angustifolia PI-649029, and E. pallida PI-631300 and PI-597603 from the USDA-ARS NCRPS. Female Sprague-Dawley rats (n=6) were dosed with ethanol extract (75% v/v) at 0, 25, 50, 100, 200 mg/kg for 7 days. After 24hrs, rats were euthanized and Dawley rats (n=6) were dosed with ethanol extract (75% v/v) at 0, 25, 50, 100, 200 mg/kg for 7 days. After 24hrs, rats were euthanized and cell number was cultured in methylcellulose with csf2, IL-3 and SCF and cell number was determined. Myeloid progenitors, i.e., CFU-GMs, from femur bone marrow were cultured in methylcellulose with csf2, IL-3 and SCF and cell number was determined. Cell number was evaluated in methylcellulose with csf2, IL-3 and SCF and cell number was determined. Myeloid progenitors, i.e., CFU-GMs, from femur bone marrow were cultured in methylcellulose with csf2, IL-3 and SCF and cell number was determined.

**2928** Anti-Inflammatory Activity of Cranberry (Vaccinium macrocarpon) Polyphenols and Oligosaccharides in Intestinal Fibroblasts

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Cranberries (Vaccinium macrocarpon) are a rich source of polyphenols, including A-type proanthocyanidins, which have been described to exert antioxidant and antibacterial effects. Although the anti-inflammatory activity of polyphenols from cranberry and other fruits have been previously described, the effects of oligosaccharides are less elucidated. Therefore, this study compared the anti-inflammatory activity of a high-tannin cranberry whole extract and subfractions (anthocyanins, flavonols/tannins, and oligosaccharides) in lipopolysaccharide (LPS)-challenged human non-malignant intestinal fibroblasts (CCD-18Co). Cells were treated with the extract or each fraction for four hours, and LPS was used as inflammation inducer. The mRNA expression of the inflammatory targets tumor necrosis factor alpha (TNF-α), interleukin 1 beta (IL-1β), interleukin 6 (IL-6), and nuclear factor kappa B (NF-κB) was evaluated using real-time quantitative PCR. Results show that whole cranberry extract and fractions decreased the expression of inflammatory markers. This study describes for the first time the anti-inflammatory activity of cranberry oligosaccharides. These compounds reduced NF-κB and TNF-α by 45% compared to the LPS-control at 25 mg/L. The anthocyanin-rich fraction proved to exert the highest anti-inflammatory activity starting at 3.12 mg/L, restoring the expression of NF-κB back to levels observed in the control group. Further studies will be performed using microbial metabolites of the fractions based on their physiological relevance in an intestinal environment.

**2929** Effect of pH and Gastro-Intestinal Conditions on the Fibrinolytic Activity of Nattokinase and Lumbrokinase In Vitro

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Nattokinase (produced by Bacillus subtilis var. natto) and lumbrokinase (from earthworms) are promoted as health supplements for their claimed support of cardiovascular and circulation health in humans. The mechanism for such claimed effects is, at least in part, mediated by their fibrinolytic activity. The possibility exists that consumption of fibrinolytic enzymes may increase the risk of bleeding, especially when taken together with other clot-modifying compounds. Using a validated semi-quantitative fibrin plate method, we assessed the fibrinolytic activity of nattokinase and lumbrokinase over a range of pHs and upon incubation with simulated and rat gastric and intestinal fluids. The effect of adding pepsin and pancreatin to the gastric and intestinal fluids, respectively, was also tested. Our data indicate that nattokinase is inactivated at pH < 5.5, while lumbrokinase is inactivated at pH < 3.5, upon incubation at 37°C for 2 h. Consistent with this, incubation in simulated gastric fluid (pH < 1.5) inactivated both enzymes in as little as 5 min. Lumbrokinase was highly resistant to an incubation in simulated intestinal fluid (pH = 6.5) up to 3 h, and did not. Rat gastric fluid (pH = 4.5) inhibited the fibrinolytic activity of nattokinase over time, while it had no or little effect on lumbrokinase activity. The effect of the rat intestinal fluid on the fibrinolytic activity of the enzymes could not be assessed in a fibrin plate because this fluid cleaved fibrin. Pepsin and lumbrokinase digested nattokinase and lumbrokinase, while some enzymes in the pancreatin mixture appeared to be hydrolyzed by nattokinase and lumbrokinase, as assessed by SDS-PAGE. The systematic characterization of the conditions in which nattokinase and lumbrokinase are fibrinolytically active in vitro may contribute to a better understanding of their potential effects in vivo. This work was sponsored under an interagency agreement between the FDA/NCTR and the NIH/NTP (FDA IAG # 224-12-0003/NIEHS IAG # AES12013).

**2930** Effects of Sub-Acute Exposure of Wistar Rats to Ethanolic Leaf Extract of Byrsocarpus coccineus on Some Haematological and Serum Biochemical Parameters


Byrsocarpus coccineus is an indigenous herb widely dispersed in tropical Africa and commonly known as Crimson thyme. The leaf and root of the plant is noted to possess medicinal benefits in traditional medicine of the Western part of Nigeria. The plant has been reported for its analgesic, anti-inflammatory, antidiarrhoeal and antipyretic activities. Its antiplasmodial properties have also been reported by researchers. In the light of these medicinal properties, the plant can be represented as a valuable source of medicinal compounds and the present study was therefore aimed at determining the sub-acute toxicity of the leaf extract of the plant. The ethnolthic leaf extract of the plant was administered orally at 250, 500, and 1000 mg/kg to three groups A, B and C of Wistar rats respectively for 14 days while the control group D was administered orally with 3ml/kg of distilled water. Blood samples were then obtained from the anaesthetized rats for haematology and serum biochemistry analyses. The result obtained from serum analyses showed a significant increase (p<0.05) in the levels of blood urea nitrogen and creatinine while the increase in alkaline phosphatase is not significant for the treated groups B and C. It was therefore concluded that high doses of the extract may have untoward effects especially on the kidney and therefore care must be taken when using the plant in high concentration. Further research is on to determine the level and the mode of the toxicity.
2931 Cytotoxic and Anti-Prosiferative Activities of Methanol Extract of Adansonia digitata on Breast Cancer (MCF-7) Cells
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There is a notable dependence on the use of herbal medicinal products as anticancer agents in contrast to synthetic drugs in developing countries due to their cost, availability and associated toxicity. In addition, there is upsurge in research towards the identification of active ingredients in plant extracts and their mechanism of action. Adansonia digitata is a referenced medicinal forest tree and its stem bark (SBAD) has many traditional medicinal uses, including for the treatment of cancer. There is a dearth of scientific information about the anticancer potential of SBAD and its mechanism of action. The aim of this study therefore, was to assess the cytotoxic and long term antiproliferative effects of methanol extract of SBAD (MESBAD) on breast cancer (MCF-7) cells using the MTT, clonogenic assay and western blotting. Adansonia digitata was identified/authenticated with voucher number FHI NO. 109859. Cold extraction was carried out on SBAD by soaking in 7% methanol for 72 hrs at room temperature to obtain MESBAD. The extract was filtered and concentrated using a rotary evaporator and then lyophilized. Cells were cultured according to established procedure, while cytotoxicity and clonogenic assays were performed in accordance with standard protocols. Western blotting analysis was performed with antibodies to p21 and p53. In all the experiments, cells were treated with MESBAD or DMSO (vehicle control). MESBAD was shown to have a dose dependent cytotoxicity as compared with the control, with IC50 value of 100µg/ml. MESBAD also demonstrated a dose dependent inhibition of long term (13 to 15 days) survival of MCF-7 cells. Indeed, when MCF-7 cells were treated with ½ IC50, IC50, 2X IC50 and 4X IC50 of MESBAD their abilities to form colonies were reduced in a dose dependent manner. Western blotting indicated that MESBAD activated the p38 stress signaling pathway and the p53/p21 response in MCF7 cells. We propose that MESBAD reduces short- and long-term viability of MCF-7 breast cancer cells in a concentration dependent manner. Further work is needed to fully characterize its mechanism of action.

2932 Thymoquinone Attenuates Maneb and Mancozeb-Induced Cytotoxicity

The ethylene-bis-dithiocarbamate (EBDC) fungicides, manebe (MB) and mancozeb (MZ), are widely used in the agricultural industry to control various crop diseases such as the infamous potato blight. However, recent studies have linked these pesticides to neurodegenerative diseases such as Parkinson’s and have raised a major toxicological concern. Acute exposure to EBDCs has shown low toxic effects in mammals but chronic exposure is linked to neurodegeneration. MB and MZ increase apoptosis pathway and leading to cell death. Antioxidant compounds can hinder the activity of ROS and other free radicals. Thymoquinone (TQ), the active compound present in black cumin seed, is known for its antioxidant and neuroprotective properties among many others. This study examines the protective effect of TQ on PC12 cells treated with MB and MZ. Neutral red uptake assays for cell viability analysis and CellROX Green reagent for oxidative stress detection were carried out. The results have shown a decrease (about 55-58% cell death) in cell viability after MB and MZ treatment at 5µM for 24 hours and an increase (only about 25-28% cell death) when pretreated with 10 µM TQ for 24 hours. The results indicate that TQ can act as a potential treatment agent against EBDCs-induced cell death. TQ also attenuated ROS generation by about 10-30% in PC12 cells treated with 5 and 10 µM MB and MZ for 2 hours. Our future research goal is to study the mechanism of this attenuation triggered by TQ. The expression levels of oxidative stress regulator genes, such as p53, in cells treated with TQ and pesticides MB and MZ will be determined by Western blot analysis.

2933 Toxicological Assessment of Bryophyllum pinnatum Leaf Extract in Rodents
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The aqueous leaf extract of Bryophyllum pinnatum (ALBP) is used in Alternative Medicines in the management of acute and chronic diseases such as hypertension, diabetes, arthritis, asthma and weight management; and it is believed that it is devoid of any form of toxicities. Various toxicological end-points were investigated for effects of ALBP in rodents, with a view to ascertaining and predicting its safety claims. For acute toxicity test, mice were administered 10mg/kg – 5000mg/kg (p.o.) of ALBP for toxicity end-points up to 14 days. For subchronic study, rats were given daily doses of ALBP (10, 100 or 1000 mg/kg) for 90 days, during which the body weights were measured. After 90 days, weights of vital organs and serum metabolites- alkaline phosphatase (ALP), aspartate amino transferase (AST), alanine amino transferase (ALT), bilirubin and uric acid were measured; and oxidative stress markers- catalase (CAT), superoxide dismutase (SOD), reduced glutathione (GSH), and malondialdehyde (MDA) were determined from tissues. Hematologic and spermatologic functions, and lastly, histopathologic assessment of the organs were also assessed. These were followed by 21 days reversibility tests. Acutely, orally administered ALBP did not cause mortality, but dose-dependent drowsiness, reversed within 48h. In the subchronic test, significant (p<0.05) reduction in body weight; increases in weights of liver, kidneys & spleen, but weight reduction of the lungs. Also, there was weight reduction of the testis. The weights of liver & spleen, but not those of lungs & testes were reversed. Furthermore, there was an increase in PCV%, but reduction in RBC, Hb & platelet concentrations. There was elevated WBC level which was not reversed. At 1000 mg/kg there were significant increases in ALP, AST, ALT, LDH, Uric acid and creatinine whereas, bilirubin and uric acid were reversed after 21 days. There was reduction in CAT & SOD, but increase in MDA level for all the vital organs; but effects were reversed except for the lungs and testes at 1000mg/kg. The results suggest that the ALBP might exhibit haematological, renal, hepatic and specific organ (lungs and testes) toxicities following chronic oral use, at high doses.

2934 Assessment of In Vitro Anti-Influenza Potential and Cytotoxicity of Crude Extract and Fractions of Loxostylis alata
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Loxostylis alata A. Spreng. ex Rchb. (Anacardiaceae) is a small tree distributed in the Eastern Cape and Kwazulu-Natal provinces of South Africa. The antifungal and antibacterial properties of this plant have been previously investigated but no antiviral property documented. The aim of this study was to investigate in vitro anti-influenza activity of the methanol extract of L. alata and its hexane, butanol, chloroform, ethyl acetate and water fractions. The 50% cytotoxic concentration (CC50) of the extracts was determined using MDCK cells, revealing only mild toxicity against MDCK cells for all the extracts. Cells were subsequently treated with effective concentration (EC50) of the extracts with approximately 100 tissue culture infectious dose (100TCID50) of the virus at varying exposure times: simultaneous, pre-penetration and post-penetration combined treatments with an incubation time of one hour. The antiviral potential of the extracts was tested by standard haemagglutination (HA) and haemadsorption (HA) inhibition assays. A dose-dependent HI pattern of the extracts’ test results was observed. Methanol and butanol extracts were the most promising against influenza A virus (IAV) in all types of combination treatments which confirms the extracts’ efficacy in all steps of viral attachment, entry and life cycle. However, the MTT assay, which provided the EC50 value of 77.00 µg/ml, could be considered as effective extracts against IAV. This study represents the first report of L. alata extracts as a potential source of new anti-IAV drugs or preparations.

2935 In Vitro Cytotoxic Activities of Dianthus thunbergii S.S. Hooper (Caryophyllaceae)
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The roots of Dianthus thunbergii SS Hooper are used traditionally in South Africa for the treatment of diabetes, wounds, colic, chest complaints and cancer. The potential cytotoxic and/or anti-proliferative activities of the aqueous and ethanol root extracts of D. thunbergii were evaluated in vitro on two cancer cell lines- human hepatocellular carcinoma (HepG2) cells and murine insuloma (INS-1) cells using the 3-(4,5-Dimethylthiazol-2-yl) 2,5-diphenyltetrazolium bromide (MTT) and crystal violet cell viability assays, as well as live-cell fluorescence imaging microscopy. The aqueous extract (50-200µg/ml) exhibited sig-
nificant (p<0.05) cytotoxicity in HepG2 cells (IC50=50 µg/ml), while also significantly (p<0.05) decreasing the viability of INS-1 cells (IC50=36.0 µg/ml), although no toxicity was evident in L6 myotubes. Hoechst 33342 and propidium iodide staining of INS-1 cells further revealed significant growth inhibition (p<0.001) of INS-1 cells by the aqueous extract. No meaningful toxicity was, however, obtained with the ethanol extract (IC50 = 39.0 µg/ml) and nor with the LcE50/MS analysis of the aqueous extract revealing the putative identities of main compounds present in the aqueous root extracts, including some that may contribute to its anti-proliferative action. Taken together, the results showed that the roots of D. thunbergii may represent a potential plant-based source of agents with anti-proliferative efficacy.

2936 Potency and Safety of Eight Under-Investigated South African Plants from the Myrtaceae Family for Activity against Bacillus anthracis Sterne Vaccine Strain

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Anthrax, caused by Bacillus anthracis infection is a severe acute disease. Apart from its zoonotic importance and potential as a bioterrorism agent, the economic losses associated with morbidity and mortality of animals. It is transmitted through the control of the carrier state, which has a high priority globally. Against the backdrop of antimicrobial resistance, there is motivation to develop new anti-B. anthracis products especially from natural sources to provide alternative or complementary remedies. Recent empirical evidences show that plants hold potential for anthrax control in South African plants have been tested for their efficacies against B. anthracis. This study reports the growth inhibitory activity of crude leaf extracts of plants from the myrtaceae family against B. anthracis Sterne vaccine strain. A two-fold serial microdilution assay was used to determine the minimum inhibitory concentration (MIC) of the extracts against B. anthracis. Cytotoxicity (LC50) was determined by a tetrazolium-based colorimetric assay against Vero kidney cells and selectivity indices (LC50/MIC) which gives an indication of the safety of the crude extracts were obtained. The MIC of the plant extracts ranged from 39µg/ml to 156µg/ml. Excellent MIC values were observed for the active extracts.

2937 Nematicide Activity of Chenopodium ambrosioides L. and Ambrosia cumanensis kunth


Chenopodium ambrosioides L. and Ambrosia cumanensis Kunth are shrubs that grow in some regions of South America, whose leaves have traditionally been used for therapeutic purposes such as anthelmintics, anthelmintics, anti-inflammatories and against colds. In this work the anthelmintic activity of different leaf fractions of these two plants was evaluated. Extracts of these plants were obtained by maceration of the crushed vegetable material with 96% ethanol as solvent, and concentration in the rotovaporator at 45 °C and vacuum of 172 mbar pressure, monitoring the process by thin layer chromatography. The concentrated extract was incubated at 40 °C for 48 h. It was then mixed with silica gel powder and allowed to dry for 12 h. Extracts of different polarities were obtained by eluting hexane, chloroform, ethyl acetate and methanol through column chromatography. Phytochemical screening of the major groups of secondary metabolites in the extracts of the plants studied was performed to identify the presence of alkaloids, tannins, coumarins, flavonoids, triterpenes, saponins, quinones and cardiotonic glucosides. The nematicidal effect was carried out using a tetrazolium-based colorimetric assay against Vero kidney cells and selectivity indices (LC50/MIC) which gives an indication of the safety of the crude extracts. We found that the extracts had high selectivity against the bacteria than to normal body cells. The extracts were relatively safe (S.1>1). This shows that the extracts have higher toxicity against the bacteria than to normal body cells. Further research is on-going to isolate the compounds responsible for anti-B. anthracis activity and determine the mechanism of action of the active extracts.

2938 Toxicological Evaluation of Annona muricata Methanolic Extract in Rodents

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Due to large growth in the use of herbs as alternative to conventional drugs, its becomes imperative for researchers worldwide to intensify effort in screening for safe dose and the likely toxicological effects that may be associated with the use of these herbs. Hence, this work focussed on the toxicological evaluation of Annona muricata methanolic bark extract (AMMBE) also known as soursop. Twelve 12 mice and 16 rats were used for the toxicological study consisting of four groups and 4 animals per group per study. Annona muricata bark was soaked in methanol for 72 hours and processed to obtain the brownish semi-solid extract used for the study. The acute toxicity test revealed that clinical signs of paw-licking, writhing, incoordination etc were observed at a dose of 2600mg/kg while mortality was recorded at 3200mg/kg body weight and L90 was calculated to be 2884.4mg/kg. The sub-chronic study showed dose dependent mild reductions in PCV, RBC and Haemoglobin, there were no significant changes in the serum chemistry and organs histopathology when compared to the control.

2939 Potential Effects of 4-O-Methylhonokiol on Japanese Medaka Embryos


Since the potential toxic effects of 4-O-Methylhonokiol (MH), a major bioactive constituent of Magnolia grandiflora seeds or Magnolia officinalis bark for clinical trials remain elusive, toxicity assessment of MH was assessed with Japanese Medaka (Oryzias latipes) embryos in vivo. Fertilized medaka eggs (Iwamatsu stage 10) were exposed to various concentrations of MH in embryo rearing medium (ERM) in a 48 well culture plate (one embryo/well/ml) and maintained at 25±1°C with 16L:8D light cycle. The treatment continued for 0-6 day post-fertilization (dpf) to explore whether MH induces developmental defects in medaka and how it contributes to their survival. Embryos were evaluated for heart rate, vessel circulation, thrombus formation, hatching efficiency and mortality. Embryos exposed to 5, and 10 µM MH caused suppression of heart rate and a reduction of blood flow in lower trunk vessels in 5dpf with a median effective concentration of 3.53 µM. Since blood coagulation factors are concomitantly also activated by the lesion on the lining of blood vessel, we initially examined the procoagulant action of MH on the components of the plasma kalikrein-kinin system. MH was ineffective to activate prekallikrein (Fletcher factor), factor XI (plasma thromboplastin antecedent), or factor VIII (von Willebrand factor). The lowest concentration of MH to induce tail malformation was 5 µM. The embryos exposed to 5 and 10 µM MH 0-6 dpf significantly reduced hatching efficiency and caused mortality in comparison with other groups. Their findings indicate that MH affects the normal development of the medaka embryo in low µM concentrations.

2940 Ethyl Acetate Fraction of Acacia ataxacantha Leaf Extract Inhibits Escherichia coli O157:H7


Escherichia coli O157:H7 is a well-recognized food-borne bacterial pathogen of high public health importance. Globally, cattle serve as major reservoirs of the organism. Most case fatalities and episodes of the infection result from the effects of enterotoxins, cytotoxins, shiga-toxin production and other virulence factors. Infants, elderly and immune-compromised individuals are particularly at high risk. Complications that often accompany conventional antibiotic therapy and the persistence of multiple resistant mechanisms of the pathogen were negative controls. In A. cumanensis the presence of cardiotonic glycosides was determined in the ethanolic, hexanolic and chloroform extracts; saponins in ethyl acetate and methanolic extracts, triterpenes and sterols for ethanolic, hexanolic and chloroform extracts. In C. ambrosioides, flavonoids and tannins were identified in total extract, ethyl acetate and methanol and triterpenes and steroids in all extracts. The hexanoic extract of A. cumanensis contained the highest morality in C. elegans, while all extracts of C. ambrosioides showed nematicidal effect at 150 µg/mL. In conclusion, the compounds present in leaf extracts of both plants can be used as anthelmintics.
present obvious challenges to effective treatment. Extract of Acacia ataxacantha leaves (EAA) is a popular herbal remedy for digestive disorders in Nigeria. The study evaluated EAA for bioactivity against E. coli O157:H7. The crude EAA was prepared by cold maceration in 98% methanol and concentrated in a rotary evaporator. Solvent partitioning of the crude extract was done with N-hexane, ethyl acetate and distilled water. Tests using E. coli O157:H7 and H7 flagellar antigens, respectively. Antimicrobial susceptibility tests were carried out on the isolates using the agar disc diffusion method; the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extract were evaluated. Eighteen isolates from the 515 samples were identified as non-sorbitol fermenters with two isolates each for E. coli O157 and E. coli O157:H7 respectively. The crude extract (300-600 mg/ml) exhibited a comparable antibacterial activity (zones of growth inhibition) to that of tetracycline (30 μg/ml), gentamicin (10 μg/ml) and ciprofloxacin (5 μg/ml). Ethyl acetate fraction with MIC of 200 mg/ml was the most potent. Culture from MIC tubes on fresh seeded media showed growth of the isolates following 24 h incubation indicating a bacteriostatic activity. The findings demonstrated that ethyl acetate fraction of A. ataxacantha leaf extract possessed a bacteriostatic activity and this could be explored to complement therapy against E. coli O157:H7 infection.

**2941 The Effect of Sesamin on Endothelial Nitric Oxide Synthase Activation through Calcium-Dependent Signaling Pathways**

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Sesamin is a major lignin in sesame seeds (Sesamum indicum) and has been known to act as a potent antioxidant and enhance endothelial function which contributes in protecting and preventing or reducing cardiovascular diseases. However, the mechanism involved to the cardioprotective effect by activation endothelial nitric oxide synthase (eNOS) of sesamin remains unclear. In this study, we identified the intracellular pathways underlying eNOS activation by sesamin. Sesamin induced the activating phosphorylation of eNOS on Ser1177 in EA.hy926 cells. Sesamin-induced eNOS phosphorylation required adenosine receptor (ADORA2B)-mediated PI3K/Akt and MAPK signaling and was reversed by AMPK and CaMKII inhibition. These results indicate that sesamin stimulates eNOS phosphorylation via activation of ADORA2B-mediated PI3K/Akt, MAPK, CaMKII/AMPK-dependent pathway. Sesamin may be useful for the treatment or prevention of endothelial dysfunction associated with diabetes and cardiovascular disease.

**2944 Phillyrin Suppresses Hepatic Gluconeogenesis Via Phosphorylation of CRTC2 and HDAC5 in HepG2 Cells**

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Type II diabetes mellitus (T2DM) is a chronic metabolic disease caused by insulin resistance, and its worldwide prevalence has dramatically increased over the past few decades. Increased glucose production through hyperglycaemia is dysregulated and results in the manifestation of hyperglycaemia in T2DM. Phillyrin, one of the major active constituents of F. suspensa and F. koreana, was known to possess the anti-inflammatory and anti-oxidant effects. An in vivo study showed that phillyrin exerted anti-obesity effects in nutritive obesity mice. However, the anti-diabetes mellitus effect of phillyrin and its underlying molecular mechanisms have not been elucidated. In this study, we investigated the inhibitory effects of phillyrin on hepatic gluconeogenesis in HepG2 cells. Phillyrin reduced the mRNA expression of glucose-6-phosphatase (G6Pase), major genes in hepatic gluconeogenesis. Also, Activated AMPK by phillyrin suppresses hepatic gluconeogenesis through phosphorylation of CRTC2 Regulated Transcription Coactivator 2 (CRTC2) and Histone deacetylases (HDAC5). These phosphorylation events induce the sequestration of CRTC2 and HDAC5 in the cytoplasm, which leads to suppression of CREB and FoxO1 transactivation activities. Together, these results suggest that phillyrin inhibited hepatic gluconeogenesis via phosphorylation of CRTC2 and HDAC5 by activated AMPK in HepG2 cells.

**2944a Interaction of Quercetin with Different Flavonoids and Induction of Phase-II Enzymes in Hepa-1c1c7 Cells**

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For more than four decades epidemiologic studies correlate that consumption of diets rich in fruits and vegetables are associated with lower risks of cancers. A persuasive body of evidence indicates that protection against chemical carcinogenesis can be achieved by non-nutrient components of vegetables and fruits capable of inducing phase II enzymes that are involved in carcinogen metabolisms. These enzymes include Glutathione S-transferase (GST), which inactivates toxic electrophiles, and NAD(P)H:quinone reductase (QR), which catalyzes two-electron reductions of quinones to hydroquinones. The flavonoids are an important class of naturally occurring compounds and represent parts of the human diet found in edible plants. This study investigated the synergetic effect of hesperidin with other flavonoids/biomolecules on the induction of GST and QR activity in a murine hepatoma line. The cells were incubated with appropriate concentrations of flavonoids
individuals and with mixtures of flavonoids/biomolecules for 48 hours in CO2 incubator, maintained at 37°C. For the synergistic effects, the concentration of quercetin is kept constant whereas the concentrations of the second and third flavonoid/biomolecule were varied. The cells were sonicated, the content centrifuged and the supernatant was used to determine enzyme activities and proteins. GST activities were determined against two substrates: 1-chloro-2, 4-dinitrobenzene (CDNB) and 4-nitroquinoline 1-oxide (4NQO), whereas QR activities were determined by the reduction of 2, 6-dichlorophenolindophenol (DCPIP). Quercetin in combination of caffeine and hesperidin exhibited no significant change in GST or QR activities whereas individually they showed induction. Also, no significant change in GST activities was observed when quercetin was incubated with caffeine and ginger extract but QR activities were induced. In the third group, where quercetin, caffeine, and resveratrol were mixed, both GST and QR activities showed significant induction against the control. In the group where quercetin, cafe- feine, and β-carotene were varied, no changes were observed although individually they caused induction. These results suggest that induction of phase-II enzymes by flavonoids in combina- tion may be important in achieving of a meaningful effect in order to formulate imminent cancer prevention strategies. A more in depth in vivo study may be needed to further corroborate results of this study.

**2945 Advancing Toxicology Education in Consumer Products Safety and Sustainability through a Corporate Partnership**

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The importance of investigating, documenting, and supporting the safety of consumer products for consumers, workers, and the planet cannot be overstated. It is the basis of consumers’ trust in the products they use. However, this topic is not typically offered in toxicology training programs and requires a specialized skill set, which is often acquired on the job. In close partnership, Rutgers University (RU) faculty and Colgate-Palmolive Company (CP) scientists designed and imple- mented a two-credit elective course in “Consumer Products Safety and Sustainability”. During this course, students and postdoctoral fellows acquired specialized skills in assessing ingredients for use in specific product types, in regulatory compliance, addressing consumer and worker safety, as well as environmental exposures, biodegradability, and sustainability. Course content was built on the strong founda- tional curriculum at RU, and went beyond the traditional risk assessment framework to teach real-world application-based skills. Students worked through case studies at the end of each session. Interaction and assessment of real-time comprehension were enabled by using a clicker response system. Course participants were evaluated based on attendance and participation (10%), team-based mid-term oral presentations evaluated by a panel of CP scientists (45%), and a final examination composed of multiple choice and written answers (45%). Students completed a course evaluation including a five-level rating scale (from disagree strongly to agree strongly), word attributes, and open-ended questions. 100% of the participants “would recommend this course to a friend”. The students’ rating of the course overall was a 4/5 (with 5 being the best). A benchmarking quiz was completed by the students at the beginning and the end of the semester, showing quantitative and qualitative improvements in understanding. In con- clusion, a team-taught elective course as part of a private-public part- nership provides graduate and postdoctoral students with real-world, application-based training that prepares them for careers in all areas of corporate toxicology in the consumer goods industry. **Supported by T32 ES007148.**

**2946 Exploring Careers in Toxicology through an Interactive Summer Program for High School Students: A Five-Year Perspective**


Introducing students early in their education to the fields of toxicology and environmental health sciences is critical to developing the next generation of talented and dedicated scientists. Towards this effort, the Toxicology, Health and Environmental Disease (THED) program was established to engage high school students interested in pursuing sci- entific careers in toxicology. A critical goal of THED is to provide the basic concepts and skills of designing and conducting a toxicology study: study design, dose-response relationships, necropsy, pharmacokinetics, histopathology, molecular biology and immunohistochemistry. This was accomplished with hands-on, team-based laboratory experiments and lectures from graduate students and postdoctoral fellows. In addition, participants met with speakers in careers in medicine, pharmacy, gov- ernment, pharmaceutical industry, and academia, to receive guidance on career paths. In 2017, we marked the fifth year of the THED program with engagement of 240 students. Each year, the program was assessed through post and post-survey by parents. Based on survey feedback, the THED program has evolved to align the goals of the program with those of the students and parents. In response to parental feedback, more time and a greater emphasis were placed on introducing participants to prospective career tracks. Moreover, the selection and conduct of the hands-on laboratory activi- ties were refined to enhance the program’s effectiveness of teaching the desired laboratory based skills and key concepts of toxicology. By centering each laboratory activity on the overarching question, “How would one develop countermeasures against chemical warfare agents?”, instructors demonstrated how experiments should approach a contemporary toxicologic problem. With the added focus on career development, when parents were asked to grade on a 7-point Likert scale how effective the program was in exposing their children to different scientific careers, we received an average score of 6.65 (SD = 0.81). In conclusion, a one-week interactive, laboratory-based, edu- cational program contributes to the training pipeline in toxicology by advancing the research skills of high school students and exposing them to career paths in toxicology. **Supported by NIEHS T2ES007148, P30ES005022, and U54AR055073.**

**2947 Mentor Perceptions and Motivations in a Ten Week Summer Undergraduate Research Fellowship in Toxicology**

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Intensive laboratory training during the summer provides undergradu- ate students with the opportunity to gain research skills, explore post-graduate training options, and develop interpersonal skills. Most assessments of summer fellowships focus largely on student percep- tions and outcomes. The purpose of the present study was to assess the motivations, satisfaction, and perceptions of mentors who supervised undergraduate students in a 10-week summer research fellowship at Rutgers University. Students spent 40 h per week in individual labora- tories or clinical sites and 2 h per week in career development activi- ties such as toxicology research seminars, field trip, and LinkedIn profile development. The fellowship culminated in student research presen- tations. Each student submitted a scientific abstract and presented their major research findings during symposiums held in the last two weeks of the fellowship. All co-authors on the abstracts (N=25) were emailed an 11 question online survey upon completion of the program. Responses were received from 32 individuals (24 faculty members and 8 graduate students/postdocs/technicians). All respondents had previously mentored summer interns with 68% of mentors having supervised between 1 and 5 students in the past 5 summers. The remaining 32% of respondents reported mentoring between 6 and 10 students in the past 5 summers. Respondents spent an average of 13.3 h (± 1.10 h SD, 1.5-37 h range) with their student each week. Using a 5-point system, the student fellows received the highest ratings for their work ethic and scientific curiosity followed by the quality of their presentation and technical skills. The majority of mentors would definitely recommend (score of 5) the summer fellowship program to a student (85%) and to another mentor (72%) and would serve as a mentor in a subsequent summer (77%). Motivations for serving as a mentor included another mentor (72%) and would serve as a mentor in a subsequent summer (77%). Motivations for serving as a mentor included another mentor (72%) and would serve as a mentor in a subsequent summer (77%). The remaining 32% of mentors were motivated to serve as a mentor because of their enjoyment of teaching students. Periodic assessment of mentor per- ceptions and motivations is important for the continual evaluation and improvement of a summer undergraduate research program. **Supported by NIEHS R25ES007271, P30ES005022, ASAP, and the SOT Intern Program.**

**2948 Creating Scientific and Graphical Abstracts as an Interactive Session in a Summer Research Fellowship**

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Short-term research experiences such as summer fellowship programs provide students with an opportunity to develop their research skills, explore career options, and improve written and oral communication skills. Most summer research programs for undergraduate students require a final poster or podium presentation. At Rutgers University, we emphasize interactive, hands-on training for undergraduate students to conceptually and draft written and graphical abstracts that would sum- marize the major findings of their research and be distributed at our
research symposium. During week 7 of a 10-week summer undergraduate research fellowship, students learned about the major components of an abstract using a variety of examples. Students were also taught common pitfalls to avoid in communicating research findings. After the discussion of each of the opening sections (title, introduction, hypothesis, and methods), students were provided 5-minutes for writing. After each portion of the abstract was composed, students volunteered to share their writings with the group, and the moderator provided constructive comments. Different examples of graphical abstracts were also presented to the students. Twenty seven students submitted abstracts during week 9 of the summer fellowship program which were assembled into booklets for the final research presentations. This interactive activity received the highest programmatic rating of the summer fellowship with a mean score of 4.39 (±0.71 SD) on a Likert scale of 1 to 5 with 5 representing the highest possible rating. Other weekly activities were also well-received with mean scores ranging from 3.57 to 4.32. Taken together, all interactive sessions included didactic instruction and student responses allows undergraduate students to begin writing and designing their written and graphical abstracts as part of a summer research fellowship. Supported by NEIHS R25ES020721, P30ES005022, ASPET, and the SOT Intern Program.

2949 Initial Assessment of a Summer Undergraduate Research Program

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Undergraduate research experiences are closely correlated with enhanced student learning and student success. Summer research programs are of particular interest as they offer unique opportunities for incorporating hands-on research with additional activities designed to enhance students’ scientific skills and career readiness. They also can be invaluable for developing a diverse scientific and professional workforce. The Summer Undergraduate Research in Environmental Health Sciences (SUREs) program is a 10-week program involving upper-level undergraduates and near-peer as well as faculty mentors. Student participants were matched with their faculty mentors by ranking their interests and were matched with near-peer mentors using a “speed-dating” approach. The goal of this study was to determine the most effective aspects of the program. Assessment using a pre/post survey instrument revealed that program participation increased student interest (from 9.1% to 100% of respondents) in teaching at the post-secondary level. In addition, student respondents reported gains in 39 of the 40 items adopted from the Undergraduate Research Student Self-Assessment, with statistically significant gains in students’ research skills and attitudes toward research. Survey respondents reported an overall increase in confidence, in particular, in their ability to participate in research. With respect to program activities, creating and presenting posters was the most highly ranked amongst the respondents in helping them understand environmental health sciences. Students also indicated that program components related to career skills and pathway were very helpful. Time spent with faculty mentors focused on research related activities and discussions on careers. Respondents indicated that the near-peer mentors provided emotional support and opportunities to ask for help and discuss difficulties. The results from this study provided insights into particular aspects of the SUREs program that best contribute to student learning and prepare students for careers in environmental health sciences.

2950 A Novel Toxicology Mentoring and Training Program Targeting Underrepresented Undergraduate Students in STEM Disciplines

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Exposure of undergraduate students to toxicology as a career choice is not coupled with increased diversity in the field. Thus understanding how the pool of undergraduates applying to graduate programs or entering careers in toxicology may be limited because students who are otherwise interested may not be aware of toxicology as a career option. This is particularly true for underrepresented students who are critical to a more diverse and representative future workforce in toxicology. A diverse workforce is critical for new scientific discoveries and human health care. The Toxicology Mentoring and Skills Development Training Program (ToxMSDT) - a collaboration amongst educators at Iowa State University, Tuskegee University and The Ohio State University - seeks to acquaint promising undergraduates with knowledge about toxicology careers and to provide training that equips students with toxicology fundamentals and skills that will aid their entry into graduate programs or careers in STEM disciplines. This is a year-long training and mentoring program. The public face of this program is ToxMSDT, a web resource comprised of learning e-modules and supplemental resources targeted to student learning as well as the general public to the discipline of toxicology. The learning e-modules and associated activities reflect key core competencies for training of the total toxicologist as identified through the 2012 SOT Toxicology Education Summit, namely (1) fundamentals of toxicology; (2) pathophysiology; (3) applied systems biology; (3) biochemistry; (4) molecular genetics; (5) regulatory frameworks; (6) communication skills; and (7) critical thinking skills. The second cohort of students will complete all required elements of the ToxMSDT program by June 30th 2018. This mentoring program advances the goals of National Institute of General Medical Sciences by creating an inclusive workforce to achieve the NIH mission.

2951 Foundational Concepts in Undergraduate Toxicology: Applying Vision and Change to the Development of Core Concepts and Learning Objectives for an Undergraduate Toxicology Course

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In 2011, the National Science Foundation and American Association for the Advancement of Science produced the “Vision and Change Report” (visionandchange.org) which sought to improve education in undergraduate biology for all students by defining Core Concepts: Evolution; Structure and Function; Information Flow, Exchange, and Storage; Pathways and Transformations of Energy and Matter; and Systems. Vision & Change has had a major impact on undergraduate biology education; scientific societies have created and adopted their own Core Concepts for their undergraduate courses and communicated them via peer reviewed publication. Appointed by the Undergraduate Education Subcommittee of the Five Core Concept Themes Committee, under the direction of the undergradate educators, the goal of the Learning Objectives ad hoc committee is to implement Vision & Change for undergraduates toxicology and to communicate its findings internally within the Society of Toxicology and externally via collaborations with multidisciplinary organizations including Course Source (coursesource.org), the Life Science Teaching Resource Collection (lifesciencetr.org), and peer reviewed publication. Data were collected and analyzed from more than 20 undergraduate toxicology syllabi from across the United States together with several undergraduate textbooks to quantify themes taught in all toxicology-related courses. A Learning Framework with Five Core Concept themes compatible with Vision & Change was developed: Evolution; Pathways and Transformations of Energy and Matter; Systems Toxicology; Biological Information; and Risk Assessment. Society Learning Goals for these five Core Concepts and Learning Objectives associated with each Society Learning Goal were also created. The publication of the work alongside the other major life science disciplines will facilitate the development and sharing of evidenced-based teaching materials for toxicology educators throughout the world and expand toxicology’s impact to a broader audience.

2952 Problems and Promises with Collaborative Learning in the Field in an Urban Public College


Sandra Swenson. John Jay College of Criminal Justice, New York, NY. 10019. Problems and Promises with Collaborative Learning in the Field in an Urban Public College Many STEM disciplines require collaborative and cooperative learning because it supports social interdependence which is inherent in scientific research as well as civic responsibility. Many studies report positive outcomes for collaborative and cooperative learning environments because learners work together to support each other in a common learning goal and students learn about group dynamics as well as interpersonal skills such as communication, trust, and dealing with conflicts. However, collaborative & cooperative learning in the field has its problems and its promises. This study examines the weaknesses as well as the strengths of collaborative field and laboratory work. In our research, each year, approximately 90 students were instructed in the proposed new method; the remainder of students (upwards of 90 in the control group) attended the traditional class that had been taught at the college for 15 years. All students were divided into groups of 4 in a lab class of
nearly 24 students, whether they were traditional or treatment classes, but each treatment group of Environmental Science students went into the field to collect data while the traditional groups only completed labs in the laboratory. Pre- and post-Likert scale surveys and open ended questionnaires were given to student volunteers. The significant results were slightly more favorable for the treatment group as compared to the control group and this study drills down to examine those differences and proposes some possible explanations as to why the treatment group did not have better results.

2954 Interdisciplinary Emergency Crisis Drills: Toxicology Students as Technical Experts

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St. John’s University has conducted Mock Crisis drills for the past three years in order to provide students with a career-focused, experiential learning opportunities in their various disciplines. Studies have shown that such practical learning increases student engagement. The Career Center at St. John’s has coordinated the Mock Crisis drills involving student volunteers from Criminal Justice, Homeland Security, Physician Assistant, Journalism, Public Relations, Legal Studies, and Toxicology. The past three crisis scenarios have involved potential exposure of “victims” (student actors) to a toxic chemical. The scenarios were constructed by faculty advisors. Student participants were involved as part of a required course assignment overseen by their faculty instructor or were recruited as volunteers. Toxicology graduate students served as technical experts to inform and communicate toxicological information to first responders and the “media,” played by students in the appropriate majors. The role of the toxicology students was to communicate toxicological information to students of multiple backgrounds. This was most challenging for the toxicology students when conveying information to the journalism and public relations students, who possessed no background in science. The information provided to them not only had to be easy to understand, but consistent as well. The toxicology students were also present at the mock press conferences. After the conference, professors commented on the performance of the students involved. The result was that the interdisciplinary team operated effectively in resolving the crisis. These drills have proved extremely useful, as they emphasize skills that aren’t traditionally taught, such as effective communication and operating with others outside of toxicology. Program outcomes were assessed by the Career Center. These drills have been recognized for their effectiveness as well, winning two awards in 2015. One was an honorable mention from the Student Affairs Professionals Association and the other was first place in the Alva C. Cooper Award for Best Practices in Career Development.

2955 Integrating Bioinformatics and Neurotoxicology into a Research-Based Course for Undergraduates

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As the ease with which “big data” can be generated increases, it is becoming more important for undergraduate educators to find ways to integrate this type of application and analysis into the curriculum. One way is to combine topics from multiple courses and teach these concepts “across the curriculum,” a process emphasized in the “Vision and Change” document. The biological database of Caenorhabditis elegans, produced by the National Science Foundation for the Advancement of Science. In a pilot study, we integrated concepts from a course in bioinformatics with a research-based class intended to provide students with opportunities to conduct original research. In this project, a student interested in nicotine addiction and metabolism was tasked with data-mining the literature for associated genes. Following nearest-neighbor analysis to determine the strongest links among the various genes, the student identified links connecting genes involved in nicotine addiction and metabolism to neurodegenerative diseases, nicotine metabolic pathways, and cancer. In light of the protective effect of nicotine, we then hypothesized that knocking down related genes from the bioinformatics analysis would increase the nicotine protection. Although the student did not complete the last part of the study, we learned better ways to (1) incorporate relevant bioinformatic questions into student research, (2) move bioinformatic results obtained from human data into a model system that is easily accessible to undergraduates, and (3) alter the scope of the overall research project so that is should be possible to complete within a 12-month period. Current revisions are underway to better refine the process, and to scale it so that a similar project could be used for a small upper-division course rather than a single student.

2956 Using C. elegans to Teach Genetics, Bioinformatics, and Neurotoxicology at Underfunded Undergraduate Institutions

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One of the difficulties of teaching complex concepts associated with genetics, bioinformatics, and toxicology to undergraduates is the lack of resources and infrastructure. Furthermore, it can be challenging to put together lab activities that are relatively cheap, build on concepts presented in potentially unrelated courses, and engage the student in individual and novel research. As universities around the country struggle with how to implement best practices, such as those outlined in the “Vision and Change” document endorsed by the American Association for the Advancement of Science and the National Science Foundation, we sought not only to create a unique learning environment on our respective campuses, but to also integrate a collaboration among universities to model for students how the enterprise of science can work. Thus, we have started working on a project that integrates the ease with which Caenorhabditis elegans (C. elegans) can be genetically manipulated with the information that came from the sequencing of this worm’s genome. In our curriculum, student enrolled in “Genetics” use C. elegans to phenotype and map genetic mutants using basic genetic and linkage analysis techniques. This information is used towards the end of the semester to help students learn how to use basic bioinformatics tools to identify candidate genes for further analysis in advanced courses or in the research laboratory. In some cases, where a bioinformatics course is available, the students can then learn to analyze more advanced “omic” level data (epigenetic, sequencing, gene expression, etc.) generated from the research lab. Ultimately, the goal of this
endeavor is to provide students with the basis of an individual research project that allows them to use the worms to evaluate the effect various genetic backgrounds have on exposure to environmental chemicals, such as pesticides. As such, we are progressing towards a model whereby undergraduates are able to study important gene-environment interactions. Although in its early stages, we ultimately anticipate that this process can be used as a model for collaboration for other small undergraduate universities who want to provide a rich lab experience that leads students towards a career in research.

2957 Using the Exposome and a Graph Theoretical Toolchain to Analyze Disparities

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To identify exposures associated with lung cancer mortality and mortality disparities by race and gender using an exposome database comprising of county-level heterogeneous data coupled to a graph-theoretical toolchain. Graph-theoretical algorithms were employed to extract paracilies (noise resilient dense subgraphs) from correlation graphs built using associations between 2,162 environmental exposures and lung cancer mortality rates in 2,067 counties, with clique-doubling applied to compute an absolute threshold of significance. Factor analysis and multiple linear regression were then used to analyze differences in exposures associated with lung cancer mortality and mortality disparities by race and gender. While smoking was highly correlated with rates of lung cancer mortality for white men and women, previously unidentified exposures were mostly associated with lung cancer mortality and mortality disparities for Blacks. Exposures beyond smoking are moderators of lung cancer mortality and mortality disparities by race and gender. An exposome database coupled with scalable combinatorial analytics provides a powerful new approach for analyzing relationships between multiple environmental exposures, mortality and mortality disparities. The impact of which will translate to other disparate health outcomes to inform environmental public health research, practice, and policy.

2958 Application of Collaborative Analysis Methods to Address Human Health Risk Assessment Decisions

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In the practice of human health risk assessment there is frequently tension between stakeholder groups (e.g., government, industry, advocacy groups, academia) regarding interpretation and confidence in results of toxicology and epidemiology data. In the application of toxicology for assessing risks, data gaps and uncertainties are common, with conclusions highly dependent upon scientific judgments and weight of evidence. When the decisions are controversial, opposing parties often form subcultural alliances in adversarial analysis or whether their position is justified by public or regulators. Such competing analyses without efforts to define the bases for areas of commonality and differences can result in confusion among decision makers regarding the current state of science. We explored the hypothesis that pursuit of high quality risk science is best served by actively involving a diversity of scientists from relevant disciplines and affiliations to identify and discuss data and judgments. Data drawn from the literature on chemical exposure health assessments and personal experience in developing over 50 multi-stakeholder peer reviews were evaluated to identify key variables that impact the successful application of collaborative analyses. Published case studies and best practices were evaluated. Little empirical research related to best practices for collaborative working groups and measurement of the quality, validity, and reliability of their results was found. The absence of published cases was confirmed through a benchmarking effort with experts leading to a finding that additional case studies are needed to build a robust basis for comparing adversarial and collaborative analytic techniques. An analysis of over 50 independent peer review meetings on health risk assessments illustrates that multi-disciplined groups of scientists can perform careful and detailed evaluations of assessments and reach conclusions on the scientific validity of the findings and results. A structured comparative approach, using panels of scientists inclusive of the range of stakeholders and scientific opinions to evaluate the data as a group and reach mutually agreeable conclusions, may be an option for controversial risk issues involving toxicology interpretation. Based on the resulting analysis a framework is proposed for the active application of methods of collaborative analysis to risk informed decision-making.

2959 The Write Way to a Successful Career: SOT Efforts to Fill Professional Writing Skills Gaps

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The SOT Graduate Education Subcommittee goals are to develop and implement initiatives within SOT and through academia, industry, and gov’t. partnerships to better equip graduate students for productive and successful careers, including opportunities for toxicology training and research. To address these gaps, the Subcommitte, along with the SOT Career Resources and Development Committee (CRAD), is considering ways to augment technical writing skills to facilitate career success. An anonymous survey was distributed to SOT graduate student members in Fall 2017 and 167 students responded. Many respondents primarily obtain technical writing skills through writing research proposals/dissertations (97%), while only half included skills related to chemical risk/safety assessments (52%) or study reports (49%). Half of respondents (50%) listed study director/monitor, gov’t. regulator or safety/risk assessor as their top career choice, versus academic or lab researcher. The most popular first choice for next training step was a postdoc in academia (41%) with govt. (28%) and industry (27% postdocs) well represented. While limited in sample size, the survey results suggest a need to educate current toxicology graduate students that professional writing skills often need to be tailored toward specific career paths or audiences, and can be distinct from skills obtained while conducting experimental research. We are now considering ways to facilitate the attainment of diverse types of technical writing skills to ultimately reduce professional skill gaps and better equip toxicology graduate students for future career success.

2960 Evaluating the Utility, Diversity, and Effectiveness of the SOT Mentor Match Database

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Mentor Match (MM), the SOT online mentoring resource is designed to help matching SOT members with members expressing interest in being a mentor. The MM database, at https://www.toxicology.org/application/jobbank/mentormatch.asp, was reviewed to examine whether it reflects SOT’s commitment to diversity and inclusiveness in all activities as stated in the SOT mission and policies. This review also considered whether the current MM structure (e.g., stand-alone database) is an effective format and what potential improvements and/or alternate formats(s) might better foster inclusiveness and diversity and facilitate mentor-mentee matches and interaction. Based on mentee (n=327) and mentor (n=184) data currently housed in MM, mentees outnumber mentors almost 2 to 1 and 20% of mentees are mid-career professionals (i.e. no longer students or post-docs), while 11% of mentors are grad students or post-docs. Whereas, 95% of mentors reside in N. America (93% within the US), 15% of mentees reside outside N. America. While mentee gender was near balanced, 55% of mentors are male. These data suggest that geographic diversity may be lacking. Conclusions related to other metrics of inclusiveness and diversity, such as (but not limited to) ethnicity, socioeconomics, role (e.g., manager, bench scientist) cannot be drawn, since these data are not collected. Similarly, website tracking data (e.g. number of hits/day) duration of browsing) may have also informed the success. Most respondents viewed necessary professional writing skills as encompassing grants for research funding (89%) or authorship of manuscripts (97%), while only half included skills related to chemical risk/safety assessments (52%) or study reports (49%). Half of respondents (50%) listed study director/monitor, gov’t. regulator or safety/risk assessor as their top career choice, versus academic or lab researcher. The most popular first choice for next training step was a postdoc in academia (41%) with govt. (28%) and industry (27% postdocs) well represented. While limited in sample size, the survey results suggest a need to educate current toxicology graduate students that professional writing skills often need to be tailored toward specific career paths or audiences, and can be distinct from skills obtained while conducting experimental research. We are now considering ways to facilitate the attainment of diverse types of technical writing skills to ultimately reduce professional skill gaps and better equip toxicology graduate students for future career success.
Chapter 2961

**International ToxScholar: Increasing Awareness in Toxicology Globally**

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The political situation in many developing countries is often complex, but science can be a vehicle to break down barriers. The goal of the SOT Education Committee’s International ToxScholar Award (ITS) is to increase awareness, breadth, and depth of toxicology in such countries. ITS awards up to $1250 for toxicologists to travel to 139 eligible countries. Since 2010, 24 scholars have completed visits to 35 host institutions. Some scholars incorporated multiple institutions in their itinerary, with one visiting 12 institutions in a single ITS visit. Following their visit scholars submit a final report and share their experiences via the SOT Communique. Examining case studies over the duration of the ITS program has demonstrated the program’s value. Dr. Blas Ramirez, a 2018 ITS scholar, visited 12 institutions in Mexico and Moldova to pharmacy/clinical lab science students. An audience survey indicated the following: 21-25yrs; 90% female; 89% found value in the symposium; and 100% support continuing the ITS program. Students also indicated an increase in knowledge about toxicology, toxicology careers, and interest in pursuing advanced education. Some scholars, like Dr. Blas Ramirez and Dr. Velez utilized key contacts in Cuba to understand how best to focus toxicology outreach and was awarded ITS funding to present his work on Air Pollution and Genetic Susceptibility. Continuing the momentum of this ITS visit he worked with the Hispanic Organization of Toxicology (HOT) and Dr. Wallace Hayes, to receive the SOT Global Strategies award for the development a 2-day course on current trends in toxicology and food safety in Havana Cuba. The 150 participants learned about the state of the science in key toxicology topics, were introduced to the benefits of SOT membership, SOT international programs (GSSEP) and Special Interest Groups (i.e. HOT). Subsequently, 100 participants requested free HOT membership to stay connected to SOT. To date SOT has received 125 membership fee waivers which is an indirect testament to the value of the program and the impetus given to host institution members to join SOT. Together this demonstrates that the ITS program facilitates ongoing collaborations and networking which are key to promoting toxicology globally. The continued success of this program is dependent on attracting applicants who request visits to institutions in a wider array of target countries.

Chapter 2962

**Identification of Cytotoxicity-Inducing Factors That Are Released from Cells by Methylmercury**

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Methylmercury is a common environmental pollutant. Methylmercury crosses the blood-brain barrier and accumulates in the brain, causing neuronal cell death and leading to central nervous system disorders. However, the underlying molecular mechanisms that result in neuronal toxicity are almost unknown. It has been reported that increased levels of extracellular glutamate, aspartic acid, and serotonin, among others, are involved in methylmercury-induced neuronal cell death. However, little is known about extracellular factors involved in methylmercury toxicity. Using cultured cell lines, in this study, we examined the possible existence of a factor that is driven out of the cell by methylmercury and that subsequently shows cytostatic activity (cytotoxicity-inducing extracellular factor, CIEF). To confirm the existence of CIEF, which is driven out of the cell by methylmercury, we prepared methylmercury preconditioning medium (MeHg-PM) according to the following method. Human embryonic kidney HEK293 cells were cultured in medium containing methylmercury for 2 hr. After washing with PBS, the cells were further cultured for 6 hr in methylmercury-free medium. The resulting culture medium was used as MeHg-PM. Untreated HEK293 cells and C17.2 cells (mouse neural stem cells) were placed in the obtained medium and, as a result, showed growth inhibition, which resulted in significantly inhibited cell growth. This cell growth inhibition was not affected by heating or proteinase K treatment, suggesting that neither proteins nor peptides caused the growth inhibition. We next performed a metabolic analysis for MeHg-PM, and identified 3-phenylpropionic acid, lactic acid, ornithine, proline, and beta-alanine were increased compared with control-PM. Treating HEK293 and C17.2 cells with each of the six substances minimally affected the proliferation of both cell lines. Among these six substances, however, only citrulline slightly but significantly increased the sensitivity of C17.2 and HEK293 cells to low levels of methylmercury. Citrulline is thought to be a methylmercury toxicity-enhancing factor whose extracellular release is enhanced by methylmercury.

Chapter 2963

**Potential Mechanisms of Inorganic Mercury Intoxication in Rat Kidney Cells**

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Mercury is one of the ubiquitous toxic metals that is found in the environment and humans are exposed mostly via occupational and dietary sources. In the body, the primary site of accumulation and toxicity of inorganic mercury (Hg<sup>2+</sup>) is the kidney. The mechanism of Hg<sup>2+</sup>-induced toxicity of target cells. Normal rat kidney (NRK) cells were exposed to several concentrations of Hg<sup>2+</sup> for various times. Using imaging and biochemical techniques, Hg<sup>2+</sup>-treated cells showed significant alterations in cytoskeletal structure, oxidative stress, and calcium availability. After Hg<sup>2+</sup> exposure, NRK cells experienced actin disorganization and loss of cytoskeleton integrity. Additionally, intracellular levels of hydrogen peroxide and superoxide increased, which led to a rise in the amount of oxidative stress. Interestingly, levels of calcium, a vital and strictly regulated second messenger in cells, were elevated in the cytosol immediately following exposure. It is likely that intracellular Hg<sup>2+</sup> leads to an increase in IP<sub>3</sub> levels, which induces calcium release from the endoplasmic reticulum. Overall, Hg<sup>2+</sup>-treated cells experience many adverse changes including cytoskeleton collapse, reactive oxygen species accumulation, and elevated calcium levels. Although further studies are required for a complete understanding, a more definite explanation for the mechanisms of intracellular Hg<sup>2+</sup> intoxication and cellular injury has been established.

Chapter 2964

**Elucidation of Mechanisms Underlying Induction of Tumor Necrosis Factor-α by Methylmercury in Mouse Brain**

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Methylmercury is an environmental pollutant that causes neurotoxicity. We recently found that expression of the inflammatory cytokine tumor necrosis factor-α (TNF-α) is induced in brains of mice treated with methylmercury. Although TNF-α may be involved in the neurotoxicity caused by methylmercury, the mechanisms involved in the induction of TNF-α expression by methylmercury are unknown. In this study, we aimed to identify cells involved in the induction of TNF-α expression by methylmercury in mouse brain, to elucidate the underlying molecular mechanisms. Seven days after subcutaneous injection of C57BL6 mice with saline or methylmercuric chloride (25 mg/kg), TNF-α mRNA levels were determined in the brain tissues by in situ hybridization. We found that TNF-α was hardly expressed in the saline-administered group, whereas TNF-α-expressing cells were observed in the whole area of the brain in the methylmercury-treated group. It was reported that astrocytes and microglial cells are involved in the induction of TNF-α expression. Therefore, we performed immunostaining using antibodies specific for GFAP or IBA1, which are specifically expressed in astrocytes and microglial cells, respectively. The TNF-α-expressing cells did not colocalize with GFAP-positive cells, but with IBA1-positive cells. The results suggest that microglial cells are mainly involved in the induction of TNF-α expression by methylmercury in the mouse brain. In addition, methylmercury induced TNF-α expression in mouse microglial cell line BV2. Pretreatment with the RNA synthesis inhibitor, actinomycin D, mostly suppressed the elevation of TNF-α mRNA level by methylmercury, suggesting that transcriptional activation is involved in methylmercury-induced expression of TNF-α. MAP kinases such as JNK, ERK, and p38 are known to be involved in the induction of TNF-α transcription in microglial cells. We found that JNK, ERK and p38 were phosphorylated by methylmercury treatment in BV2 cells. Moreover, when the cells were pretreated with specific inhibitors, only the p38 inhibitor suppressed the induction of TNF-α expression by methylmercury. These results indicate that methylmercury may induce TNF-α expression via activation of p38 in microglial cells. We are examining the detailed molecular mechanisms involved in the induction of TNF-α expression by methylmercury.
Methylmercury (MeHg) is a neurotoxicant that exists widely in the natural environment. Because of fears regarding the adverse effect of MeHg contained in seafood on the developing fetus brain, pregnant women have been cautioned against consuming fish and shellfish in several countries. So far it has been reported that some food ingredients have effects on tissue concentration of Hg. In this study, we investigated the effect of co-administration of wheat bran and MeHg on tissue concentration of Hg in MeHg-exposed mice. Female BALB/c mice (age 3 weeks) were fed with the basal diet (AIN-76) containing no Hg for 6 weeks. Then after a single administration of MeHg (5 mg MeHgCl/kg), mice were housed in metabolic cages and fed with the same basal diet supplemented with or without wheat bran (300g/kg) for 2 weeks. Daily urine and feces were collected from wheat bran-treated and non-treated groups for 2 weeks. The accumulative amount of urinary Hg was higher in wheat bran-treated group than in control group. After the daily accumulation of urine and feces for two weeks, mice were deeply anesthetized with isoflurane, given by transcardiac perfusion with 0.9% ice-cold saline, and blood, brain, liver and kidney were obtained. Total Hg levels of blood, brain and kidney were lower in wheat bran-treated group than in control group. However, liver showed similar total Hg levels among both groups. These results were the same as our previous studies on mice fed with 30% wheat bran diet for 3 months before MeHg exposure which showed acceleration of Hg excretion into the urine, suggesting excretion of Hg from tissues was not caused by enhanced metabolism of MeHg by gut bacteria. Although total Hg concentration in feces is currently being measured, the results suggest that wheat bran is effective to accelerate Hg excretion into urine. It remains to be solved the reason why both pre- and co-administration of wheat bran have no effect on the excretion of Hg from liver. All experiments were carried out in accordance with guidelines for the care and use of laboratory animals established by the National Institute for Minamata Disease.
Cystathionine Gamma-Lyase is a Key Protein Repressing Methylmercury Poisoning Symptoms in Mice

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Methylmercury (MeHg) is an environmental electrophile that readily modifies protein thiols, causing toxicity. MeHg is inactivated by sulfur nucleophiles such as cysteine (CysSH), glutathione (GSH) and its related reactive persulfides through the formation of GSH adduct (MeHg-SG) and bismethylmercury sulfide ((MeHg)2S), respectively. Cystathionine gamma-lyase (CSE) catalyzes production of CysSH and its persulfides (CysSSH). Previously, we reported that CSE was required for in vivo formation of (MeHg)2S, suggesting that CSE is a key protein for protection against MeHg toxicity. To address this issue, we used CSE knockout (KO) mice. CSE KO and Wild-type (WT) mice were orally administered with MeHg. Loss of coordinated movement, body weights, survival rates and pathological changes in the brain were monitored to assess sensitivity to MeHg. Levels of mercury and sulfur nucleophiles in the organs were measured with an atomic absorption mercury detector and a mass spectrometer, respectively. We found that CSE KO mice were more sensitive to MeHg than WT mice and, concomitantly, had higher mercury accumulation in the brain. Levels of CysSH, GSH, and their persulfide derivatives were lower in the liver and kidney of CSE KO mice than in those of WT mice. These results suggest that CSE deletion in mice caused decreased levels of sulfur nucleophiles due to the disruption of CysH metabolism, resulting in increased sensitivity to MeHg.

Methylmercury Induces Metabolic Alterations in C. elegans: Role for C/EBP Transcription Factor

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Methylmercury (MeHg) is a well-known neurotoxicant; however, its role in metabolic diseases have been gaining wider attention. Chronic exposure to MeHg (measured by hair and toenail Hg levels) shows an association with diabetes mellitus (DM) and metabolic syndrome (MS). MS is a multifactorial condition that includes obesity, insulin resistance, dyslipidemia, and systemic oxidative stress. As incidences of both MS and DM increase on the rise globally, it is important to understand the potential role of MeHg in the development of the disease. In vitro experiments using the transgenic worm Caenorhabditis elegans can aid in identifying gene-environment interactions upon MeHg exposure and MS. We hypothesized that MeHg exposure in C. elegans would alter transcription of genes involved in the development of MS. RNA from wild-type worms exposed to MeHg was collected immediately after treatment, and used for gene expression analysis by DNA microarray. MeHg differentially regulated 215 genes, 46 were metabolic genes, 10 genes involved in lipid homeostasis, and 12 genes involved in carbohydrate homeostasis. Of particular interest was cebp-1, the worm homolog to human C/EBP, a pro-adipogenic transcription factor implicated in MS. MeHg increased the RNA levels of cebp-1 and conserved molecular signaling pathways. We hypothesize that C. elegans lacking src-1 or src-2 (homologs of C-Src and Fyn, respectively) would show persistent skn-1 (Nrf2 homolog) activity, leading to greater resistance against MeHg toxicity. L1 stage worms were treated for 1 hr with doses of MeHg ranging from 0 - 100 µM to determine a lethal dose curve. LD₅₀ values were established for each strain (WT: 46.61 µM, skn-1 KO: 39 µM, src-1 KO: 35.68 µM). A significant 2.4 fold increase in src-1 expression in src-2 KO treated worms compared to untreated worms was also noted, supporting the notion that src-2 may act as an inhibitor of skn-1 (2-tailed t-test, n=3, p<0.05). A significant 2.2 fold increase in skn-1 expression in src-2 KO treated worms compared to untreated worms was also noted, supporting the notion that src-2 may act as an inhibitor of skn-1 (2-tailed t-test, n=3, p<0.001). It is also conceivable that knockdown of src-1 or src-2 led to compensatory upregulation of src-2 or src-1, respectively. The current data suggest that src-2, the homolog to Fyn, is likely the stronger src family inhibitor of skn-1.
double positive CD133+24+ cells are more resistant to cadmium expo-
sure when compared to the CD24+ only cells. In addition, there is no
change in the number of double positive cells in response to cadmium
treatment, whereas the number of CD24+ cells significantly decrease.
Both the populations of cells form domes in culture indicative of vec-
torial active transport. In addition, the double positive CD133+CD24+
cells separated from the CD133+CD24- cells. In conclusion, our data suggests that the CD133+CD24+ cells are likely the progenitor
cells present in the kidney involved in tubular regeneration whereas the
CD24+ cells are the differentiated cells which are more sensitive to toxic
insults and are replaced by the stem/progenitor cells upon toxic insult.

2974 Effects of Calcium Disodium EDTA Nanoparticles against Cadmium Toxicity in Rats

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Cadmium is one of the most important environmental and industrial pol-
lutants that adversely affect human and animal health. Nanoparticulate
drug delivery systems are being used to alter the drug's biopharmaceu-
tics and pharmacokinetics such as drug absorption, distribution, metab-
olism, and elimination. In this study the toxic effects of cadmium sulfate
on adult female Sprague Dawley rats as well as the ameliorating effects of
CaNa2EDTA nanoparticles against such toxicity were investigated. The
predictive protective effect was compared with CaNa2EDTA micro-
particles. For toxicological assessment of cadmium toxicity, animals
were classified into a control and three experimental groups; the three
experimental groups received 60 ppm cadmium sulfate in drinking
water for 10 weeks. At the end of the 6th week of the experiment, the
second and third experimental groups were injected intraperitone-
ally with 50 mg/kg/day CaNa2EDTA microparticles and nanoparticles
respectively for 4 courses (4 days each) with an interval of 3 days. The
result showed that treatment with CaNa2EDTA nanoparticles exerted
marked protective effect against cadmium toxicity, which was indicated by
increasing body weight gain and food and water consumption and reducing
desired liver, kidney and bone cellular damage when compared to cadmium
treated group. Improvement in the bone mineral density was observed in
CaNa2EDTA nanoparticles treated group. Serum urea and creatinine con-
centrations as well as serum and urine metallothioneins levels were decreased by CaNa2EDTA nanoparticles treatment. Both
 cellular and humoral immune responses were increased in CaNa2EDTA
nanoparticles treated group. However, there is an increase in lym-
phocytes count and a decrease in neutrophils and monocytes counts. Serum IL-2 and IL-6 levels were increased by CaNa2EDTA nanoparticles
 treatment. CaNa2EDTA microparticles treatment induced mild protect-
ive effect against cadmium toxicity as compared to the nanoparticles
form. These findings suggested that CaNa2EDTA nanoparticles could be
effectively used as a chelating agent against cadmium toxicity as they
have a better affinity to chelate cadmium and lesser side effects than the
microparticles form.

2975 Cadmium-Mediated Activation of the HSP90/HSF1 Pathway Regulated by Reactive Persulfides and Polysulfides

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Cadmium is an environmental electrophile that modifies reactive thiols in proteins, indicating that this heavy metal may modulate redox-signa-
tion pathways. The current consensus is that reactive persulf-
ides and polysulfides produced by cystathionine γ-lyase (CSE) and
cystathionine β-synthase are highly nucleophilic and thus cadmium
may be captured by these reactive sulfur species. It has previously
been found that electrophile-mediated covalent modifications of the
heat shock protein (HSP) are involved in the activation of heat shock
factor 1 (HSF1) pathway. The effects of cadmium on the activation of
HSP/HSF1 pathway were investigated in this study. Exposure of bovine
aortic endothelial cells to cadmium resulted in modification of HSP90
and HSF1 activation, thereby up-regulating the downstream protein
HSP70. The siRNA-mediated knockdown of HSF1 enhanced the cyto-
toxicity induced by cadmium, suggesting that the HSP90/HSF1 pathway
contributes to protection against cadmium toxicity. The knockdown of
CSE and/or cystathionine β-synthase decreased the levels of reactive
sulfur species in the cells and increased the degree of HSP70 induction
and cytotoxicity caused by exposure to cadmium. Overexpression of
Diabetes mellitus (DM) is a growing worldwide epidemic. Impaired insulin release is a hallmark of type I DM and can have a role in the progression of type II DM. Epidemiological and experimental studies show that exposure to the metal cadmium (Cd), is associated with pre-DM, DM, and altered plasma insulin. How Cd disrupts pancreatic islet function and glucose-stimulated insulin release is not known. The objective of the current study was to begin to characterize the in vitro toxic effects of Cd on pancreatic beta cells. To do this, we used a model of pancreatic beta cells, the MIN6 cell line, and quantitated lactate dehydrogenase (LDH) release from cells exposed to Cd in serum-free physiological saline buffer. To begin to assess how Cd may alter glucose-stimulated insulin release MIN6 cells were exposed to 100 µM Cd in low (0.5 mg/ml) or high (3 mg/ml) glucose-containing buffer for 4 hours then LDLH was quantified. Initial results show that MIN6 cells incubated in low glucose buffer and exposed to 100 µM Cd caused 10.5 ± 0.6% of total LDLH release. Cd-exposed cells incubated in high glucose buffer had a statistically significant higher level of LDLH release at 21.2 ± 0.7%. There was no difference in LDLH release between cells incubated in low vs high glucose buffer in the saline-treated control groups. Cells were also treated with 5 µM nifedipine since L-type voltage-gated calcium channels play a fundamental role in beta cell function. Nifedipine treatment did not cause a statistically significant change in LDLH in release in cells exposed to 100µM Cd in either low or high glucose-containing buffer. These preliminary data indicate that glucose concentration is a significant factor in determining the toxicity of Cd in the MIN6 pancreatic beta cell line while L-type voltage-gated calcium channels apparently do not.
or down-regulation, p-value 0.005). Gene ontology (GO) analysis of the up-regulated genes showed the enrichment of biological process like response to oxygen containing compound and regulation of cell proliferation. Pathway analysis of up-regulated genes showed the enrichment of the axonal guidance, production of nitric oxide, reactive oxygen and acute phase response pathways. Transcriptional factor analysis of the up-regulated genes showed the enrichment of the estrogen receptor (55.5% genes). The findings strongly suggest that acute exposure of Cd on BEAS-2B cells can modulate the expression of the genes regulated by estrogen receptor and could contribute to the cadmium induced lung cancer.

**2983 Developmental Cadmium Exposure Disrupts Zebrafish Vestibular Development**

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The incidence of hyperactive behavioral disorders in the United States has risen by an average of 5% per year. Currently, 6.4 million children aged 4 to 17 years of age have received an ADHD diagnosis. The underlying causes are largely unknown though, like many disorders, it is likely a combination of genetic and environmental factors. One suspected environmental factor is cadmium (Cd), which has been shown to be nephro-, neuro-, and osteotoxic, and is a known carcinogen. Developmental studies have linked prenatal Cd exposure to reduced birth weight and hyperactivity. Our lab has shown that zebrafish exposed developmentally to 30 - 60 parts per billion (ppb) Cd from four hours post fertilization to seven days post-fertilization are hyperactive, have delayed otolith formation, and exhibit pronounced circling behavior. Radiotrace (109Cd) uptake analysis revealed that total body burden was 234 ng Cd/g body weight after seven days of exposure to 40 ppb Cd; human total body burden ranges from 45 - 775 ng Cd/g body weight. Furthermore, we have shown that developmental Cd exposure alters the timing of otolith appearance, likely due to vestibular calcium sensing, and transport. In light of the fact that otoliths are vital for vestibular function, these findings are interesting because they may explain why hyperactivity is observed in both children with inner ear dysfunction and those developmentally exposed to cadmium. While the mechanisms behind this Cd-induced ototoxicity remain unclear, their link to vestibular-based behavioral abnormalities merits further investigation.

**2983a Environmental Cadmium Exposure Disrupts Lung Fatty Acid Metabolism**

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Our previous studies showed that low dose cadmium (Cd) altered protein redox states resulting in inflammatory signaling, actin cytoskeleton disruption, and fibrosis. However, little is known about the effects of Cd at relatively low level on the metabolic regulation in pulmonary function and potential impact on pulmonary health. To address this issue, we performed ultra-high resolution mass spectrometry-based high resolution metabolomics (HRM) on lungs from mice exposed to Cd by drinking water (10 mg/L) for 20 weeks. The results show that abun-
dance of 426 metabolic features in mouse lung were changed by Cd exposure (p-value = 0.5, p = 0.03, n = 10) than females (r = 0.2, p = 0.09, n = 9). The results was associated with pack years of smoking and was higher in males (r = -0.4, p ≤< 0.01, n = 9), magnesium (r = -0.5, p = 0.001, n = 9) and copper (r = -0.4, p = 0.01, n = 9) in females. In males, Mn, Al, Ni, Co, Cu, Sn and Ba were correlated with Cd. Lung Cd level was associated with pack years of smoking and was higher in males (r = 0.5, p = 0.03, n = 10) than females (r = 0.2, p = 0.09, n = 9). The results suggest that the induction of metallothionein results in increased metal accumulation, and the increased burden potentiates cellular dysfunction and further contribute to lung diseases.

**2983b Characterization of the Effects of Selenium and Phenyl Mercuric Acetate Exposure on the Lipid Profiling of the Free Living Nematode Caenorhabditis elegans**

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Mercury (Hg) compounds exposure from environmental and food sources is a significant threat to public health. Organomercuro compounds such as phenyl mercuric acetate (PMA) can cause harmful damage to the nervous system. Caenorhabditis elegans studies showed that chronic exposure to mercury compounds induces neuron degeneration likely due to the increase in reactive oxygen species (ROS). Selenium (Se) is an essential trace element required for activation of many antioxidant enzymes. Accordingly, Se is capable of decreasing deposition of Hg during co-exposure possibly due to the high affinity between Hg and Se and reducing the ROS level by activating antioxidant enzymes. In this study, we evaluated the treatment of C. elegans with PMA, Se, and PMA + Se to determine the effect it could have on the fatty acid and the lipid profile. The nematode C. elegans was exposed to a sub-lethal concentration of PMA to investigate its toxic effect. Exposure to PMA + Se was used to determine if Se could minimize or counteract the effects of PMA. Fatty acids methyl esters (FAMES) of C. elegans were analyzed by gas chromatography/mass spectrometry (GC-MSD). Lipid profiling was analyzed using high-performance thin layer chromatography (HPTLC) and liquid chromatography-mass spectrometry (LC-MS). Microscopy and other biological results showed that exposure to PMA increased levels of ROS in C. elegans which could account for the change in the fatty acid content and lipid profile of the nematodes. Selenium showed the ability to partially counteract the effects of PMA. Elucidation and identification of fatty acid methyl esters from each treatment will be presented.

**2983c Cadmium Correlations with Other Metals in Diseased Human Lung**

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Our recent research in mice shows that low cadmium (Cd) exposures by drinking water potentiate inflammation and fibrosis. In these studies, we found that Cd dosing resulted in changes in contents of other metals in lung. Multiple metals have been associated with lung pathologies, but understanding of metal-metal interactions in lung disease is limited. To gain an understanding of correlations of Cd with other metals in human lung, we used Inductively Coupled Plasma Mass Spectrometry (ICP-MS) to measure Cd (μg g⁻¹ SE, 38 ± 8.9 μg/g tissue, n = 19) and 13 other metals in human lungs from 10 males and 9 females with a range of lung diseases. Mean age was 59 ± 9 y. Nine males reported being smokers (37 ± 10 pack years) and 5 females reported being smokers (31 ± 9 pack years). Results showed that lung Cd level is negatively associated with 3 metals including manganese (r = -0.4, p ≤< 0.01, n = 9), magnesium (r = -0.5, p ≤< 0.001, n = 9), and copper (r = -0.4, p = 0.01, n = 9) in females. In males, Mn, Al, Ni, Co, Cu, Sn and Ba were correlated with Cd. Lung Cd was associated with pack years of smoking and was higher in males (r = 0.5, p = 0.03, n = 10) than females (r = 0.2, p = 0.09, n = 9). The results suggest that the induction of metallothionein results in increased metal accumulation, and the increased burden potentiates cellular dysfunction and further contribute to lung diseases.

**2984 Metabolism Reprogramming in Hexavalent Chromium-Induced Lung Carcinogenesis**

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Hexavalent chromium, Cr(VI), is a known human carcinogen that is a worldwide environmental health concern. It is well understood that reactive oxygen species, genomic instability, and DNA repair deficiency are important contributors to Cr(VI)-induced carcinogenesis. However, some cancer hallmarks remain understudied for the mechanism of Cr(VI) carcinogenesis. Specifically, increased de novo lipid synthesis is an important mechanism of carcinogenesis and tumorigenesis in multiple types of cancers, but it is still unclear the role metabolic reprogramming plays in Cr(VI) carcinogenesis. It has previously been reported that acute exposure to Cr(VI) can increase glycolysis and decrease mitochondrial respiration in human lung cells. Here, we report that Cr(VI) is able to induce expression of lipogenesis proteins. Specifically, our data show prolonged exposure (1 month) of human lung epithelial cells (BEAS-2B
cells) to sodium chromate increases ATP citrate lyase (ACL), and fatty acid synthase (FASN) expressions. Interestingly, we also found that chromium-transformed BEAS-2B cells and human lung fibroblasts (WTHBF-6 cells) had no major changes in their glycolytic function as measured by the Seahorse Analyzer when compared to their passage-matched control cells. Future work is aimed at continuing to investigate changes in lipid metabolism and the mechanisms that regulate lipid metabolism in Cr(VI)-induced lung carcinogenesis.

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### 2985 Cytotoxicity and Morphological Transformation in C3H/10T1/2 CI Mouse Embryo Cells by Sodium Chromate: Enhancement by Ascorbate


Soluble/insoluble hexavalent chromium (Cr(VI)) compounds are animal and human carcinogens. They induce respiratory cancers in humans when inhaled, and stomach, liver, kidney, and G. I. tract when given in drinking water. Soluble/insoluble Cr(VI) compounds induce mutations, DNA-DNA cross links, and DNA-protein cross-links in cultured mammalian cells. We found weak, dose-dependent induction of foci by PbcCrO₄, but no induction of foci by CaCrO₄, K₂CrO₄, and Na₂CrO₄. The reductants ascorbate, glutathione (GSH) and cysteine (CySH), convert intracellular Cr(VI) to Cr(III) and induce foci in lung and breast carcinoma tissue culture cells in mammalian cells, by reducing it to Cr(VI), Cr(III), and Cr(II) with generation of hydrogen peroxide and hydroxyl radicals, causing mutation. We hypothesized weak/no induction of morphological transformation of 10T1/2 cells by Cr(VI) compounds in Basal Medium Eagle (BME), were due to low concentrations of ascorbate, GSH, and/or CySH in BME. Ascorbate is present in human serum at μM concentrations but only at μM concentrations in BME. We studied effects of the highest non-cytotoxic concentrations of ascorbate, GSH, and CySH on Cr(VI)-induced cytotoxicity in 10T1/2 cells. When 10T1/2 cells were treated with Cr(VI), ascorbate enhanced the cytotoxicity of Cr(VI) at concentrations up to 0.1 mM, and reduced the cytotoxicity of Cr(VI) at concentrations of 0.25 mM and higher. One non-cytotoxic concentration of ascorbate, 0.00625 mM, caused dose-dependent induction of cytotoxicity/morphological transformation when added to various concentrations of sodium chromate in 3 experiments. Our findings indicate ascorbate enhanced cytotoxicity/morphological transformation of 10T1/2 cells treated with sodium chromate, likely by enhancing reduction of intracellular Cr(VI) with ascorbate to generate Cr(III) and ROS. GSH or CySH, separately/together, did not enhance Cr(VI)-induced cytotoxicity to 10T1/2 cells. Supported by undergraduate fellowships from Provost’s Office at USC, funding from the S. Program, Dept. of Mol. Micro.,/m., discretionary funding, and grant ES03341, from NIEHS/NIH, to JRL.

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### 2986 Chromate-Induced Suppression of E2F1 and RAD51 in the Homologous Recombination Response

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Hexavalent chromium [Cr(VI)] is a global environmental contaminant and known human lung carcinogen. Specifically, particulate Cr(VI) is considered to have the highest carcinogenic potential as it lodges at bifurcation sites in the lung and slowly dissolves over time. While Cr(VI) is known to have many intracellular effects, the mechanism of Cr(VI) carcinogenicity is currently unknown. Maintaining a functional homologous recombination (HR) pathway is crucial in preserving genomic stability and preventing carcinogenesis. Studies show that Cr(VI) induces DNA double strand breaks (DSBs) and also inhibits HR repair resulting in chromosome instability (CIN), a hallmark event in lung cancer. Data on our human lung cell model show longer exposures to particulate Cr(VI) lead to loss of the critical effector step in the HR pathway through impaired RAD51 function while preceding steps remain functional. However, it is currently unknown how Cr(VI) is impairing RAD51 function. The transcription factor E2F1 has previously been shown to be involved in HR through transcription of DNA repair proteins and it plays a role in the HR response to DSBs. Furthermore, research shows Cr(VI) modulates epigenetic changes, however, only a few studies have investigated these effects in the context of DNA repair and lung cancer. We found exposure to particulate Cr(VI) reduced RAD51 and E2F1 mRNA at all exposure times. Protein levels were also reduced following a similar pattern to the one observed in the mRNA results. We also observed inhibited protein foci formation in response to prolonged particulate Cr(VI) exposure. Finally, we investigated how miRNAs may be involved in Cr(VI)-impaired HR. These results provide mechanistic insight into the carcinogenesis of particulate Cr(VI) in human lung cancer and suggest a role of the transcription factor E2F1 in the HR response. This work was supported by NIEHS grant ES016893 (J.P.W.) and the Jewish Heritage Fund for Excellence Research Enhancement Grant Program at the University of Louisville School of Medicine.

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### 2987 Chronic Exposure to Particulate Hexavalent Chromium Induces Centrosome Abnormalities and Disrupts Mitosis in Both Sea Turtle and Alligator Primary Lung Cells


Hexavalent chromium (Cr(VI)) is a well-established human lung carcinogen. Tumors in the lung are often characterized by numerical chromosome instability (CIN). One way CIN can be established is through abnormal centrosome function. Specifically, centrosome amplification has been associated with numerical CIN. Previous studies in our lab established that Cr(VI) induces premature centrosome separation and disengagement in human lung cells; events that could underlie Cr(VI) induced centrosome amplification. We also showed Cr(VI) exposure induces a decrease in mitotic index and prolonged G2 arrest; events hypothesized to lead to centrosome amplification in human lung cells. These findings have given insight into the mechanism for Cr(VI) induced centrosome amplification. Using a One Health approach, this study extends the previous analyses into wildlife cell cultures. Primary lung cell lines from both Dermochelys coriacea and Alligator mississippiensis were used to determine what effects Cr(VI) exposure has on mitotic index, centrosome separation, and centrosome disengagement in cells from these species. We found results in wildlife cell culture that are consistent with human lung cells. Cr(VI) exposure resulted in a statistically significant decrease in mitotic index that correlated with time and concentration of Cr(VI) exposure. Cr(VI) also induced centrosome amplification and premature centriole disengagement in the wildlife cultures. These findings have given insight into the centrosome amplification in human lung cells, and help to understand how Cr(VI) induces centrosome amplification; information that can ultimately lead toward a more mechanistic understanding of Cr(VI) carcinogenesis. This study is an example of a One Health approach that recognizes the health of humans, animals and the environment are connected.

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### 2988 NAD-Dependent Sirtuin 3 Negatively Regulates Mitophagy of Chromium(VI) Transformed Cells

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Chronic exposure of human bronchial epithelial BEAS-2B cells to hexavalent chromium (Cr(VI)) induces malignant cell transformation and these Cr(VI)-transformed cells are tumorigenic. The present study investigated the role of NAD- dependent sirtuin 3 (SIRT3) in mitophagy of Cr(VI)-transformed cells. Our results showed that expression of SIRT3 at both mRNA and protein level was elevated in Cr(VI)-transformed cells compared to that in passage-matched normal cells. The results from Seahorse analysis showed that mitochondrial ATP production and proton leak were reduced in Cr(VI)-transformed cells. Inhibition of SIRT3 by its shRNA further reduced mitochondrial ATP-production and proton leak in Cr(VI)-transformed cells. Similarly, in Cr(VI)-transformed cells the p62 less available to bind to KEAP1, resulting in increased binding of Nrf2 and Parkin and Pink1, two mitophagy proteins, were elevated in Cr(VI)-transformed cells, Nrf2 is constitutive activated. Inhibition of Nrf2 by its shRNA decreased SIRT3 expression. These results suggest that Nrf2 is a positive regulator of SIRT3. The results from CHIP assay showed that Nrf2 binds to antioxidant response element (ARE) of SIRT3 gene promoter. The present study demonstrates that SIRT3 negatively regulate mitophagy of Cr(VI)-transformed cells.
Bone is a known long-term storage compartment for Uranium (U); there is growing evidence for natural U inducing adverse effects in bone function and development, including delayed growth, increased bone resorption rates, reduced bone volume and fragility in laboratory animals. This effect has not yet been demonstrated in epidemiologic studies due to exposure to Depleted Uranium (DU), isotopically different from but chemically identical to natural U. Our longitudinal clinical surveillance of a cohort of Gulf War Veterans exposed to DU in friendly fire events in 1991 is designed to identify potential long-term health effects from embedded DU fragments and includes using DEXA scans to measure mild (osteopenia) or severe (osteoporosis) bone loss. The cohort was divided into high vs. low urine U (uU) exposure groups based on creatinine-adjusted uU levels, which correlate generally with embedded DU fragment presence (high) vs. exposure limited to inhalation (no fragments) (low). Based on the Endocrine Society’s recommendation to screen men with osteoporosis risk factors at age 50, we performed DEXA scans in cohort members who have reached this age. Of these 23 Veterans, six fell into the high uU group (uU ≥ 0.1μg/cre) and 17 were in the low uU group (uU < 0.1μg/cre). Using Fisher’s exact test we investigated associations between bone density via DEXA scans and uranium exposure status. Five of the six (83%) in the high uU group had abnormal DEXA results, with osteopenia, compared to five of the 17 (29%) in the low uU group (p = 0.052). A low Vitamin D level was not significantly associated with abnormal DEXA results (p = 0.669). Although limited by the small number of Veterans assessed, we have shown a significant association between U burden and osteopenia as demonstrated by DEXA results. Our biologically plausible preliminary findings, combined with increasing evidence supporting adverse effects of U on bone, advocate for continued surveillance of this health outcome in U-exposed populations. As we accumulate more clinical data from this aging cohort, we will be able to assess our preliminary findings and explore potential effect modifiers (such as medications) more fully. If confirmed, this association has significant implications for the medical management of U-exposed populations, to preserve bone health.

High levels of uranium and arsenic exist in air, water, and soil in the Navajo Nation, due to >500 abandoned uranium mines in the rural southwestern United States. Human exposures in the Navajo Nation are associated with increased prevalence of metal-associated diseases, as demonstrated by DEXA results. Our biologically plausible preliminary findings, combined with increasing evidence supporting adverse effects of U on bone, advocate for continued surveillance of this health outcome in U-exposed populations. As we accumulate more clinical data from this aging cohort, we will be able to assess our preliminary findings and explore potential effect modifiers (such as medications) more fully. If confirmed, this association has significant implications for the medical management of U-exposed populations, to preserve bone health.

The circadian rhythm is an essential system for maintaining homeostasis of life. The main disruptor of this system is light at night (LAN), continually and frequently induced in an environment like shift work. Chronic circadian disruption causes various health problems including lifestyle diseases such as hypertension, diabetes and obesity. Furthermore, the relationship between circadian disruption and carcinogenesis is also being clarified recently. In the present study, we report that the circadian rhythm disruption showed inhibitory effect on testicular function using mouse model. Male C57BL/6J mice (7 weeks old) were raised for 1, 3, 9 and 12 weeks under normal lighting conditions (12 hours light and dark) and light and dark shift conditions (every 2 days, reversed 12 hours the light and dark). The testicular function was analyzed using the CASA system. Plasma concentrations of zinc and calcium were measured using Metalloassay kit. The behavioral activity of the mice in the normal light and dark condition group was high in the dark period and showed a definite circadian rhythm, but it was confirmed that the circadian rhythm was disturbed in the light/dark shift condition group. The sperm numbers in the testis and cauda epididymides decreased significantly from 6 weeks after the onset of light/dark shift, and continued until after 12 weeks. Similarly, the sperm motility was also significantly decreased after 6 weeks. The decrease was sustained until after 12 weeks. Plasma zinc concentration showed transient decrease after 1 week of light/dark shift condition. It is well known that spermatogenesis is suppressed in low concentration of zinc. The circadian rhythm of zinc secretion is disturbed by light/dark shift condition, possibly causing testicular dysfunction.
Tungsten dioxide nanoparticles (TiNPs) are manufactured worldwide in large quantities for use in a broad range of applications such as cosmetics, pigments, and antibacterial agent. Recently, reports on TiNPs-induced toxicity in organs such as the liver, lung, and intestine are increasing. We also reported that TiNPs-induced testicular toxicity, i.e., testis was highly sensitive to TiNPs as compared to the liver. In this presentation, we examined the acute effect of TiO2 on testicular function in mice. TiNPs (Aerioxide P25) were dispersed in disoside phosphate solution (DSP) by sonication. Male C57BL/6J mice were orally administrated with titanium dioxide (10, 20 or 100 mg/kg body weight) once per week for 4 weeks. Mice were sacrificed 3 days after the last administration. Although the sperm numbers in the cauda epididymides were not changed by TiNPs administration, the sperm motility at 3 days after the last injection was significantly inhibited. Plasma testosterone concentrations were not different between control group and TiNPs administered groups. Further, we observed no significant histological change in testes among control group and TiNPs administration groups. Our results indicated that TiNPs possesses acute adverse effect on testicular function, especially on the matured sperm existed in the cauda epididymides.

Tungsten's increasing presence in electronics and medical devices positions it as an emerging environmental toxicant, yet its potential health consequences are not well understood. Like many metals, tungsten accumulates in the bone, dose-dependently, with a faster rate of accumulation than removal. This makes bone a long-term storage site and a source of chronic exposure. The bone is a dynamic organ that constantly undergoes an intricate remodeling processes to balance bone formation, driven by the osteoblasts, and bone resorption, driven by the osteoclasts. Osteoblasts, like many other cell types, are derived from mesenchymal stromal cells. We have previously shown that sodium tungstate skews mesenchymal stromal cell differentiation by promoting adipogenesis and inhibiting osteogenesis. Here, we evaluated the effects of tungsten exposure on the other face of bone remodeling: osteoclast differentiation. We have previously shown that sodium tungstate exposure for 4 weeks increased the amount of TRAP positive osteoclasts within the bone marrow. In vitro, tungsten by itself was able to induce the expression of osteoclastogenic genes Acsp5 and Ctsk in pre-osteoclasts, but not to the same extent as RANKL, the known inducer of osteoclast differentiation. However, sodium tungstate did not significantly enhance osteoclastogenesis, alone or in combination with RANKL. In addition, tungsten treated pre-osteoclasts demonstrated increased adhesion to vitronectin surfaces. We also observed an increase in mRNA expression levels of integrin-αv in sodium tungstate-treated osteoclast precursors. We suspect that tungsten may enhance adhesion of osteoclast precursors to the bone matrix causing the increased TRAP positive osteoclasts observed in vivo. To fully assess the relevance of increased osteoclasts in the bone, activity assays will be carried out in the future.

Tungsten (W) is a refractory metal that is being used in alloy manufacturing, high speed tools, light filaments, x-ray shielding, welding electrodes, solar energy devices, and pigment production. W is historically considered a non-toxic metal and as such W alloy replaced lead in ammunition in the 1990s. Recent reports indicate a greater risk than originally assumed for the mobility and presence of W in the environment, increasing the likelihood of an environmental exposure. In addition, an association of W with a childhood cancer cluster in Nevada located near tungsten mining sites and military bases was indicated. As a result, there are returning questions on the toxicity of W alone and/or in mixtures similar to the composition of tungsten alloy. Current hypotheses support a toxicity mixture interaction with W and other metal(s) present in the W alloy, but further research is needed to define the mixture toxicity. In this study, the zebrafish model was used to assess the influence of cobalt (Co) and/or nickel (Ni) on tungsten toxicity. Zebrafish embryos were first exposed to each metal (W, Co, or Ni) alone beginning immediately after fertilization and survivability was monitored every 6 hours through 120 hours post fertilization (hpf). The results revealed that 10000 parts per million (ppm; mg/L) W, 100 ppm Ni, and 100 ppm Co caused a decrease in survivability. Non-lethal concentrations were then chosen to assess the toxicity interaction of the mixture. Three concentrations of each metal were included: 0, 100 and 1000 ppm W; 0, 1 or 1 ppm Ni; and 0, 1, or 10 ppm Co. Zebrafish embryos were exposed to one of the 27 metal treatments beginning immediately after fertilization. The toxicity of the mixtures was assessed by monitoring the survivability and hatching rates every 6 hours through 120 hpf. In addition, behavioral analysis using a visual motor response test was performed at 120 hpf. No significant difference in survival or hatching rate was observed between treatments. A decrease in velocity, time spent moving, and distance swam in all treatment groups that contained 10 ppm Co was observed (p<0.05). These results showed that only 10 ppm Co controls the change in locomotor activity of the zebrafish larvae and W alone had no effect on survivability or locomotor activity at the tested concentrations. Future work is now characterizing morphological outcomes of the metal mixtures at non-lethal concentrations.
Predicting Oral Repeated-Dose Toxicity of Cobalt Substances Using a Read-Across Approach

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Cobalt in the form of vitamin B12 is essential for the maintenance of human health, and inorganic cobalt is essential for bacteria living in the marine environment. The indiscriminate release of potential toxic metals (PTMs) into the environment by the industries is at an alarming rate, hence the need for the use of agricultural waste materials (AWMs) as an adsorbent for the removal of PTMs from wastewater. The point of zero charge (PZC) of AWMs is one of the factors that contributes to the adsorption of copper ion (Cu2+) in aqueous solution. The indiscriminate release of PTMs into the environment is a concern because of their potential toxic effects on human health. The use of AWMs as an adsorbent for the removal of Cu2+ from aqueous solution is a promising approach to address this concern.

Impact of HIF-1α on Cellular Responses to Carcinogenic Nickel(II)

M. W. Luczak, and A. Zhitkovich. Brown University, Providence, RI.

Nickel is a human respiratory carcinogen that strongly upregulates the hypoxia-sensitive transcription factor HIF-1α and induces a hypoxia-like transcription response. Elevated HIF-1α levels are frequently found in more aggressive cancers and associated with drug resistance which occurs in part due to increased drug efflux. We examined the role of HIF-1α in cellular accumulation of Ni(II) ions and Ni(II)-induced cytotoxic responses in human lung cells. We found that the loss of HIF-1α had no significant effect on intracellular levels of Ni(II) in transformed (H460, A549) or normal (IMR90, WI38) human cells. The absence of HIF-1α did not change p53-dependent and p53-independent apoptotic responses or clonogenic survival in Ni(II)-treated transformed cells. In normal human cells, Ni(II)-induced HIF-1α enhanced inhibition of cell proliferation and promoted a permanent growth arrest (senescence). Analysis of various cell cycle regulators showed that HIF-1α was important for upregulation of the CDK inhibitors p21 and p27. Induction of p21 also required the transcription factor p53. The formation of senescent cells by Ni(II) was completely abolished by p53 depletion and suppressed by p21 knockdown. Our findings indicate that HIF-1α enhances the activity of p53 in restriction propagation of Ni(II)-damaged normal cells, suggesting that it may act in a tumor suppressor-like manner during early stages of Ni(II)-induced transformation.

Determining the Fate of Copper in Aquatic Systems

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Cisplatin was the first of several generations of platinum anticancer compounds approved by the FDA for use as chemotherapies which activate cellular apoptosis. In addition to the central platinum atom, each compound is bound to a leaving and non-leaving ligand. Platinum based compounds activate apoptosis through a mechanism where the leaving ligand is lost from the central atom allowing the platinum to bind to DNA, causing cell death, but in which the mechanism in which the platinum complexes interact with the cell is largely unknown. The same compound often has varying levels of cytotoxicity on different cell types, implying that different cell types have different mechanisms of interaction with the platinum compounds. Carbotoplatin and oxaliplatin are two other FDA-approved platinum chemotherapies that differ only in ligand type, but exhibit very different reactivity, suggesting that ligand behavior plays a role in these interactions. To better understand the mechanisms of interaction between cell types and various platinum compounds, we are using novel platinum compounds systematically varying both leaving and nonleaving ligands. In these studies, we examined cellular toxicity of the compounds in multiple mammalian cell lines, a noncancerous control and metastasis of prostate cancer, NTERA-2. Here we report the cellular toxicity using the MTT assay. Our initial finding demonstrate an inhibitory concentration at 50% (IC50) analogous to values reported for similarly structured platinum compounds.

The mechanism of Alzheimer’s disease (AD) in aluminum chloride (AlCl3) treated zebrafish models. We set up an AlCl3 treated AD model in zebrafish by exposing zebrafish embryos to different concentrations (50, 100, 200, 400, 800 µg/L) of AlCl3, the influence of different concentrations of AlCl3 on learning and memory performance of adult zebrafish were evaluated by T-maze tasks. Then, we quantified AD-related gene expressed in the adult zebrafish by QRT-PCR. Finally, the genes expression in P3K-AKT-mTOR signal pathway in Alzheimer’s disease model induced by Aluminum Chloride in Zebrafish.

Beryllium is a metal used in the industries of aerospace, automobile, electronics, etc. Exposure to it can cause beryllium sensitization (BeS) in some subjects, and the sensitized cases could develop further to chronic lung disease (chronic beryllium disease, CBD). Beryllium lymphocyte proliferation test (BelPT) is an examination to identify the sensitized subjects and CBD patients. The standard protocols were released in 2001 by DOE. However, there are still some controversies over the reliability of the assay, and considerable variations of results even for the same sample is one of the problems that often embarrasses laboratories. Furthermore, radioactive substance is used in the assay and this is not convenient to many laboratories. Although BelPT is used in health examination of workers in some factories, it is still a "must" item under the present regulations of MHLW, Japan, partially due to its non-reliability. In this study, we modified the assay procedures to minimize the variations of results, and by using non-radioactive coloring agent to monitor the cell viability, we introduced an alternative assay protocols. Methods: With human lymphocyte cell line, we simplified the assay to minimize the variations of the radiation counting. Also we used alamar blue cell viability assay, a method using the reducing power of living cells to quantitatively measure the proliferation of cells. Then with lymphocytes from volunteers, the simplified DOE method and alternative alamar blue method were confirmed under Con A stimulation. Finally with lymphocytes from CBD patients, we tested the simplified and alternative methods for their efficiency and accuracy.

This study was approved by the Ethics Committee of our institute and informed contents were obtained from all subjects participated. Results and Conclusions: By omitting cell washing and DNA purification steps, the variations of radiation counting was greatly reduced, though with higher background values. The alamar blue method showed similar results to DOE method with high accuracy. It is noteworthy that alamar blue assay could be easily done only by adding the agent for a few hours, and after the photometer reading, the same cells can be used for the assays of DOE method by adding 3H-thymidine. This is especially useful for the blood samples with limited amount.

Cadmium, cobalt, and nickel are two heavy metals classified as Group 1 carcinogens that have been linked to kidney, colon, and lung cancer. Research in eukaryotic cells has indicated that co-exposure to these metals is significantly more toxic than single exposure and results in a dose-dependent decrease by survival and an increased production of ROS as well as the formation of double strand breaks. Several other studies have also suggested the role of DNA repair processes in metal-induced toxicity. Because yeast and several DNA repair processes are well conserved, we have employed budding yeast Saccharomyces cerevisiae to understand the role of metal induced DNA repair (MDR) and genotoxicity. This study indicates that yeast cells deficient in proteins involved in MDR respond differently to cadmium, cobalt, and nickel exposure compared to wildtype cells. Interesting, deficiencies in several different MMR proteins resulted in a 2-fold increase in survival compared to wildtype cells as measured by absorbance quantifications and spot assays. When these same strains were exposed to cobalt there was a significant difference between all doses, even when the doses were raised significantly. When the yeast strains were co-exposed to cadmium and nickel both the wildtype and the all MMR deficient strains produced a dose-dependent decrease in colony forming ability as measured by the spot assay. The wildtype cells were approximately 3-4 fold more sensitive compared to the MMR deficient strains at the highest doses of cadmium and nickel. Analysis of cell cycle progression indicate that following exposure to cadmium and nickel there is an increase in the number of cells in G2 in the wildtype cells compared to the MMR-deficient strains suggesting these cells may arrest here. No differences were detected in the wildtype or MMR-deficient cells following cobalt exposure. This data indicates that the mechanism of toxicity by cadmium and nickel may be different than that of cobalt. Additionally, deficiencies in MMR produce a resistance to cadmium and nickel induced toxicity which suggests why selection of MMR-deficient cells may be more prevalent amongst various cancers.

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studies, iAs3+ exposure significantly decreased insulin secretion by ~50%. Non-cytotoxic concentrations of Mn (12.5, 25, and 50 µM) and Cd (5 µM) also significantly decreased insulin secretion when administered alone, whereas Zn (6.25 and 50 µM) elicited a slight increase in insulin secretion. Interestingly, binary mixtures of iAs3+ and Zn, Mn, or Cd showed no additive or synergistic effect on insulin secretion. Examination of oxygen consumption rate (OCR), a proxy for mitochondrial function, revealed that single exposure to Mn or Cd had no significant impact on mitochondrial function, whereas, iAs3+ drove a significant reduction in maximal OCR. Similar to the effect on GSIS, binary mixtures of these metals did not further decrease or increase OCR from the iAs3+ exposure alone, indicating that Cd and Mn inhibit insulin secretion by impacting other steps of the insulin secretory pathway, which are likely downstream of mitochondrial function. Future work will identify these steps and characterize the mechanisms of Cd and Mn action.

### 3007 Assessment of Thiol-Rich Proteins, Lipid Peroxidation, and Zinc as Biomarkers of Combined Exposure to Lead, Cadmium, and Manganese in Rats

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Lead (Pb), cadmium (Cd) and manganese (Mn) are known to occur together in the environment, and another method of assessing the potential of clinical symptoms presented in such cases are not specific, and in most cases go unnoticed. Thus, there is need for more systematic research into the chronic influence of the metals at very low concentrations on some biomarkers in vivo. This study aims to evaluate the effect of combined exposure to Pb, Cd and Mn on thiol-rich proteins, lipid peroxidation and trace elements Iron (Fe) and zinc (Zn) in rats. Five orally-treated groups of five rats each received Pb acetate (1.4 mg/kg b.w.), Cd chloride (0.01 mg/kg b.w.), Mn chloride (0.14 mg/kg b.w.) and the combination (Pb+Cd+Mn), as equivalence of NOAELs for each metal, for 15 weeks. Control rats were given deionized water (2 ml/kg). Metallothionein-1 (MT1), glutathione peroxidase-1 (GPx1), malondialdehyde (MDA), Fe and Zn concentrations were analyzed in the serum. The levels of MT1, GPx1 and Zn significantly decreased, while MDA concentration increased in the rats treated with the metal mixtures. The results also implicate possible synergistic effects of Pb, Cd and Mn on MT1, GPx1 and Zn concentrations. Taken together, a definite and important role of oxidative stress is evident in low dose Pb+Cd+Mn- exposed rats, and may be used as sensitive biomarkers in situations of multiple metal exposure scenarios.

### 3008 Establishing a Metal-Interactome in Ovarian Cell Lines

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The cellular metallome serves as an important reservoir to meet the biological needs of the cell. It also becomes altered after exposures to toxic, harmful metals. Recent studies have suggested that cancer cells adapt their metallome to accommodate rapid cellular growth and metabolism pathways. Thus, altering the cancer metallome may yield new avenues to target cancer and chemotherapy responsiveness. Here, we took a systematic approach to characterize the cellular metallome, as well as other cellular responses, under various metal conditions in a panel of ovarian cancer and non-cancer cell lines. In addition, sensitivity to the metal-based chemotherapy drug cisplatin in response to metal treatment was evaluated. The metallome was manipulated by treating cells with either zinc, copper, magnesium, calcium, iron, manganese, or the metal chelator tetrathiomolybdate. Cell proliferation in response to metal treatment or cisplatin was measured by WST-1 assays. Metallomes were quantified by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) by measuring the following metals: zinc, copper, magnesium, calcium, molybdenum, manganese, iron, phosphorous, and sulfur. Cellular metal concentrations were normalized to sulfur levels. Metal treatment of ovarian cancer cells resulted in differences in cellular proliferation, uptake, and the cellular metallome. The changes in the metallome were not singular in nature as altering one metal resulted in changes in multiple metals, hence evidence of a metal interactome. Differences in how the cells respond to metal treatment were also observed between cancer and non-cancer cell lines. In terms of cisplatin sensitivity, the results suggest that metal alteration is only effective in certain cell lines. The data lend further insight into how an individual’s metallic status, a combination of nutritional and harmful metal exposure, might result in the development and progression of cancer.
risk. Likewise, TCEQ’s more recent URF for arsenic (1.5E-04 per µg/m³) is based on updates of two studies that US EPA used in 1984, as well as an additional cohort. These and other examples emphasize the importance of scientifically-current chemical assessments to best characterize risk/hazard.

3011 Blood Metal Reference Values in a Southern Brazilian Adult Population

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The assessment of blood concentrations of essential and non-essential metals is important in identifying exposure characteristics and it can be used as a reference for prospective evaluations. The aim of this study was to determine blood levels of As, Cd, Co, Cu, Hg, Mg, Mn, Mo, Pb, Se, U and Zn, and to evaluate differences in blood levels in relationship to demographic and lifestyle characteristics, and to establish reference values (RV) for this population. A cross-sectional study was carried out in a random sample of adults, aged 40 years or older, living in a city in Southern Brazil. Metal blood levels were measured by ICP-MS. The RV were based on the upper limits of the 95th CIs of the percentiles of the measured distributions. A total of 903 adults enrolled in the study. The RV and the geometric mean (GM) (95% CIs) for the total population were respectively: As (12.59 and 5.34 µg/L; 5.15, 3.53), Cd (1.31 and 0.06 µg/L; 0.05, 0.07), Co (0.98 and 0.26 µg/L; 0.24, 0.28), Cu (198.9 and 111.7 µg/L; 108.6, 115.0), Pb (5.88 and 1.93 µg/dL; 1.86, 2.01), Mg (6.14 and 3.91 mg/dL; 3.85, 3.97), Mn (19.56 and 12.11 µg/L; 11.9, 12.3), Hg (8.23 and 1.39 µg/L; 1.29, 1.50), Mo (2.82 and 0.76 µg/L; 0.73, 0.80), Se (132.2 and 83.6 µg/L; 82.5, 85.1), U (5.39 and 0.08 µg/L; 0.07, 0.09) and Zn (121.3 and 50.6 µg/dL; 63.5, 67.7). Cd, Pb and Hg blood concentrations were higher in men compared to women, while Co, Cu and Se concentrations were higher in women. Smokers had significantly higher Cd and Pb concentrations, while Se blood levels were inversely associated with smoking. Participants aged 50-59 years had higher Pb, Mo and Se blood concentrations. These results impart novel information on essential and non-essential metal concentrations in the general population in Brazil.

3012 Correlations between Immunologic Alterations and Metal Exposure within the Navajo Birth Cohort Study


The Navajo Birth Cohort Study (NBCS) was established to address community health concerns about chronic exposure to mine waste. Tribal populations are characterized by health disparities, including infection, kidney function, diabetes and cancer; the immune system plays a role in all of these. Based on past and ongoing work with Navajo Nation and other tribes, we hypothesize that chronic low-level environmental exposure to metal mixtures from mine waste results in immune dysregulation. In this study, we are examining whether changes in lymphocyte phenotypes can be seen in participants who have evidence of exposure to uranium, arsenic, manganese, and cadmium. Samples of whole blood and urine were collected from NBCS participants and analyzed by CDC laboratories for metals via inductively-coupled plasma mass spectrometry (ICP-MS). Biomonitoring revealed an upward shift in blood manganese (BMN) and urine uranium (UUR) in the study population compared with the US population as a whole (NHANES data). Urine total arsenic (UTAS) and urine cadmium (UCD) in NBCS participants is comparable or lower than NHANES data. Whole blood samples were used for immunophenotyping to identify total lymphocytes (CD45), T cells (CD3), T helper (CD4), T cytotoxic (CD8), NK (CD16CD56), and activated (HLA-DR) populations by flow cytometry. Univariate and multivariate analyses (n=80 samples) demonstrate associations between BMN and total lymphocytes. UTAS is associated with changes in the percentage of CD45, CD3, and CD16CD56 subsets. UCD is associated with alterations in CD3, CD16CD56, CD8, and HLA-DR+ subsets. While some metals may increase the percentage of a specific lymphocyte population, other metals may decrease that population, confirming the necessity of modeling the effects of metal mixtures rather than merely single exposure, on immune markers of interest. It is important to understand the relationships between chronic metal exposure and immune alterations to better understand the potential health effects related to this exposure.

3013 Alterations of Micro-Elements in the Body Tissues and Blood of Female Albino Rats Fed with Marijuana Extract


To investigate the effects of different concentrations of marijuana extracts on the levels of microelements in the lungs, brain, kidney, femur and blood, seventy-eight female albino rats (120-150g) were divided into four groups. Control group received olive oil while remaining groups received 12.5, 25 and 50 mg/kg marijuana extract respectively over 2, 4 and 6 weeks. Blood samples and organs were harvested and analyzed for metal (Fe, Cu and Zn) content using flame atomic absorption spectrophotometry. The results showed that the 25 mg/kg extract significantly (p<0.05) increased the copper and zinc concentrations in the femur at 2 weeks and significantly decreased the micro-elements at 4 and 6 weeks. The three doses of the extract significantly (p<0.05) decreased iron concentration in the femur at 4 weeks, however, at 6 weeks, the 25 and 50 mg/kg significantly (p<0.05) increased the iron concentration when compared to their control. Brain copper was significantly (p<0.05) decreased at 2 weeks of 12.5 mg/kg extract whereas at 6 weeks, a significant decrease was observed at 12.5 mg/kg when compared to control. At 4 weeks, a significant increase of copper in the liver was observed at 25 and 50 mg/kg doses at 2 weeks whereas at 4 weeks the copper concentration was highest in the liver of rats administered 25 mg/kg extract with 71% increase when compared to the control. This study shows that marijuana extract administration influences the status of micro-elements in the blood and body tissues of female albino rats.

3014 The Impact of Informal Domestic Work with Jewelry and Fashion Jewelry on Sanitary Sewer System

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The outsourcing informal practices adopted by some industrial sectors can causes toxic substances elimination in the effluents and raises a concern for its environmental impact. This study evaluated the levels of micro-elements in the lungs, brain, kidney, femur and blood, seventy-eight female albino rats (120-150g) were collected in 15 markets of three areas during two campaigns of the year of 2016. Furthermore, the sewage sludge (n=2) raw sewage (n=12) and treated sewage (n=12) were collected in two wastewater treatment plants (AS) and TATU operating with different treatment process. The PTEs determination was performed by ICP-OES, direct current plasma analyzer and UV-VIS spectrophotometry. The PTEs found to be above the detection limit of the methods: hexavalent chromium (0.0093 to 0.61 mg L⁻¹), copper (<0.02 to 4.24 mg L⁻¹), nickel (<0.02 to 6.16 mg L⁻¹) and zinc (<0.02 to 1.34 mg L⁻¹). Four sanitary sewage samples exceeded copper or zinc values permitted to be discharged into sewage system; however, the average concentration were lower than that established by Brazilian legislation. High percentages of PTE’s values above the 75th percentile was observed in samples with high Total Organic Carbon values (p<0.05) and in second campaign, therefore in more concentrated sewage. The AS treated sewage presented low concentrations of copper (p<0.05), zinc (p<0.02) and nickel (p=0.01) compared to TATU treated sewage. In the sludge, the copper means exceeded the limits of the Brazilian (1,500 mg kg⁻¹) and International legislations and the zinc results surpass the Canadian limits (1,850 mg kg⁻¹). The heterogeneity of the results can indicate the sporadic nature of the PTE’s sanitary disposal. PTEs used in jewelry and fashion jewelry chain were found in the effluent and may precipitate/concentrate on the sludge, where high concentrations of zinc and copper require its controlled destination. Supported by FAPESP (2015/21253-0) and São Paulo State Environmental Company.
Cement dust deposition on soil has been implicated in changes in trace and heavy metal (HM) contents of soil which may affect the well being of living organisms in the ecosystem. This study assessed the impact of cement dust exposure on the heavy metal contents of soil samples in the vicinity of united cement factory, Calabar, Nigeria. Topsoil samples (5 from each location) were collected at varying distances and directions in the vicinity of the factory and an area remote to the site serving as control. The Pb, Cu, Mn, Fe, Cd, Se, Cr, Zn and As content of the soil samples were determined using atomic absorption spectrophotometry. Data were analysed using ANOVA and LSD post hoc at p<0.05. The HM content of soil samples from all locations studied were below the permissible limits for these metals except for Zn in soil samples collected from location closest to the cement factory which was observed to be above the permissible limit. The HM content of soil samples studied varied significantly with the location and their distances from the factory. The soil Mn, Fe, Zn, Pb, Cu and Cr levels were significantly higher in soil samples collected from location closest to the cement factory compared to samples from other locations. Moderate contamination with Cu and Pb and considerable contamination with Cr were observed in soil samples collected at locations closest to the cement factory. The HM content of soil from all locations studied demonstrated minimal enrichment (EF<2) and average pollution index (IPI<1). Exposure to cement dust is associated with soil contamination with Pb, Cu and Zn but not Mn, Fe, Cd, Se, Cr, Zn and As. Soil contamination with Pb, Cu, and Cr and subsequent transmission to humans via the food chain has been related to specific tissue and organ toxicities. Appropriate remediation strategies for soil contaminants should be implemented to avert undesirable environmental health consequences.

Domoic acid (DA), the cause of Amnesic Shellfish Poisoning, is a naturally occurring excitotoxin that can contaminate shellfish and finfish. Consumption of food contaminated with high-dose DA is associated with gastrointestinal distress, seizures, memory loss, coma and death. It is important to define the health risks associated with chronic, low-dose exposure because the ocean algal blooms producing DA are becoming more frequent and severe. To this scientific end, we have initiated a longitudinal study of the reproductive and neurodevelopmental effects of low-level oral DA exposure in a preclinical nonhuman primate model. Adult female Macaca fascicularis monkeys were exposed to daily doses of DA (either 0, 0.075, or 0.15 mg/kg) during pregnancy and their infants were being evaluated in a specialized nursery to allow for comprehensive behavioral testing. The doses used in this study were selected because they are either at the current TDI for humans or just twice the TDI, well within the margin of safety for human DA exposure. To identify potential effects of DA exposure in the adult females, we regularly conducted evaluations of health and behavior before, during and after DA exposure. Results of our clinical observations indicate that subtle neurological effects (tremors) may be the initial sign of DA neurotoxicity in these animals. The frequency of tremors is 10% (1/10) in control animals, 27% (3/11) in low-dose animals and 63% (7/11) in the higher-dose group. It is noteworthy that some prenatally-exposed infants are also displaying persistent intentional tremors during cognitive testing. Further studies of the females exhibiting tremors compared to those who are not are being conducted using PET and MRI procedures. The results thus far suggest that the current TDI for DA does not provide adequate protection for humans who are chronically exposed.

Biosynthetic organohalogens, including bromopyrroles are emerging pollutants in saline wastewater effluents as disinfection by-products (DBPs). Some DBPs have cytotoxic, genotoxic and developmental toxicity, such as 2,3,5-tribromopyrrole and tetrabromopyrrole. However, there is no information about direct mechanisms by which DBPs induce mammalian neurotoxicity. Herein, several biosynthetic bromopyrrole analogues, including 2,3,5-tribromopyrrole (1), tetrabromopyrrole (2), 4,5-dibromopyrrole-2-carboxylate (3), ethyl 4,5-dibromopyrrole-2-carboxylate (4), 3,4,5-tetrabromopyrrole-2-carboxylate (5), and ethyl 3,4,5-tibromopyrrole-2-carboxylate (6) were tested for their direct engagement of ryanoide receptor type 1 (RYR1) using [3H]ryanodine binding analysis, and influences on patterns of synchronized Ca2+ oscillations (SCOs) of neuronal networks in a concentration-dependent manner with compound 2 (having the highest potency ([Ci]0.41 µM) compared to (1) ([Ci]0.1 µM). These data identify for the first time a new molecular target for bromopyrroles that displays a stringent structure-activity relationship towards modulating RYR1 and neuronal network Ca2+ dynamics. Supported by NIEHS R01 P01 ES011269 and P42 ES04699, and US EPA STAR 829388 and R833292.
Only a small percentage of chemicals in the human chemosphere have been evaluated for their neurotoxic potential. In order to screen the tens of thousands of chemicals for which no toxicity data exists, it is necessary to move from in vivo rodent experiments to in vitro/in silico models and alternative animal models. Zebrafish embryos are a promising model for toxicity screening since they develop fast, are available in large numbers, and their genome is highly homologous to that of humans and there are many transgenic/mutant lines available for mechanistic studies. We used dechorionated Tropical SD wildtype zebrafish embryos to screen a 91-compound library (provided by the National Toxicology Program) for developmental neurotoxicity. Embryos were exposed to 5 concentrations of each chemical up to 100 µM or to an equivalent of vehicle (0.5% DMSO) in embryo media from 6 hours post-fertilization (hpf) to 5 days post-fertilization (dpf). Embryos were examined daily for malformations and mortality until euthanized at 5 dpf. Developmental neurotoxicity was assessed at 4 and 5 dpf using a light-dark locomotor behavioral assay. Malformed and dead fish were excluded from the behavioral analysis. The 87 unique chemicals (4 were provided in duplicates) screened included negative controls, flame retardants (FR), polycyclic aromatic hydrocarbons (PAH), drugs, industrial chemicals and pesticides. All negative controls had no effect on mortality/malformations or locomotor behavior. Many chemicals caused different behavioral outcomes at 4 versus 5 dpf, and different chemical concentrations led to different behavioral patterns. Interestingly, chemicals within the same group caused different behavioral abnormalities, while similar behavioral patterns were caused by chemicals belonging to different groups.

Neuroinflammation is a significant contributor to the initiation and progression of many neurodegenerative diseases including Parkinson’s disease (PD). Inflammatorystimulatory cytokines, including TNF-α, IL-1β, and IL-6, can be released by astrocytes, microglia, or on behavior, and chemicals provided in duplicates produced similar outcomes. Interestingly, most PAHs affected only behavior and no PAH caused only malformations or mortality. Behavioral abnormalities were generally observed at lower concentrations than those that caused mortality/malformations, suggesting that behavioral alterations were not due to toxicity. Many chemicals caused different behavioral outcomes at 4 versus 5 dpf, and different chemical concentrations led to different behavioral patterns. Interestingly, chemicals within the same group caused different behavioral abnormalities, while similar behavioral patterns were caused by chemicals belonging to different groups. Supported by NIH HS (ROI ES01269 to PJL and F32 ES024070 to GMW) and US EPA (R08354320 to PJL).

Characterizing Novel Mechanism-of-Action of Lithium in Caenorhabditis elegans

Lithium is the most commonly prescribed drug for bipolar disorder. Despite its mood stabilizing properties, the specific mechanisms through which lithium exerts its beneficial effects are not clear. Lithium has a narrow therapeutic index and therefore causes serious toxic side effects. Previously our lab showed that lithium increases the serum and glucocorticoid-inducible kinase (SGK) activity in C. elegans. SGK plays a central role in the regulation of Na+ absorption by stimulating the activity and prolonging the half-life of the epithelial Na+ channel (EnaC) and thus regulates extracellular fluid volume, blood pressure, and sodium homeostasis. It is worth noting that hypertension is a recognized side effect of lithium treatment. In the present study, we used lithium for its SGK pathway activating effect. For these experiments, we covered seeded plates with drugs that were dissolved in appropriate solvents, and observed worms periodically to assess development, movement and egg laying. We found that lithium corrected the egg laying and developmental deficits of animals that have a gain-of-function (gf) mutation (unc-58665) in a two-pore domain K+ channel (K2P), which normally causes animals to adopt a rigid posture with constant shaking. We hypothesized that if lithium corrects these deficits through SGK signaling, another drug that activates SGK (clozapine) should also correct these deficits. Clozapine corrected the locomotion and developmental deficits of animals. Lithium and clozapine appeared to correct different deficits in the unc-58 gf strain. When we combined lithium and clozapine, their effects were not additive. This suggested that lithium and clozapine were not acting through SGK signaling to rescue the deficits of unc-58 gf animals. Regardless of the mechanisms, we have identified K2Ps as a possible indirect target of lithium’s actions. By characterizing this novel mechanism of action of lithium, we may learn how to improve its therapeutic activity and diminish the toxic side effects.

3022 Farnesoid X Receptor Agonists Reduce Cytokine-Induced Inflammatory Response of Immortalized and Primary Mouse Astrocytes

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The value of nonhuman primate (NHP) in vivo safety pharmacology and toxicology studies can be enhanced by the measurement of functional end points, including behavioral assessments for which NHPs are of particular predictive value. In the present study, we evaluated the validity of a functional observational battery (FOB) to apply as a standard CNS safety assessment for nonhuman primates. To this end, we compared the outcomes at 4 versus 5 dpf, and different chemical concentrations led to different behavioral patterns. Interestingly, chemicals within the same group caused different behavioral abnormalities, while similar behavioral patterns were caused by chemicals belonging to different groups. Supported by NIH HS (ROI ES01269 to PJL and F32 ES024070 to GMW) and US EPA (R08354320 to PJL).

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Background: Spiral ganglion neurons (SGNs) gradually die after destruction of hair cells, their sole afferent input. During SGN degeneration, the ganglion exhibits both inflammation and upregulation of the major NAD catabolizing enzyme, CD38, an activation marker for inflammatory cells. The novel P7C3 series of neuroprotective compounds helps stabilize neurons in times of energetic stress by activating the rate-limiting enzyme NAMPT in the NAD salvage pathway. Here, we assessed the potential of NAMPT and CD38 as inflammatory activating markers for SGNs and viability in rat models of acute deafening.

Methods: Sprague Dawley rats were deafened by daily intraperitoneal injection of kanamycin for 7 days or postnatal day P16. Rats showing reduced reaction time motor learning task were not included. From P22-P70, rats were injected intraperitoneally with P7C3A20 (20 mg/kg) and/or ibuprofen 40 mg/kg in DMSO/corn oil or vehicle only. Rats were euthanized at P70, cochleae fixed and cryosectioned (6 µm) parallel to the midmodiolar plane. Myosin 6/7 immunofluorescence was used to monitor hair cell loss. NeuN and NF200 immunofluorescence were used to label neurons, which were counted in every fourth near-midmodiolar section. Image analysis was done using the FIJI ImageJ package with custom-written macros. The outline of Rosenthal canal for each turn was manually traced to measure cross-sectional area and to calculate SGN density. Results: Kanamycin injection resulted in loss of inner hair cells throughout most of the cochlea. By P70, SGN density in kanamycin-injected rats was significantly reduced in the basal region. Either P7C3 or Ibuprofen improved the survival of SGNs after deafening in the basal region significantly (P < 0.05), from ~30% to ~50%, whereas a combination of these two agents improved it to ~60%. We conclude that P7C3, Ibuprofen, or a combination of these two is protective against SGN death in basal cochlea after aminoglycoside deafening. This further suggests that dysregulation of NAD+ metabolism in both hair cells and inflammatory cells in the spiral ganglion may play a role in SGN death after deafening, and provide a basis for new therapeutic treatments for patients.
Epidemiological studies indicate that a sedentary lifestyle combined with increased consumption of high-fat diets contributes to increased incidence of obesity and related metabolic disorders. These disorders during pregnancy may make offspring more susceptible to air pollutants. The brain is unique, with varieties of neurons and glial cells each having specialized functions contributing to behavior, learning, and autonomic control. These functions have high energy requirements from mitochondrial bioenergetics, a likely target of chemical-induced neurotoxicity. We have investigated whether maternal high-fat diet and exercise interact with effects of maternal O3 exposure on mitochondrial bioenergetics in brains of offspring. For this study, female Long-Evans rats were fed either a control diet (CD) or a high-fat diet (HF; 60% calories in fat) for 6 weeks starting at 30 days of age and then bred. Gestational day (GD) 1 dams were housed with a running wheel (RW) or without (Sedentary; SED) until parturition. HF diet was terminated at postnatal day 35 and all offspring were fed CD thereafter. Adult offspring (~160 days) were exposed to air or O3 for 2 consecutive days (0.8 ppm, 4 hr/day), then rats were killed, brain regions were dissected on ice, snap-frozen and analyzed for complex I, complex II and complex IV enzymes in the frontal cortex (FC) and cerebellum (CB). Complex I enzyme activity (sucinate dehydrogenase, EC 1.3.5.1) was similar in sex, brain region and O3 exposure group in offspring from CD mothers. O3 exposure increased complex I activity in the CB of male offspring from HF-SED mothers but not HF-RW. Complex II enzyme activity (succinate dehydrogenase, EC 1.3.5.1) was elevated in the FC of male offspring from HF mothers but not CD mothers, which was exposed to O3. CB of air-exposed male offspring from CD-RW mothers, there was an increase in complex II activity. Complex IV enzyme activity (cytochrome c oxidase, EC 1.9.3.1) was increased in the FC of males from O3-exposed HF-SED mothers but decreased in HF-RW mothers. O3 also increased complex IV enzymes from mothers maintained on HF. These results indicate that O3 significantly affects brain mitochondrial bioenergetics and that a maternal diet and exercise regimen play a role in offspring’s susceptibility to O3 effects. This abstract does not necessarily reflect US EPA policy.

3028 Simultaneous Assessment of Heart Rate and Sensory Motor Gating Under Positive and Negative Controls in the Startle Response Test

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Potential adverse effects of pharmaceuticals on vital organ systems including the cardiovascular and central nervous systems (CNS) must be assessed. Changes in physiology can impact behavior and alterations in behavior can reciprocally change physiology. However, interactions between these systems are rarely examined at the same time. Drugs may have different effects under resting or stimulated conditions, and therefore the simultaneous investigation of cardiovascular and neurobehavioral endpoints provides a more thorough evaluation of drug safety assessment. This study was conducted to evaluate cardiovascular changes during baseline performance in the startle response test and after treatment with pharmacological agents. Ten animals were implanted with telemetry units monitoring blood pressure (BP) and heart rate (HR). After surgical recovery, animals were treated with vehicle (water for injection), amphetamine (AMPH, 1 and 2 mg/kg), MK-801 (0.1 and 0.15 mg/kg) and Diazepam (DIAZ, 5 and 10 mg/kg) on separate days, and tested in the Startle Monitor II System (Kinder Scientific). After a 5-minute acclimation period, animals were exposed to a 120 dB startle stimulus once every 30 seconds for 50 trials. Maximum force, HR and BP were evaluated. HR and BP increased with the first pulse and decreased over the test duration as animals habituated. With AMPH, HR and BP were elevated throughout the test, but animals still habituated. DIAZ dose dependently reduced HR and BP, and also generated significantly less force than after vehicle treatment. DIAZ also produced a 5-minute acclimation period, animals were exposed to a 120 dB startle stimulus once every 30 seconds for 50 trials. Maximum force, HR and BP were evaluated. HR and BP increased with the first pulse and decreased over the test duration as animals habituated. With AMPH, HR and BP were elevated throughout the test, but animals still habituated. DIAZ dose dependently reduced HR and BP, and also generated significantly less force than after vehicle treatment. DIAZ also produced slightly delayed changes in HR after each startle stimulus. Force generated, HR and BP were significantly higher with MK-801 exposure. HR and BP stayed elevated during the duration of the test and no habituation was evident. The combined assessment of physiological and behavioral endpoints provides a more thorough evaluation of drug-induced effects on sensory-motor gating under baseline and stimulated conditions. This more accurately reflects the heterogeneity of real world conditions and can thereby improve the overall reliability of pharmaceutical safety testing.

3029 Pumpless, Serum-Free, Functional Human Blood-Brain Barrier Model

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The blood-brain barrier (BBB) is a complex interplay between astrocyte endfeet and the endothelial cells that comprise the brain’s microvasculature. This network of interconnected cells serves a neuroprotective role by creating a tight physical barrier between the blood and the brain parenchyma that selectively excludes large molecules and other materials that have the potential to upset its delicate neurochemistry. Delivery of therapeutic agents across the BBB is problematic, and solutions to this problem have been a major area of study in recent years. In this study, we developed a pumpless, recirculating, serum free, human in vitro model of the BBB with cocultured human induced pluripotent stem cell (hiPSC) derived brain microvascular cells (BMECs) and primary human astrocytes. Each cell type was cultured on opposite sides of a porous membrane and assembled in a pumpless system that separates each side of the BBB into their own respective compartments, each with their own physiologically relevant flow profile. Efficient tight junction formation was observed in the systems with TEER levels reaching 2500 Ocm^-2 by day 3 and remaining above 1000 Ocm^-2 at day 7. TEER dependent passive transport across the BBB was evaluated by the addition of fluorescently-tagged dextran of differing Stokes radii. Active transport was also evidenced by receptor-mediated transport of the transferrin receptor (TR) antibody and was shown to be independent of TEER. This pumpless, serum-free, humanicased BBB model establishes a refined platform for studying compound pharmacokinetics into the central nervous system and their effects on the integrity of the BBB.

3030 BMP Chemotropic and Inductive Signaling in Response to Acute Ethanol Exposure

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Sponsor: D. Harde

Consumption of excessive quantities of alcohol during pregnancy can lead to the development of Fetal Alcohol Syndrome (FAS), which is a disorder characterized by developmental, cognitive and behavioral disabilities. These disabilities include effects on cellular differentiation and chemotropic activities in a number of different tissues. Bone Morphogenetic Proteins (BMPs), members of the TGF-β superfamily, play important roles in cellular differentiation as well as in chemotropic activities, including axon orientation and monocyte chemotaxis. Stimulation with BMP7 activates distinct intracellular signaling pathways that lead to either gene transcriptional events, through Smad-dependent signaling, or regulation of cytoskeletal organization, through PI3K- dependent mechanisms. This study explores the effects of Ethanol (EtOH) exposure on BMP-7-evoked, transcriptional and chemotropic signaling to determine if the developmental effects of EtOH, as observed in FAS, might be due to perturbation of BMP signaling and reveal whether the chemotactic activity of BMPs might be protective against the morphogenetic effects of EtOH. In order to study the influence of EtOH on BMP signaling, the C2C12 mouse myoblast cell line was utilized due to its abundance of BMP-7 and the ability to differentiate into muscle and respond to BMPs. The C2C12 cell line was selected for this study due to the ease of differentiation into muscle cells and the ability to respond to BMPs. In this study, the C2C12 cell line was differentiated into muscle cells and then exposed to varying concentrations of EtOH to determine the effects on BMP signaling. The effects of EtOH exposure on BMP signaling were determined using a variety of techniques, including Western Blotting and RT-qPCR. The results of this study showed that EtOH exposure significantly inhibited BMP signaling, as evidenced by a decrease in the expression of BMP target genes, including Smad2 and Smad3. These findings suggest that EtOH exposure interferes with the subcellular localization of these signaling mediators. The relationship between BMP7 signaling and EtOH-induced morphogenetic changes over an acute time course was explored. EtOH exposure appeared to have no effect on BMP7-stimulated Smad phosphorylation. EtOH treatment alone caused a dose-dependent decrease in Akt phosphorylation (pAkt) at all concentrations except 7.5%. PP2A partially rescued the effect of EtOH exposure on pAkt at low concentrations indicating that BMPs may provide a protective effect against the acute morphological effects of EtOH exposure.

3027 The Interaction of Maternal High-Fat Diet and Exercise on Brain Mitochondrial Bioenergetics in Rat Offspring after Ozone Exposure


This more accurately reflects the heterogeneity of real world conditions and can thereby improve the overall reliability of pharmaceutical safety testing.
New Insights into the Role Microglia Play in the Etiology of Gulf War Illness

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Gulf War Illness (GWI) is a multi-symptom disorder with symptoms: persistent headaches, chronic fatigue, memory loss/confusion, skin and GI problems. These features are characteristic of persistent sickness behavior, known to result from underlying microglial neuroinflammation. Chronic exposure to corticosterone (CORT), at levels associated with high physiological stress, can prime the CNS to mount an exacerbated neuroinflammatory response (increase in proinflammatory cytokines/chemokines) following systemic exposure to neurotoxins/inflammagens. When we administered CORT (200 mg/L 0.6% ETOH in drinking water) for 7 days prior to exposure to sarin surrogate, disopropyl fluoroephosphate (DFP; 4 mg/kg, i.p.), a heightened neuroinflammatory response was observed without astrogliosis or neurodegeneration 6-72 hours after DFP exposure. While the underlying early symptoms of GWI, they did not address the persistent episodic bouts of sickness behavior that characterize the long-term nature of GWI. As the phenotype is punctuated by symptom flares-ups, systemic exposure to lipopolysaccharide (LPS - a bacterial mimic; 0.5 mg/kg, s.c.) was used to challenge the GWI phenotype 24h following DFP treatment. CORT pretreatment primes the neuroinflammatory response to produce augmented LPS-induced inflammation and a single dose of DFP significantly exacerbated this effect. Here pretreatment with CSF1R inhibitor Pexidartinib (PLX) (290 mg/kg chow ad libitum over 28d), a compound that has been demonstrated to "eliminate" or reduce microglia in brain, was used to investigate the role microglia play in the pathogenesis of this neuroinflammatory disorder. The well documented reduction in DFP-induced mortality by CORT pretreatment was further reduced by PLX in CORT DFP (7%-36%) groups and eliminated in the DFP alone group (0%-71%). CORT pretreatment caused significant thymic involution in both control (35%) and PLX (47%) groups with PLX groups showing reduced thymic weights (11% and 27%, respectively). Brain cytokine/chemokine levels measured by qPCR revealed large increases in IL6 & GGM in response to LPS in PLX pretreated groups (LPS: 125% & 122%; CORT: 178% & 181%; CORT DFP 194% & 194%), respectively) revealing a potential proinflammatory effect of PLX. Findings potentially point to not only a non-microglial origin of these cytokines in this model, but also a protective role of microglia in certain neuroinflammatory conditions.

Using Public Data to Develop an Open-Access Computational Knime Workflow to Identify Any Cholinergic Compound

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One goal of high-throughput data mining in the life sciences is to align compounds with potential mechanistic targets for further in-depth study. As proof-of-concept using two-dimensional structural scaffolding, we sought to identify any unknown compound that might specifically interact with the cholinergic nervous system. The sympathetic and parasympathetic cholinergic nervous systems encompass the foundation of signaling at the mammalian neuromuscular junction. Neurotransmission is mediated by acetylcholine (ACh) via nicotinic (nAChR) and muscarinic (mAChR) ACh receptors and acetylcholines- terase (ACHE). Given the extent of public data from prior studies under- taken on these target proteins, we hypothesized that the cholinergic potential of any novel compound could be computationally predicted using big data mining based on the presence of structural motifs (scafolded) across the known universe of cholinergic agents. Herein we show the development of a computerized workflow to capture and cluster virtually all (~19,000) compounds known to target the cholinergic system. From these, we identified 453 scaffolds that explain the structural diversity of known cholinergins. Using the Tanimoto similarity of these scaffolds to a compound as a covariate within a random forest machine-learning algorithm, we can predict the likelihood a novel compound will target any component of the cholinergic system (nAChR, mAChR or ACHE) with exquisite sensitivity (99.3%) and high accuracy (94%). By building similar models for each separate component of the cholinergic system, we can additionally predict the likelihood of a compound interacting with a specific target (nAChR, mAChR or ACHE) with similarly high sensitivity and accuracy (>96%). In conclusion, our findings demonstrate that it is possible to implement computerized workflows to gather public data, identify key structural scaffolds that describe the known cholinergic agents, and develop automated prediction models to calculate the likelihood of a previously uncharacterized compound interacting with the cholinergic nervous system.

Doxorubicin-Induced Neuromotor Impairments in Male Athymic NCr Mice: Partial Protection by Phenylaminoethyl Selenide

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There is considerable research characterizing cardiotoxicity of the anti-tumor drug doxorubicin (DOX), while few studies have focused on neuro- motor and cognitive impairment. DOX has been shown to induce fine-motor deficits in humans and motor neuron degeneration, in vitro. The athymic T-cell deficient NCr (nu/nu) mouse is a frequently used model of cancer growth and chemotherapeutic treatment, but there have been few studies assessing neuromotor function in this strain and none investigating DOX-induced motor impairments and neuroprotection afforded by the antioxidant phenylaminoethyl selenide (PAESe). Male NCr mice were initially tested on rotarod and voluntary wheel running. After initial testing, they were assigned to exposure groups to see if there did not differ prior to treatment. Mice were then administered four weekly i.v. doses of 5mg/kg DOX, 10mg/kg PAESe, a CSFR1 inhibitor Pexidartinib (PLX) (290 mg/kg chow ad libitum over 28d), a compound that has been demonstrated to “eliminate” or reduce microglia in brain, was used to investigate the role microglia play in the pathogenesis of this neuroinflammatory disorder. The well documented reduction in DFP-induced mortality by CORT pretreatment was further reduced by PLX in CORT DFP (7%-36%) groups and eliminated in the DFP alone group (0%-71%). CORT pretreatment caused significant thymic involution in both control (35%) and PLX (47%) groups with PLX groups showing reduced thymic weights (11% and 27%, respectively). Brain cytokine/chemokine levels measured by qPCR revealed large increases in IL6 & GGM in response to LPS in PLX pretreated groups (LPS: 125% & 122%; CORT: 178% & 181%; CORT DFP 194% & 194%), respectively) revealing a potential proinflammatory effect of PLX. Findings potentially point to not only a non-microglial origin of these cytokines in this model, but also a protective role of microglia in certain neuroinflammatory conditions.

Neurotoxic Mechanisms of the Novel Psychoactive Substance Methoxetamine in Rat and Human In Vitro Models

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The use of new psychoactive substances (NPS) is steadily increasing. One of the commonly used NPS is the ketamine analogue methoxetamine (MXE). Although adverse effects have been reported for MXE, there is limited data available on its neurotoxicological mechanism(s) of action. To increase insight in the neurotoxicological profile of MXE, the aim of this study was to investigate the effect(s) of ketamine and its analogue MXE on calcium homeostasis, neuronal activity and monoamine transporters in different rat and human in vitro models. We therefore investigated the effects of ketamine and MXE on several neurotransmitter receptors and voltage-gated calcium channels (VGCCs) using single cell intracellular calcium [Ca2+]i imaging in rat primary cortical cells, human SH-SY5Y cells and human induced pluripotent stem cell (hiPSC)-derived neurons. We also investigated effects on neuronal activity in rat primary cortical cells and hiPSC-derived neurons grown on micro-electrode arrays (MEA). Finally, effects on monoamine transporters were assessed in human embryonic kidney (HEK293) cells transfected with human monoamine transporters. In human SH-SY5Y cells, MXE (10 μM) slightly inhibited the K+ and acetylcholine-evoked increase in [Ca2+]i. In rat primary cortical cells, MXE (10 μM) increased the glutamate-evoked increase in [Ca2+]i whereas ketamine (10 μM) was without effect. MXE and ketamine did not affect VGCCs, but inhibited spontaneous neuronal activity with IC50 values between 10 - 100 μM. Finally, MXE potently inhibited uptake via monoamine transporters (DAT, NET and SERT) with IC50 values in the low micromolar range. Our combined in vitro data indicate that the neurotoxicological profile of MXE that can aid the risk assessment of MXE and provides a rapid screening approach that is also useful to assess the hazard of other emerging NPS. This work was funded by the Dutch Poisons Information Center and the Faculty of Veterinary Medicine (Utrecht University).
Gulf War Illness (GWI) is a multi-symptom disorder with similarities to the features of sickle cell behavior. Previously, we have demonstrated that, like sickle cell behavior, GWI is associated with underlying neuroinflammation. In particular, toxic exposures experienced by soldiers during the Gulf War such as pesticides and nerve agents, as well as physiological stress, have led to a chronic, primed neuroinflammatory state that results in an exacerbated response to subsequent inflammatory challenges. The significant elaboration offlammatory cytokines related to the neuroinflammatory priming observed in our GWI mouse model indicates that this illness may be the result of long-term alterations in the brain’s resident immune cells, namely microglia. Here, we have investigated the potential role of microglia in GWI using the CX3CR1−/− mouse strain and minocycline, an anti-inflammatory drug with effects on microglia. Adult male C57BL/6J or CX3CR1−/− mice were exposed to our GWI model consisting of corticosterone (CORT) in the drinking water at levels associated with high physiological stress for 7 days followed by exposure to the nerve agent surrogate, diisopropyl fluorophosphate (DFP), on day 8 and a subsequent immune challenge with lipopolysaccharide (LPS) on day 10. C57BL/6J mice that were given minocycline received a single dose 30 minutes prior to LPS. To test whether minocy- cline or CX3CR1−/− disrupted LPS-induced inflammation, an additional cohort of animals was exposed to LPS exposure with or without DFP on day 8 in place of DFP. Interestingly, neither CX3CR1−/− nor minocycline-treated mice exhibited major differences in cytokine mRNA expression following CORT+LPS exposure compared to controls. However, both CX3CR1−/− and minocycline treatment removed the contribution of DFP to the GWI phenotype, reducing cytokine mRNA expression to levels comparable to CORT+LPS treatment. The recovery of the GWI phenotype to CORT+LPS levels is clinically significant, because this condition mimics a “healthy sick” state in which stress may potentiate inflammatory conditions. These results suggest that microglia play a crucial role in the development/maintenance of GWI particularly in response to DFP and that drugs with modulatory effects on microglia show promise for the treatment of veterans suffering with GWI.

Mitochondrial bioenergetics play a key role in the mechanisms of neurodegenerative disorders and chemical induced neurotoxicity. However, mitochondrial bioenergetic parameters have not been systematically evaluated within the multiple brain regions in sedentary versus active lifestyle following environmental pollutant exposures. In the present study, we measured complex I, complex II and complex IV enzymes in Brain Mitochondrial Bioenergetics following Ozone Exposure in Sedentary Versus Active Lifestyle of Female Long-Evans Rats

Mitochondrial bioenergetics play a key role in the mechanisms of neurodegenerative disorders and chemical induced neurotoxicity. However, mitochondrial bioenergetic parameters have not been systematically evaluated within the multiple brain regions in sedentary versus active lifestyle following environmental pollutant exposures. In the present study, we measured complex I, complex II and complex IV enzymes in sedentary and active with continuous access to running wheels starting at age of postnatal day (PND) 22 until the age of PND 100 and subjected to Ozone (O3) exposure. A similar effect was observed in HIP, but however, there was an increase of activity in active animals following 4 weeks of exposure in mice. Based on these premises we tested the hypotheses, chronic tobacco smoke exposure to mice for 4 weeks can impact Slc40a1 mediated iron exporter function at the neurovascular unit. Both male and female mice were divided into two groups, chronically exposed via direct inhalation of side-stream smoke) to either tobacco smoke mixed with oxygenated air or oxygenated air alone following 4 weeks of exposure. Two cigarettes/hr, 6 times/day -7 days/week mice were sacrificed and brain tissue collected. Western blot analysis of total brain homogenate was conducted. Interestingly, results revealed the unaltered levels of Slc40a1 in male mice while significant downregulation in female mice. Although needs further validation by downstream relevant assays, this gender difference in Slc40a1 expression and possibly iron shuttling is indeed a novel finding suggesting that tobacco smoke could differentially impact the cerebrovascular system (and possibly CNS functionality) based on gender.

Relevance of Gender in Tobacco Smoking-Dependent Dysregulation of the Iron Exporter Slc40a1

Cerebral iron homeostasis is crucial to maintain optimal CNS functionality. Iron is a vital cofactor involved in the processes of energy production, thus alteration of its transmembrane efflux can impact cell and mitochondrial viability. Specifically, the excess of iron resulting from unwaranted intracellular accumulation can react with oxygen to form cytotoxic free radicals leading to cellular damage. Previously, the functional carrier family Slc40a1 (1 or ferroportin 1) of a transmembrane iron exporter, has been well characterized in enterocytes, hepatocytes and reticuloendothelial system. Activity of this transporter is linked to Nh2-2e pathway to regulate cellular iron metabolism and redox balance. Our previous findings demonstrated that pharmacological inhibition of Nh2 by Snithiophosphate produces a significant upregulation of the mitochondrial Slc40a1 (both gene and protein) in various in vitro models of mouse and human blood-brain barrier endothelium. We have also shown that, tobacco smoking progressively downregulated Nh2 in total brain homogenates following 4 weeks of exposure in mice. Based on these premises we tested the hypothesis, chronic tobacco smoke exposure to mice for 4 weeks can impact Slc40a1 mediated iron exporter function at the neurovascular unit.

Is BMAA an Agent of ALS? A Preliminary Study in Zebrafish

Amyotrophic lateral sclerosis (ALS) is a progressive and fatal neurodegenerative disease with a prevalence of about 3.9 out of 100,000 people. Individuals with ALS are usually diagnosed between the ages of 40 and 70, and have an average life expectancy of three years post-diagnosis. The disease is characterized by motor neuron degeneration, leading to muscle atrophy, paralysis, organ systems failure, and eventual death. Ten percent of ALS cases are familial, with the remaining 90% classified as sporadic. While many gene mutations have been associated with ALS, it is likely that a combination of genetic and environmental factors contribute to the disease. β-methylamino-L-alanine (BMAA) is a non-protein amino acid produced by cyanobacteria, which has been implicated in several neurodegenerative diseases, including ALS. It has been postulated that exposure to BMAA could lead to formation of protein aggregates, oxidative stress, or sequestration of glutamate receptors, mechanisms that have been implicated in the etiology of ALS. However, no causal relationship between BMAA exposure and the onset of ALS has been proven. We are leveraging a transgenic zebrafish line carrying the sod1 G93R mutation to explore the possible connection between chronic and acute exposure to BMAA and ALS-like phenotypes. Wild type (WT) and sod1 mutant embryos were exposed to BMAA from six hours post-fertilization to seven days post fertilization (dpf). BMAA exposure in WT fish was associated with reduced density of neuromuscular junctions (NMJ), as determined by immunohistochemistry, and hyperactive-like behavior. By contrast BMAA did not elicit a significant behavioral change in the sod1 mutants, leading us to speculate that increases in superoxide radicals in sod1 mutants might ameliorate BMAA neurotoxic properties. Overall, our preliminary results suggest that, although some effects of neurotoxin exposure were seen, these short-term developmental exposures are not sufficient to make definitive conclusions about any possible role of BMAA in the etiology of ALS. Long-term exposures are underway to more closely assess the onset and severity of ALS-like symptoms in zebrafish exposed to BMAA and to examine motoneuron viability and function over time, and how such potential compromises might reveal mechanisms involved in neuro-muscular degeneration in ALS when analyzed by behavioral, histological and proteomic assays.

2,4,6-Tribromophenol (TBP, CAS No. 118-79-6) is a brominated chemical used in the production of flame retardant and wood preservative. TBP is found in marine environments, where it is incorporated in shellfish that may be consumed by predatory fish. It is also created as a byproduct during food processing and water treatment. TBP is a bio-active and bio-concentrated endocrine disruptor that interferes with estrogen and thyroid hormone signaling. Estrogen and thyroid hormones regulate important barrier functions, including the blood-brain barrier. The blood-brain barrier is a selectively permeable barrier composed of microvessels made of endothelial cells that are anchored by tight-junctions and express ATP Binding Cassette (ABC) transporters that actively remove toxic endobiotics and xenobiotics from the brain. In this study, we examined the effect of TBP exposure on the transport activity of three well-characterized ABC efflux transporters: P-glycoprotein (P-gp, ABCB1), Breast Cancer Resistance Protein (BCRP, ABCG2), and Multidrug Resistance-associated Protein 2 (MRP2, ABCG2), in freshly isolated rat brain microvessels from Sprague Dawley rats (Taconic, retired male breeders). To establish a dose response and time course, we treated the ex-vivo microvessels with 1-100 nM TBP for 1-3 hours and then measured transport activity. Transport activity was quantified using a confocal microscopy based assay to measure the steady-state luminal accumulation of a transporter-specific fluorescent substrate. We found TBP exposure resulted in a time and dose dependent decrease in P-gp and BCRP transport activity. We saw no change in MRP2 transport activity under identical conditions, indicating selectivity and capillary integrity. Preliminary studies inhibiting transcription and translation suggest that the TBP-dependent decrease in transport activity is through a non-genomic pathway (signaling). Our future studies will involve immunohistochemistry to determine protein localization, and western blots to quantify protein expression after exposure to TBP. Furthermore, we will measure the activity of P-gp and BCRP after in-vivo dosing with TBP. Our work is meaningful because decreases in transporter activity at the blood-brain barrier can reduce neuroprotection and increase CNS exposure to potentially toxic endobiotics and xenobiotics. Supported by the Intramural Research Program at NCI/NIEHS.

S. Rellick. West Virginia University, Morgantown, WV. Sponsor: T. Nurkiewicz

There is concern among residents living near well sites as to whether or not hydraulic fracturing can impact drinking water by releasing chemical additives into the surrounding ground, potentially contaminating both ground water and drinking water. We evaluated the effects of both acute and chronic exposure BTEX on neuronal cells. The chemical we are currently investigating consists of components of raw petroleum products, collectively referred to as BTEX (benzene, toluene, ethylbenzene and xylene), to determine its effects on neuronal cell viability and mitochondrial function in a neuronal cell culture model using the XFe96 Extracellular Flux Analyzer and Calcein AM assays. We will also expand our studies to a murine model of ischemic stroke. We observed that low concentrations of BTEX that are not toxic to neurons leads to an increase in mitochondrial activity. In a pilot animal experiment, we observed that animals will drink water containing BTEX, and can drink the water for 7 days with no visible health effects. Preliminary data suggest that mice receiving an IP injection of BTEX have increased infarction in the cortex and striatum. The increase in mitochondrial activity with low concentrations of BTEX may increase mitochondrial activity initially, but a sec- ondary insult or injury may prevent cells from being rescued and to this damage, resulting in enhanced cell death. We intend to chronically expose mice to low concentrations of BTEX in drinking water, and then induce a transient middle cerebral artery occlusion to see if exposure to BTEX leads to a worse outcome.
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**3043 Brain Lesions Detected by TSPO Positron Emission Tomography (PET) Are Highly Correlated with Neuroinflammation and Neuronal Necrosis in a Rat Model of Acute Organophosphate (OP) Intoxication**


Current medical countermeasures for acute OP poisoning do not protect against persistent neurodegeneration and cognitive impairment, underscoring the need for preclinical models that enable longitudinal monitoring of novel therapies. Previous studies demonstrated neuroinflammation co-occurred with high progesterone levels in acute intoxication with the OP diisopropylfluorophosphate (DFP), suggesting potential mechanistic relevance. The goal of the current study was to map the spatiotemporal progression of neuroinflammation following acute DFP intoxication, and evaluate PET imaging with the TSPO radioligand [18F]-PBR111 as a non-invasive tool for the longitudinal assessment of acute DFP intoxication-induced neuroinflammation. Adult male Sprague Dawley rats were treated with pyridostigmine (0.1 mg/kg, i.m.) prior to administration of DFP (4 mg/kg s.c.), atropine sulfate (2 mg/kg, i.p.) and Z-PAM (25 mg/kg, i.m.). This paradigm elicited moderate-to-severe seizure behavior in study animals. PET expression in the brain was imaged using a Siemens F120 or Inveon DPET microPET scanner; anatomic registration was obtained using a Bruker 7T MRI. Animals were imaged prior to DFP administration (baseline), and at 3, 7, 14, 21, or 28 days post-DFP intoxication. DFP significantly increased TSPO labeling within the hippocampus, thalamus, amygdala and piriform cortex (100-200% increase of protein tyrosine phosphatase 110) uptake was highly correlated with histopathological assessment of neuroinflammation by glial fibrillary acidic protein (GFAP) and ionized calcium-binding adapter molecule 1 (IBA1) immunostaining and with neuronal necrosis as determined by hematoxylin and eosin staining. The results demonstrate that TSPO PET imaging with [18F]-PBR111 can provide quantitative assessments of neuroinflammation following acute DFP intoxication, which may prove useful for longitudinal evaluation of novel therapies. Supported by NIH CounterACT program (NS079202).

**3044 Nicotine and E-Cigarette Exposure Alters Brain Glucose Utilization in Ischemic Stroke**

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Use of electronic cigarettes (eCig) is a growing health concern in both smoking and nonsmoking populations and rigorous studies are needed to investigate the effects of the nicotine exposure via eCig on the neurovascular unit (NVU) and stroke outcome. Previous studies by our lab have shown that nicotine exposure significantly decreases glucose transport across the blood-brain barrier (BBB) in ischemia-reperfusion conditions. In the present study, we investigated the effects of acute & chronic nicotine and eCig vaping exposure on neuronal & brain glucose utilization. Methods: In vitro primary cortical neurons were exposed to nicotine (10 µM) & cotinine (5 µM) for 1 or 5 days and then subjected to 2 h oxygen-glucose deprivation (OGD) followed by 24 h reperfusion to mimic ischemic conditions. Neuronal glucose utilization was measured by radiolabeled 2-deoxy-D-glucose (DG) uptake. Immunohistochemistry for glutamatergic (GLUT1, GLUT3) and nicotinic acetylcholine receptors (nAChRs) expression was determined using the Western blot assay. Results: In vivo and in vitro, nicotine and cotinine exposure significantly decreases neuronal glucose utilization in OGD-reperfusion conditions which were reversed by a non-specific nicotinic acetylcholine receptor (nAChR) antagonist, mecamylamine (20 µM). Nicotine & cotinine also decreases neuronal GLUT1 & GLUT3 expression which were correlated with a7 nAChR upregulation in OGD-reperfusion. Nicotine decreases neuronal viability (in a dose-dependent manner) in normoxic and OGD-reperfusion conditions. E-cig exposure for 7 and 14 days also decreases deoxy-D-glucose uptake under normoxic and OGD-reperfusion conditions in ABS experiments. Conclusions: These data support, from a cerebrovascular perspective, that nicotine & eCig vaping exposure creates an enhanced glucose deprived state at the NVU which could lead to enhanced ischemic brain injury.

**3045 Computational Proteome-Wide Screening Predicts Neurotoxic Drug-Protein Interactome for the Investigational Analytical BIA 10-2474**

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The investigational compound BIA 10-2474 (PubChem CID: 46831476), designed as a long-acting and reversible inhibitor of fatty acid amide hydrolase (a lipase and member of the serine hydrolase superfamily) for the treatment of neuropathic pain, led to the death of one participant and catheter-related deaths due to intracranial hemorrhage in a Phase I clinical trial in 2016. Although, off-target activities of BIA 10-2474 within the ~200-member serine hydrolase superfamily have been characterized as contributing factors to the observed neurotoxicity in humans (van Esbroeck et al., Science 2017), these activity-based proteomics data do not fully explain the clinical phenotype. Therefore, we performed a proteome-wide screening of BIA 10-2474 within a subset of human disease-relevant serine hydrolases currently in development. The proteome-wide screening identified novel drug-protein interactions for BIA 10-2474, providing actionable insights towards elucidation of toxicological, intracranial, inflammatory, hemorrhagic or clotting processes and/or diseases. Eleven proteins were identified as potential targets of BIA 10-2474, and the two highest-scoring proteins, Factor VII and thrombin, both essential blood-clotting factors, were predicted to be inhibited by BIA 10-2474. In vitro validation of top-scoring targets is currently in progress. In silico proteome-wide screening identified novel drug-protein interactions for BIA 10-2474, providing actionable insights towards elucidation of toxicology. Further, this methodology can facilitate off-target profiling for other small molecules currently in development.

**3046 Prenatal Exposure to DEHP Induces Premature Hippocampal Neurodegeneration**

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Phthalates are a family of synthetic chemicals that are widely used in medical, automotive and consumer products. Di-(2-ethylhexyl) phthalate (DEHP) is an endocrine disruptor that has anti-androgenic activity. This study tested an ongoing hypothesis that prenatal exposure to DEHP results in premature reproductive senescence and impaired memory in adulthood. Male CA1 mice were prenatally exposed to DEHP by dosing their mother daily with 200 µg, 500 mg or 750 mg/kg or vehicle from gestation day 11 to birth. The impact of the exposure on their reproductive and memory were examined when they reached the ages of 8 to 22 months. As early as at the ages of 8 months, the 750 mg/kg/day DEHP group of mice exhibited significantly reduced fertility (p=0.028). The same dose-dependent reduction of fertility continued until the age of 18 months. Six out of seven mice of the 750 mg/kg/day group (86%) were infertile, while only a minor drop of fertility was observed in the control mice (25%). At this age, however, all DEHP-exposed group had lower serum testosterone and higher serum estradiol levels than the control mice, indicating that DEHP-exposed mice exhibit a compromised gonadal steroidogenesis. Impacts on their memory was measured at the ages of 16-17 months. Novel object recognition test and Y-maze test found a significantly reduced memory (p<0.05) and spatial memory (p=0.04) in DEHP-exposed mice, respectively. Because hippocampal navigation and short-term memory, whether hippocampal neurons were impacted by the prenatal DEHP exposure was examined. Nissl staining showed that majority of pyramidal neurons in hippocampus were shrunken, indicating that they were undergoing a neuronal degeneration. Indeed, quantitative Spatial Light Interference Microscopy (SLIM) revealed a significantly less hippocampus pyramidal neurons in CA1 and CA2 regions of DEHP-exposed mice than controls (p<0.01). The hippocampal neurodegeneration was in part caused by inflammation and oxidative stress as COX-2 (inflammatory marker) expression and 8-OHDG (DNA oxidation marker) staining were elevated in the DEHP-exposed group. In summary, this study finds that the prenatal exposure to DEHP induces premature reproductive senescence and hippocampal neurodegeneration.
Mamelonitobin (MMB) is a small-molecule inhibitor of Janus kinase 1 and 2 (JAK1/JAK2). The oral toxicity of MMB was evaluated in a repeat dose toxicity study in rats for 26 weeks before initiating Phase 3 clinical trials in myelofibrosis. Rats were administered MMB at dose levels of 0 (vehicle), 5, 15, and 50 mg/kg/day. Following 26 weeks of administration, cohorts of animals/group were maintained for a 10 week recovery period. Assessments of neurobehavioral effects and general toxicity included, but were not limited to, functional observational battery (FOB) evaluations and neuro- and electrophysiological evaluations at Weeks 1, 4, 13, 26, and anatomic and clinical pathology. Toxicokinetic assessment was conducted for the MMB and its metabolite. Image acquisition of neuroconduction velocity (NCV) and amplitude were measured in the caudal nerve (sensory, motor and autonomic axons), digital nerve (distant extrema of the sciatic nerve) and tibial nerve (response of muscles of the rat hind paw following stimulation). Compared to the vehicle control group, after administration of 50 mg/kg/day for 13 and 26 weeks, the caudal NCV was reduced by 16% and 15%, respectively. At 26 weeks, a 7% reduction in digital NCV was noted. These changes were reversed by the end of the recovery phase. No deficit in the tibial motor nerve and no deficit in the response amplitudes occurred at 13 or 26 weeks and no deficits were observed in any cohort in the 5 and 15 mg/kg/day groups. There was no FOB or peripheral neuropathological findings related to MMB at either the terminal or recovery intervals. There were no microscopic correlates for the reported in-life electrophysiology findings. A follow-up in vitro study revealed no effect on ion channels associated with peripheral nerve conduction such as the hKv1.2, hNav1.5, heredity channels, the hKv1.1 and 1.2 potassium channels, and the hKv1.1 and 1.2 potassium channels. A 5% increase in the frequency of peripheral neuropathy, mostly grade 1 and 2 in severity, was observed in a 24-week Phase 3 clinical study.

The human cerebral cortex is organized in a complex 3-dimensional (3D) structure comprising different neural cell types. The coordinated work of these different cell types is key for brain function and homeostasis. Recently, much work has been focused on obtaining 3D brain organoids in an attempt to better recapitulate the brain development/function in vitro. However, current protocols may lead to variable organoid size and function, making the use of these powerful tools impractical in an investigative toxicology setting. Here we describe the development of a highly homogenous human induced pluripotent Stem cell (hiPSC)-derived cortical spheroid screening platform in 384 well format, composed of cortical neurons and astrocytes. Immunofluorescence analysis indicated that these derived neurons and astrocytes display key markers of cellular identity as well as maturity, such as synaptic proteins and glutamate transporters. Viability assays carried out with compounds with known mechanism of action indicated scalability and feasibility of the assays, with results comparable to a standard 2D model employing the same culture composition. Kinetic, high throughput calcium flux analysis performed in a in a Fluorescent Imaging Plate Reader (FLPR) highlighted that the spheroids present quantifiable, robust and uniform spontaneous calcium oscillations, measured. The calcium signal was modified with excitatory and inhibitory modulators coherently and in a highly reproducible fashion, confirming the presence of a functionally integrated glutamatergic/GABAergic circuit. High speed confocal imaging confirmed homogenous calcium oscillations at the cellular level, whereas multielectrode array (MEA) analysis demonstrated robust synchronous neurophysiological activity at the network level. Additionally, these cortical spheroids are amenable to immunostaining in suspension, enabling scalable high content image-based assays focused on key protein markers. Altogether, the developed 3D cortical spheroid platform can be easily implemented as a reliable high throughput screening platform to investigate complex cortical phenotypes in vitro, as a reliable high-throughput screening platform for toxicity studies, disease modeling and drug testing.

The key initiating event for many toxicological pathways is often the binding of an electrophile to an endogenous nucleophile, such as DNA, protein, or lipids. Protection against these electrophiles is critical, especially in nervous tissues which have limited capacity for cellular regeneration. Glutathione S-transferases (GSTs) metabolize electrophiles, but their precise role in protection against electrophile-mediated tissue damage is poorly understood. This has hindered the ability to ascertain increased health risks in humans with GST genetic variants, particularly the common GSTM1 and GSTT1 null alleles. To address the limited knowledge concerning the role of GST families and individual GSTs in detoxification of environmental pollutants, we generated mouse lines lacking 14 GST genes, including members of the GSTP, GSTM, and GSTT gene families. Here we identify the essential role of GSTs in protection against both DNA and cellular damage mediated by the electrophilic neurotoxicant acrylamide, an industrial pollutant which is also found at high levels in starchy foods. Consistent with human studies, subchronic exposure of mice to acrylamide in drinking water resulted in loss of motor function, as measured by the rotarod test. However, the severity of motor impairment was dramatically increased in the GST deficient mice. Interestingly, acute acrylamide exposure resulted in strain-specific, GST-sensitive, damage to the gastrointestinal epithelium and gastroparesis, a sequela of acrylamide exposure not previously documented in humans or rodents. Together, our studies demonstrate that GSTs play a critical role in protection against tissue damage mediated by environmental electrophiles such as acrylamide. GST deficient mouse lines provide a means for investigating the increased risk of these pollutants to humans carrying GST variants, as well as methods for risk mitigation in these GST-compromised individuals.

Perfluorooctanoic acid (PFOA) and perfluorooctanoic acid (PFOA) are members of perfluorinated compounds used in a variety of products, such as stain and oil resistant coatings, aviation hydraulic fluids, fire-fighting foams, paints, adhesives, waxes, polishes, and other products. Several studies have shown detectable levels of PFOAs and PFOA in the environment. In particular, drinking water samples have been contaminated with these agents which may cause potential long-term effects for humans and animals. Researchers have determined that PFOAs and PFOAs causes toxicity to humans and animals including hepatotoxicity, nephrotoxicity, neurotoxicity, disruption to the endocrine system, and other harmful effects. A number of studies showed that PFOAs and PFOAs have been associated with induction of oxidative stress. The purpose of this study was to determine the role of Nrf2 in protection against the neurotoxicity of these agents. Nrf2 is a transcription factor that is activated in response to oxidative stress. Upon activation, Nrf2 upregulates the gene expression of several antioxidant enzymes, such as superoxide dismutase, glutathione peroxidase and reductase, catalase, and other antioxidant molecules. For the present study, Nrf2 knockout and wild-type C57BL/6 mouse astrocytes were used as in vitro models. Knockout and wild-type astrocytes were exposed to concentrations of PFOA ranging from 75-600 μM and 400-1000 μM for PFOA for 24 hours. Lactate dehydrogenase (LDH) assay was conducted to assess cell cytotoxicity. LDH release was significantly higher in Nrf2 knockout than in the wild type astrocytes for exposure to 150-600 μM concentrations of PFOA and 800-1000 μM concentrations of PFOA. Phase contrast light microscopy was used to investigate the morphological changes of exposed astrocytes. Marked toxicity was observed, such as cell debris, rounded cells, vacuolization, cell blebbing and decreases in cell number consistent with the cell viability data. We conclude that PFOAs and PFOAs are cytotoxic to astrocytes and that Nrf2 knockout cells are more sensitive to toxicity by these agents, an effect that is potentially limited to the ability to detoxify the antioxidant capability of these cells.

Bisphenol A (BPA) is one of the highest volume produced and used chemicals worldwide. Early-life exposure to BPA has been shown to alter sex-specific neural organization, neuroendocrine physiology, and behavior in laboratory animals, and impacts neural development in human. Oxidative stress is induced by an imbalanced redox state, involving either excessive generation of reactive oxygen species (ROS) or dysfunction of antioxidant system. The brain is one of organs especially vulnerable to the effects of ROS because of its high oxygen demand. Neurodegeneration has increasingly been linked with mitochondrial dysfunction and inhibition of the electron transport chain. This inhibition leads to the generation of ROS and depletion of cellular energy levels, which can consequently cause cellular damage and death mediated by oxidative stress. Mitochondrial membrane potential is an important parameter of mitochondrial function used an indicator of cell health. The aim of this study was to examine the neurotoxic effects of BPA in human neuroblastoma SH-SY5Y cells. Human neuroblastoma SH-SY5Y cells were exposed to BPA at a concentration range of 0.001 to 1000 µM for up to 24 h. Cell proliferation and viability, ROS production, mitochondrial membrane potential and intracellular glutathione (GSH) content were evaluated. Our results indicated that exposure to BPA significantly decreased cell viability in a concentration-dependent manner in SH-SY5Y cells. At low concentrations (0.001–10µM) of BPA, mitochondria were significantly increased at 3h and 6h in SH-SY5Y cells. BPA induced a significant decrease of mitochondrial membrane potential after 3 h similar to CCCP, the mitochondrial uncoupler positive control. On the other hand, a significant reduction in intracellular levels of GSH was observed at 1h and 3 h with treatment of BPA. These data suggest that alteration in cellular GSH, loss of mitochondrial membrane potential and the elevated ROS production play a contributing role to the toxic effects of BPA and that BPA adversely results in neurodegeneration. The data further indicate that the availability of GSH to the cells may be insufficient to provide protection against BPA toxicity. Thus, BPA could be a risk factor for neurodegenerative diseases. Supported by Title III.

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Synthetic cannabinoid abuse has become an international public health problem including acute intoxication and memory impairments. A recent report identified several synthetic additives in samples of “Spice/K2”, including JWH-018 (1-pentyl-3-(1-naphthoyl)indole), a synthetic ligand for the cannabinoid receptor 1 (CB1). There is only limited research on the neurotoxicity and memory impairment induced by JWH-018: The Mechanism and the Effect on Learning and Memory


Premutation carriers with CGG trinucleotide expansion in the 5’-untranslated region of FMR1 (PreCGG) have early onset neurologically deficits and high risk of developing neurodegenerative FXTAS later in life. Carriers of expressed mutations within FRY1 are susceptible to the pharmacogenetic disorder malignant hypertension (MHS) and are intolerant to heat stress. Knock in mice expressing PreCGG (170 repeats), MHS mutation T4826-RyR1, singly or in combination (double mutation; DM) were compared to congenic wild type (WT) for serum corticosterone (CTS) responses to water aversive stress (WAS), a model of psychological stress. Neuronal-astrocytic hippocampal co-cultures obtained from these genetic lines were trained in a novel place recognition memory task with numerous endogenous and xenobiotic ligands playing a key role in a number of cellular processes especially immune cell function and development. Differential effects of AHR ligands upon CD4 naïve Tcell development has been described with 2,3,7,8-tetrachlorodibenzop-dioxin (TCDD) inducing regulatory Tcells while FICZ promotes Th17 responses. Previously our lab has demonstrated prolongation of graft survival in a fully mismatched skin allograft model (Balb/c donor to C57BL/6 recipient) with intraperitoneal administration of TCDD and early rejection with FICZ treatment. In the present work we demonstrate prolonged graft survival in a minor mismatch allograft model (C57BL/6 Male to Female) with dietary supplementation with the AHR ligand, Indole-3-Carbinol (13C). 7wk old C57BL/6 female mice were maintained on semi-purified base (SPB) diet deficient of AHR-ligands or SPB diet with 13C supplementation (3ppm) for 3wks prior to transplant and for the duration of the model. AHR-mediated graft survival was correlated to decreases in IFNy producing Graft infiltrating Leukocytes. Animals maintained on SPB diet demonstrated a loss of intestinal Treg, Innate Lymphoid Cells type 3, and γδ Tcells. However, 13C supplementation led to maintenance of these mucosal immune cell populations as measured by Flow cytometry. Conversely, maintenance of animals on the AHR-deficient SPB diet led to accelerated graft rejection (Median GST of 46 vs 15.9days p<0.05). These findings were AHR-dependent as AHR null animals were found to not only reject grafts earlier than WT counterparts but were not responsive to 13C supplementation (Median GST, AHR null on AHR-deficient diet vs 13C diet were 17 vs 15.5 days respectively). These data demonstrate a novel regulatory pathway for prolongation of graft survival via dietary supplementation of naturally occurring AHR ligands. Splenocytes isolated from animals maintained on either SPB or 13C diets were collected and cultured in vitro with exposure to HY peptide. ELISA performed on the supernatants demonstrated...
a decrease in IFNg and TNFa production in those animals maintained on I3C vs SPB diet. While the underlying mechanism remains unclear, these data suggest a role for activation of the mucosal immune system via AHR stimulation and dietary AHR ligands play a novel regulatory role in modulating systemic immune responses.

3055 Modulation of the Aryl Hydrocarbon Receptor via Oxictoxine

The human population is consistently exposed to ultraviolet radiation (UVR), which has both beneficial and detrimental effects. High levels of exposure is a major contributor to the risk of developing skin cancer, yet some exposure is needed for vitamin D synthesis. In an attempt to combat the negative consequences, sunscreen use is highly encouraged, especially to vulnerable populations such as children and those living in high latitude (e.g. Scandinavia and Australia) areas with more intense exposure. While sunscreens have been shown to be effective at reducing the risk of skin cancer, there is an increasing body of literature suggesting that some UVR filters may have hormone disrupting properties, which makes further toxicological studies on these chemicals essential. We hypothesized, based on structural composition, that UVR filters may be agonists for the Aryl hydrocarbon receptor (AHR). They possessed AhR responsive reporter constructs that play a significant role in the metabolism of various exogenous and endogenous compounds, as well as regulating the immune system. When ligand binds to the Ahr, the complex acts as a transcription factor for various genes, including members of the cytochrome P450 family such as CYP1A1. Interestingly, the AhR agonist octinoxate (FICZ) is thought to be produced in the skin after UVR exposure. To determine if sunscreen ingredients can activate the AHR, we utilized the H111.1c2 luciferase hepatoma reporter cell line to screen a variety of chemicals commonly found in sunscreens. Our results indicated that none of the ingredients tested were able to activate the AHR themselves. However, when tested in conjunction with FICZ, the chemical octinoxate was shown to potentiate FICZ ability to activate the AHR.

The experiment was repeated using 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), another well-known AHR ligand. Interestingly, our results not only showed that octinoxate was unable to potentiate TCDD’s ability to activate the AHR, but they also indicated that this co-treatment completely eliminated the ability of TCDD to activate the AHR. We hypothesize that octinoxate may be directly interacting with either the ligands themselves or with cytochrome P450 enzymes that are transcribed and translated after AHR activation occurs. While there is more research to be done, these results suggest that octinoxate may be able to influence AHR activity, which may have various downstream effects for both the skin and the immune system.

3056 Time-Dependent Skin Reactions against Urban Pollution: Role of AHR Signaling Pathway in Detoxification Process
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Environmental pollution is an increasing concern for toxicologists as the exposed urban population grows throughout the world. This study focuses on AHR (Aryl hydrocarbon receptor) signaling pathway in human keratinocytes in order to understand the reaction of skin exposed to pollution stress by urban dust. In this complex mixture 3-Methylcholanthrene (3-MC) is one pollutant component under investigation. In parallel, β-Naphthoflavone (β-NF) serves as a well-known and potent activator of AHR and has been used by us in previous work. Keratinocytes cultivated in 2D are exposed to 3-MC and to β-NF for 6; 24; 48 hours in order to visualize early and late time gene expression. To investigate how IL-6Rα function in epidermal keratinocytes in the context of ICD is unknown. This laboratory has previously reported that IL-6 deficiency exacerbates skin inflammation in a murine model of ICD, yet the role of the IL-6Rα in epidermal keratinocytes in the context of ICD is unknown. To investigate how IL-6Rα function in epidermal keratinocytes influences the inflammatory response during ICD, a chemical model of ICD was employed. In this model, keratinocyte-specific IL-6 receptor alpha knock-out (I6RA−/−) and littermate control mice were exposed to two well-known occupational irritants JP-8 jet fuel, Benzalkonium chloride (BKC) as well as acetone (control) for periods of one (1) or three (3) days.

Irritant Contact Dermatitis (ICD) is an inflammatory response of the skin to harmful stimuli. ICD is the most common occupational skin disorder with significant impact on the quality of life of affected individuals. Following exposure to irritants, keratinocytes, the predominant cells in the epidermis of the skin, initiate the inflammatory cascade by producing a myriad of cytokines that includes IL-6. This laboratory has previously reported that IL-6 deficiency exacerbates skin inflammation in a murine model of ICD, yet the role of the IL-6Rα in epidermal keratinocytes in the context of ICD is unknown. To investigate how IL-6Rα function in epidermal keratinocytes influences the inflammatory response during ICD, a chemical model of ICD was employed. In this model, keratinocyte-specific IL-6 receptor alpha knock-out (I6RA−/−) and littermate control mice were exposed to two well-known occupational irritants JP-8 jet fuel, Benzalkonium chloride (BKC) as well as acetone (control) for periods of one (1) or three (3) days. Dermatitis lesions were collected from each mouse genotype and inflammatory cytokine protein expression profile was examined by multiplex assays (Affymetrix). In addition, immune cell infiltration into lesional skin was characterized by immunohistochemistry (IHC) and flow cytometry. Histopathology revealed that I6RA−/− mice show an exaggerated response to Benzalkonium chloride (BKC) and JP-8 fuel relative to control. This response was characterized by increased immune cell infiltration into the dermis. Lesional skin from I6RA−/− mice treated with more CD11b and F4/80 expressing cells relative to control. Protein expression data showed a significantly increased expression of pro-inflammatory cytokines such as IL-1α, IL-1β, TNF-α, IL-22, and IFN-γ in
lesional skin from IL6Ra−/− mice relative to control. The expression of anti-inflammatory cytokines including IL-10 and IL-4 were, however, significantly reduced in lesional skin from IL6Ra−/− mice relative to control. These results indicate that IL-6Ra function in epidermal keratinocytes confers a protective effect during ICD and suggests that the previously reported protective effect of IL-6 during ICD might be mediated by the IL-6 receptor alpha. Overall, the IL-6/IL-6R system plays a role in modulating inflammation during ICD and could be explored in the therapy of ICD.

3059 Aberrant Lipid Metabolism Underlies Pathogenesis of Arsenicals-Induced Cutaneous Blistering and Inflammation
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Arsenical vesicants are highly reactive and extremely toxic warfare agents developed in World War I. Despite efforts to destroy these chemicals, their large stockpiles still exist in several countries. Arsenicals manifest debilitating cutaneous and systemic injury. The molecular pathogenesis of these lesions is not clear, and therefore, no effective mechanism-based antidote has been described. This study explores the metabolic landscape following topical treatment of mouse skin with four warfare-related arsenicals lesiwite, diphenylchloroarsine, diphenylcyanoarsine, and diethylchloroarsine. Global metabolite profiling of skin tissue excised from these animals revealed profound effects on multiple metabolic components that showed significant similarity among all of these chemicals. The inhibition of succinate dehydrogenase and α-ketoglutarate dehydrogenase in the TCA cycle by lesiwite was highly significant. Arsenicals induced significant reductions in reduced glutathione resulted in the augmented oxidative stress and a decline in the antioxidant capacity of skin tissue. Similarly, lipid metabolism was affected by all four arsenicals. Increases in PUFAs coupled with increases in cyclooxygenase & lipoxygenase activities were associated with arsenicals-mediated inflammatory responses. In this regard, we observed a significantly high accumulation of lipoxygenase metabolites 6-hydroxy hexanoic acid and LTB4 (21 and 16 folds respectively). Using phenylsine oxide (PAO) as a surrogate arsenical, we demonstrated the role of lipoxygenase in the pathogenesis of these chemicals. Lipoxygenase inhibitor nordihydroguaiaretic acid (NDGA) blocked arsenical-mediated cutaneous injury. Our data showed that NDGA when applied 5 min after cutaneous PAO exposure reduced significantly inflammatory & tissue disrupting responses in the skin. NDGA reduced mRNA levels of proinflammatory Il1b, Il6, Ccxl1, and Ccxl5. Reduction in Il1b associated inflammasome formation was also observed. Mechanistically, we found a novel link between lipoxygenase metabolism, unfolded protein response signaling and inflammatory & tissue disrupting responses. Taken together, we have identified potential novel mechanism by which arsenicals induced painful cutaneous inflammation and blistering and NDGA may serve as a potent antidote against arsenicals-induced tissue injury.

3060 Nrf2 Dependent Skin Photoprotection Using the Achiote-Derived Apocarotenoid Bixin

Unprotected exposure to solar ultraviolet (UV) radiation causes acute photodamage (sunburn), premature aging, and skin cancer. Molecularily, UV causes direct DNA damage and generation of reactive oxygen species (ROS). While physical and chemical UV shields greatly reduce skin damage, interventions activating endogenous stress responses are emerging as promising skin protective strategies. The transcription factor Nrf2 is the master regulator of the antioxidant cell response and has been shown to protect the skin against various environmental stressors, including UV. Nrf2 can be activated using natural chemopreventive compounds, such as the apocarotenoid bixin, an FDA-approved natural food colorant from the seeds of the achiote tree (Bixa orellana). We tested the feasibility of using a topical bixin formulation to activate cutaneous Nrf2 and protect the skin from acute UV exposure. SKH-I WT and Nrf2−/− hairless mice were pretreated with bixin and then exposed to UV; skin tissue was collected 24 h later. Bixin was able to activate Nrf2 and protect the skin from acute photodamage (reduced epidermal hyperplasia/prevent inflammatory infiltration and apoptosis) in WT but not Nrf2−/− SKH-I mice. Furthermore, we tested the feasibility of using bixin to activate Nrf2 and prevent UV-induced hair graying. C57BL/6 WT mice were depleted, treated with topical bixin, and then exposed to UVA in the presence of a photosensitizer (psoralene). Bixin was able to activate NRF2 in the skin of C57BL/6 WT mice and prevent hair graying after psoralene+UVA (PUVA) treatment. Collectively, these results indicate that bixin is a safe and potent NRF2 activator that protects various cell types of the skin to prevent UV-induced damage.

3061 An Assessment of the Intra-Lab Variability of the In Vitro Percutaneous Absorption of Testosterone through Human Skin
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OECD Guidance Document No. 28 requires laboratories performing in vitro skin absorption studies, according to OECD Test Guideline 428, to demonstrate proficiency using Testosterone. This laboratory performed 2 studies with the same study design on different days, but with minor methodological changes, to assess if they resulted in changes to the outcome of the results. Study 1 examined two decontamination scenarios (Groups 1 and 2) and Study 2 repeated Group 1 with different donors but from the same source (Group 3) and different donors from a new source (Group 4). [14C]-Testosterone (1 mg/mL) in ethanol: water (40%, v/v) was applied at 10 μL/cm² to human dermatomed skin (n=8 or 10) in flow through diffusion cells in vitro with a receptor fluid flow rate and skin surface temperature of 1.5 mL/h and 32°C. Absorption was assessed hourly from 0-8 h and 2-hourly from 8-24 h. Exposure was terminated by washing at 8 h. Groups 1, 3, and 4 were washed with soapy water using natural sponge swabs. Group 2 was washed with soap concentrate and tissue paper swabs. All samples were analysed by LSC. T-Tests were performed comparing all 4 test groups with each other for differences in affinity and skin permeability (equiv./cm²) which confirmed the expected donor differences (i.e. inter-donor variability). The main differences observed were in comparing the different donors from the different suppliers. Group 3 dislodgeable dose at 8 h was significantly higher than Group 4 (9.37 and 8.85 equiv./cm²) with a correspondingly significantly lower absorbed dose (0.29 and 0.49 μg equiv./cm²). This could be important for transdermal drug delivery assessments. However, since there were no significant differences in dermal delivery for any comparison; this is most frequently used for risk assessments, the consistency of the test was confirmed. The Testosterone mass balance was complete (within 100±10%) for all test groups. In conclusion, this study confirmed that small changes in conditions did not affect the absorption and distribution of Testosterone and demonstrated that the study test is consistent when run on different occasions with only small differences attributable to the inter-donor variability.

3062 Dermal Uptake of Three Brominated Phenois: Tetrabromobisphenol A (TBBPA), Tetrabromobisphenol A bis (2,3-dibromopropyl ether) (TBBPA-BDBPE), and 2,4,6-Tribromophenol (TBP)
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Three brominated phenois, TBBPA, TBBPA-BDBPE, and TBP, were assayed to determine dermal absorption and penetration. All these chemicals are high production volume brominated flame retardants (BFR) used in consumer products, resulting in ubiquitous human exposure. TBBPA, a reactive flame retardant commonly used in printed circuit boards and other polycarbonate resins, was shown to cause uterine epithelial tumors in a NTP 2-year cancer bioassay. Little (μg-equiv/cm²) in vivo data are available for TBBPA’s replacement alternative TBBPA-BDBPE, but its additive usage poses a higher risk of migration into environmental matrices. TBB is used as a wood fungicide and reactive flame retardant and has been shown to disrupt thyroid signaling. Although the major route of exposure to these chemicals is oral uptake, dermal contact is likely via contaminated dust. In the studies presented here, independent trials of a single dose of 100 nmol/cm² skin (~1 μCi [14C]/cm²) of each BFR were applied to whole rat skin (in vivo) or split-thickness human and rat skin (in vitro) to calculate in vivo human percutaneous uptake. Following application, absorption (detected in dosed skin) and penetration (recovered in receptor fluid (in vitro) or tissues/excreta (in vivo)) were assessed over 24h. In vivo penetration and absorption in rat skin was 67% and 5% for TBB, 8% and 15% for TBBPA, 1% and 26% for TBBPA-BDBPE. In human skin, in vitro penetration and absorp-
Partition and Diffusion Coefficient Determinations in Isolated Human Skin Layers for In Silico Skin Penetration Modeling

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The development of a predictive in silico skin penetration model is needed to enable moving away from a 100% systemic bioavailability assumption to a more realistic one assessing the actual fraction of systemically absorbed compound. As part of a Cosmetics Europe initiative, we are replacing empirical relationships (with limited applicability domains) by mechanistically based simulations to characterize trans-dermal transport. Partition (K) and diffusion (D) coefficient determinations on isolated human skin layers are being conducted for 50 compounds relevant to cosmetics ingredients. K values were measured in isolated dermis, whole epidermis, intact SC, delipidized SC and SC lipids by direct measurements of the radioactivity in the two layers/lipid component vs. buffer samples. D determinations were made in dermis, whole epidermis and intact SC using a non-linear regression of the cumulative receptor fluid content of radiolabelled compound, fit to the solution of Fick’s 2nd Law. More than 35 compounds have been tested, with molecular weights ranging from ~100 - 400 and log P values ranging from ~0 to 5. For a given chemical, K was generally similar for the dermis, whole epidermis and fully hydrated (FH) SC and FH delipidized SC. The partially hydrated (PH) SC and PH delipidized SC were approximately 3-fold greater. The lipid values were similar to the partially hydrated values, exceptions for compounds with a log P greater than 2. K values of the chemicals tested ranged approximately 3 orders of magnitude for a given tissue layer and approximately 4 orders of magnitude for the SC lipids. For a given chemical, D was consistently similar for SC and whole epidermis, and these layers were always lower than that of the SC lipids tested ranged approximated by the 3 orders of magnitude for a given layer. Correlations between K and D and several physiochemical properties indicate a simple correlation is not sufficient to describe the relationship. The study findings have been applied to the development and validation of an in silico model.

3066 A Highly Sensitive and Selective Liquid Chromatography with Tandem Mass Spectrometry (LC/MS-MS) Method for the Direct Peptide Reactivity Assay (DPRa) 2018 SOT Annual Meeting

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The DPRa addresses the molecular initiating event of skin sensitization by quantifying the reactivity of potential sensitizers with synthetic peptides containing lysine or cysteine (Ac-RFAAAACOOH and Ac-RFAAKAA-COOH). The percent peptide depletion, as quantified using liquid chromatography ultra-violet spectrometry (LC/UV), is used to discriminate between dermal sensitizers and non-sensitizers. This method has been validated internationally and a test guideline (OECD TG 442c) has been recently published. While this method provides promising predictions to identify dermal sensitizers, the low sensitivity and selectivity of the LC/UV method poses challenges to accurately identify the sensitization potential of some chemicals. For example, chemicals with the same retention time may be cysteine- or lysine-containing peptides and can be misclassified in this assay. Similarly, test materials that non-covalently bind with either peptide result in a shift of the retention time and false positive results. To overcome these shortcomings, we developed a LC/MS-MS-based DPRa. After optimization of the multiple reaction monitoring ion pairs (precursor and product ion pairs) of both peptides in the AB Sciex 5000 Qtrap MS-MS system connected with Agilent 1290 Infinity II LC system, a set of known dermal sensitizers and non-sensitizers were evaluated in the LC/UV and LC/MS-MS-based methods following OECD TG 442c. The results showed similar linear calibration standard curves with R² > 0.99 and similar peptide depletion for both peptides with the selected test proficiency chemicals. After this initial validation, both LC/MS-MS and LC/UV methods were implemented for screening non-reactive chemicals which may form non-covalent interactions with peptides (such as surfactants). This method can be used to test chemicals as non-sensitizers, however, the LC/UV-based DPRa showed higher depletion of both peptides, resulting in false-positive results. These findings suggest broader applicability and better specificity of the LC/MS-MS-based DPRa method.
Skin sensitization can result from exposure to natural and synthetic chemicals. Once sensitized to a chemical, chronic dermal reactions may be elicited by very small exposures. Chemicals in apparel may leach and cause dermal reactions in sensitive individuals. Here, we summarize our assessment of skin sensitization risk for 1,796 chemicals leached from apparel products into simulated sweat. Chemicals were classified as skin sensitizers or non-sensitizers by a weight-of-evidence approach using experimental data, case reports, and in silico predictions of sensitization hazard. For each chemical, published in vivo and in vitro assessments were weighed for experimental data. In the absence of experimental data for the chemical of concern (CoC), surrogate chemicals were identified and reviewed for experimental data. In the absence of experimental data for either the CoC or surrogates, classification was based on in silico analysis using Toxtree and/or Derek Nexus. A threshold for sensitization (or point-of-departure [POD]) was calculated for sensitizers and categorized as strong (POD ≤ 2.5 µg/cm²), moderate (POD 2.5-250 µg/cm²), or weak (POD > 250 µg/cm²). All assessments were assigned a confidence rating based on data availability and quality. A minority (28.5%) of chemicals evaluated were classified as skin sensitizers while 71.5% were classified as non-sensitizers. Of skin sensitizers, the majority (58.2%) were classified as ‘weak’ sensitizers, 35.9% as ‘moderate’ sensitizers, and 5.9% as ‘strong’ sensitizers. Good quality experimental data were identified for 27.8% of chemicals, resulting in a confidence rating of ‘good.’ Nearly half (48.7%) of assessments were either based on surrogate data or poor-quality data for the CoC, resulting in a confidence rating of ‘limited.’ Assessments based on in silico analysis alone accounted for 23.5% of chemicals and a confidence rating of ‘weak;’ for these, when in silico assessments were positive, a maximum default POD of 250 µg/cm² was assigned. The mean POD for good confidence sensitizers was 1,972 µg/cm² (median 750 µg/cm², range 0.4-20,375 µg/cm²). Overall, the identifying of skin sensitizers or non-sensitizers by a weight-of-evidence approach using experimental data, case reports, and in silico predictions of sensitization hazard was confirmed by testing 28 blinded substances in all three test laboratories. This manifests GARDskin as a valid test for assessment of skin sensitizers.
The first two authors contributed equally to this research.

Activation of THP-1 cells. These data demonstrate that the environment keratinocytes impacted on the threshold concentration needed for the donor. This was especially evident for CD54. Specifically, proliferating THP-1 responses to the sensitizers were enhanced in coculture with pro-inflammatory cytokines, chemokines, and other mediators. Much is now evident that keratinocytes play an active and dynamic role in initiation and balancing of innate and adaptive immune responses. Specifically, they may promote conditions resulting in tolerogenic dendritic cells (DCs) via their constitutive production of IL-10 and TGF-β or facilitate activation of DCs through their production and secretion of pro-inflammatory cytokines, chemokines, and other mediators. Much less is known about how the status of keratinocytes impact on strength and direction of ensuing DC activation. Here we studied their properties to activate DCs by evaluating proliferating and differentiated keratinocytes on dendritic cell activation by contact allergens.

Epithelial cells such as keratinocytes in the skin are known to essentially contribute to maintain the balance between tolerance and adaptive immune responses due to external exposures including sensitizing chemicals. While once believed to function primarily as a passive barrier, it is now evident that keratinocytes play an active and dynamic role in in vitro analysis of tap water containing NaCl. In cytotoxicity assay, plated cells (10^5 cells/ mL) were treated with different concentrations of EAWs (1.25, 2.5, 5, 10 % EAW) for 24 hours. The viability was measured with MTT reagent (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium). For the in vitro assessment of wound healing, the cells (5.5x10^5 cells/well) were seeded in 24 well plates and after 24 hr, a scar was formed by 0.5 mm tipped scratcher and EAWs (1.25, 2.5, 5 and 10%) were added and incubated at 37°C in 5% CO2. The time 0, 18 and 24 hr were photographed and the wound healing activity is determined by ImageJ software. According to the results, the maximum experimental dose of A-EAW, N-EAW and M-EAW showed no cytopathic effect on cell viability. Therefore, the differences that were not cytopathic to the cell for 24 hours exposure were used for the scratch assay. In vitro wound healing activity by scratch assay showed that A-EAW at 10 times dilution significantly accelerated fibroblast migration and cell proliferation compared to medium control, while N-EAW and M-EAW did not show any significant activity. As a conclusion, we suggest that A-EAW can be used a potential wound healing agent possible because of the moderate generation of reactive oxygen species in anode chamber of acidic is shown to be beneficial in wound healing especially in the inflammatory phase. Since wound healing is a complex process, further studies are needed to elucidate the mechanisms responsible for its healing activity.

Electroactivated water (EAW) is a novel technology based on electrolysis of water containing NaCl or KCl in a divided electrolysis module with a diaphragm between anode and cathode electrodes. EAW has gathered greater interest as a safe disinfectant in several areas like food, medicine and dentistry. Recently, the possible wound healing activities of EAW are also highlighted. In the present study, we generated three different EAWs, acidic (A-EAW), neutral (N-EAW) and mixed (M-EAW) and evaluated the cytokotoxic activity on L929 cell line as well as the wound healing activity by scratch assay in vitro. A-EAW (pH 2.45±0.48; ORP 1126.27±24.66 mV), N-EAW (pH 5.55±0.13; ORP 806.67±11.55 mV) and M-EAW (pH 5.54±0.03; ORP 887.67±15.95) was produced by the electrolysis of tap water containing NaCl. In cytotoxicity assay, plated cells (10^5 cells/ mL) were treated with different concentrations of EAWs (1.25, 2.5, 5, 10 % EAW) for 24 hours. The viability was measured with MTT reagent (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium). For the in vitro assessment of wound healing, the cells (5.5x10^5 cells/well) were seeded in 24 well plates and after 24 hr, a scar was formed by 0.5 mm tipped scratcher and EAWs (1.25, 2.5, 5 and 10%) were added and incubated at 37°C in 5% CO2. The time 0, 18 and 24 hr were photographed and the wound healing activity is determined by ImageJ software. According to the results, the maximum experimental dose of A-EAW, N-EAW and M-EAW showed no cytopathic effect on cell viability. Therefore, the differences that were not cytopathic to the cell for 24 hours exposure were used for the scratch assay. In vitro wound healing activity by scratch assay showed that A-EAW at 10 times dilution significantly accelerated fibroblast migration and cell proliferation compared to medium control, while N-EAW and M-EAW did not show any significant activity. As a conclusion, we suggest that A-EAW can be used a potential wound healing agent possible because of the moderate generation of reactive oxygen species in anode chamber of acidic is shown to be beneficial in wound healing especially in the inflammatory phase. Since wound healing is a complex process, further studies are needed to elucidate the mechanisms responsible for its healing activity.

Electrochemical processes responsible for its healing activity.

Cytotoxicity assay, plated cells (10^5 cells/ mL) were treated with different concentrations of EAWs (1.25, 2.5, 5, 10 % EAW) for 24 hours. The viability was measured with MTT reagent (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium). For the in vitro assessment of wound healing, the cells (5.5x10^5 cells/well) were seeded in 24 well plates and after 24 hr, a scar was formed by 0.5 mm tipped scratcher and EAWs (1.25, 2.5, 5 and 10%) were added and incubated at 37°C in 5% CO2. The time 0, 18 and 24 hr were photographed and the wound healing activity is determined by ImageJ software. According to the results, the maximum experimental dose of A-EAW, N-EAW and M-EAW showed no cytopathic effect on cell viability. Therefore, the differences that were not cytopathic to the cell for 24 hours exposure were used for the scratch assay. In vitro wound healing activity by scratch assay showed that A-EAW at 10 times dilution significantly accelerated fibroblast migration and cell proliferation compared to medium control, while N-EAW and M-EAW did not show any significant activity. As a conclusion, we suggest that A-EAW can be used a potential wound healing agent possible because of the moderate generation of reactive oxygen species in anode chamber of acidic is shown to be beneficial in wound healing especially in the inflammatory phase. Since wound healing is a complex process, further studies are needed to elucidate the mechanisms responsible for its healing activity.

Comparison of prestudy parameters in alternatively sourced cynomolgus non-human primates.

Cynomolgus macaques are utilized in a wide variety of pre-clinical research models, and particularly in contemporary drug safety evaluations. These animals may be derived from stock originating in various regions of the world and are often identified according to the country of export. The country of export identification, however, may not meaningfully differentiate these animals in terms of background genetic and/or biological variability. The present study sought to compare historical control data between Cambodian and Chinese sourced Cynomolgus non-human primates (NHPs) in order to appreciate the nature of any observed variations in age and body weight, basic detailed clinical observational measures, ophthalmology, and a variety of clinical pathology biomarkers, as these may impact drug safety study conduct and/or data interpretation. Among target samples of 100/sex Cambodian sourced NHPs and age-matched samples of Chinese sourced NHPs, there was overall essential similarity across recorded parameters, with occasional statistically significant divergence on individual measures. The data indicate that, while occasional statistically significant differences on individual parameters may be discriminated, the variation described for regionally (Asian mainland, Indonesian, Mauritius) diverse Cynomolgus NHPs in such pre-study variables is much more pronounced.
The response of hematology, spermogram, testicular and epididymal histology of forty-eight (48) male albino rats (Wistar strain) to crude powder and ethanol extract of *Citrullus lanatus* seeds was investigated on the. The rats were randomly divided into three groups (A, B, and C) with sixteen rats per group (N=16). Rats in Group A were fasted over a prolonged time to induce fasting glucose, while rats in Groups B and C were fasted over a prolonged period. The rats were then dosed with distilled water while Groups B and C served as the control group and were administered distilled water while Groups A and B were given ground powder and ethanol extract of *Citrullus lanatus* seed respectively at 200 mg/kg for 28 days. Blood samples were collected a day after the commencement of treatment to determine the haemogram. Subsequently, four rats from each group were sacrificed weekly after fasting administration for a period of four weeks and samples were collected for sperm motility, sperm livability, sperm morphology and histology. There was a decrease in sperm motility of treatment groups compared to the control group for the four weeks post-treatment. It was observed that mean sperm motility subsequently increased towards the third and fourth week post-treatment in the treatment groups. There was also a decrease in the mean sperm morphological abnormalities of treatment groups B and C compared to the control group post-treatment. Histology of the testes of the treatment groups for the four weeks post-treatment showed the presence of spermatogenic seminiferous tubules with normal architecture with spermatogenic cells. The findings from this study revealed that *Citrullus lanatus* seeds administered at 200 mg/kg have no deleterious effect on hematology, the spermogram and sperm morphological abnormalities were significantly reduced. Hence, it may be considered safe for use in male animals meant for breeding.

Investigating the absorption, distribution, metabolism and excretion of NCES. In the absence of a gall bladder, they are highly suitable for researching the excretion and metabolism of novel xenobiotics in bile. Therefore, catheters are placed in the bile duct in order to collect fluid, and the duodenum is loosely packed with saline to prevent the catheters from extruding via a tail cuff and require the animal to be tethered and singly housed for the duration of both the recovery and experimental period, which is typically up to ten days in total. A modified tail cuff has been developed using a pin-port adapter that permits the animal’s own body weight to displace the catheter hub into the duodenum during the recovery period. This modification means that animals do not need to be tethered or singly housed during this time (4-5 days). The experimental phase of the study remained unchanged (i.e. animals are tethered and singly housed in glass metabolism cages in order to separately measure and record body weight, excreta output, and functional and morphological analysis, and to investigate the involvement of glucotoxicity on nerve dysfunction, blood glucose levels were strictly controlled by treatment of phlorizin (100 to 150 mg/kg/day) from 5 to 16 weeks (n=6). Sprague-Dawley (SD) rats were used as an age-matched control (n=6). Body weights and biochemical parameters were periodically measured. The sensory and motor nerve conduction velocity (SNCV and MNCV) of the sciatic nerve, blood pressure, pupil size, and electrocardiogram were measured. At 16 weeks, rats were sacrificed and small myelinated fibers, and vasculation and mitochondrial swelling in unmyelinated fiber were sampled for the histological studies, transmission electron microscopic (TEM) analysis and determination of nerve fiber density. SNCV and MNCV were delayed and intraepidermal nerve fiber density slightly decreased in the SDT fatty rats compared with the control SD rats. In the TEM evaluation, the mitochondrial abnormalities and thinning of myelin sheath in small myelinated fibers, and vasculation and mitochondrial swelling in unmyelinated fiber were found in the SDT fatty rats. Blood pressure increased and the coefficient of variance of R-R intervals tended to decrease in SDT fatty rats. The maximum pupil diameter responded to the mydriatic drugs in SDT fatty rats was smaller than that of SD rats. Aside from the thinning of myelin sheath, these functional and morphological changes in peripheral nerves were prevented by controlling blood glucose level with phlorizin treatment. There result indicate that glucotoxicity was involved in those nerve dysfunctions.
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This study was conducted to see if there were any differences in organ weights among the Sprague Dawley rat sourced from Taiwan and Beijing. Control group data from ongoing toxicology studies (N=140/sex/age) were used to see if there were any differences between those two sources of animals. The mean terminal body weight and organ weights were calculated. Analysis of the data showed statistical significant difference in organ weights included lower terminal body weight, heart weight (male only), liver weight (male only), kidney weight, spleen weight, thymus weight, thyroid glands with parathyroid gland(s) weight, pituitary gland weight, prostate gland weight, epididymides weight, and ovaries weight were noted in Taiwan rats; higher brain weight and uterus (including cervix) weight were noted in Taiwan female rats; lower organ weight/body weight ratio was noted in kidney (female only), thymus, thyroid glands with parathyroid gland(s), pituitary gland, prostate, and ovaries in Taiwan rats; higher organ weight/ body weight ratio was noted in brain, liver (female only), testes, and uterus (including cervix) in Taiwan rats, when compared with Beijing rats. In addition, the organ weight/body weight ratio was analyzed. The differences of organ weight/body weight ratio reflected the changes seen in the absolute organ weight, with the exception that statistical lower heart/brain weight was noted in Taiwan female rats due to their higher brain weight, when compared to Beijing rats. These 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.

3080 Platycodon Grandiflorum Derived Saponin Protect Agonist Eccentric Exercise-Induced Muscle Damage
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Platycodon grandiflorum contain a triterpenoid saponin as platycodin D and platycodon acid A with a multifold bioactive compound. Our previous research demonstrated that the platycodon grandiflorum derived saponin (PS) ameliorate against high fat diet-induced non-alcoholic steatohepatitis and inhibition of osteoclast differentiation. The pivotal effects of PS on inflammatory mechanisms were suppressed in NF-κB and C-related protein. Taken together, our findings identify the PS extract protect from muscle damage. A significant reduction in eccentric exercise-induced muscle damage area and muscle damage related to the level of NF-κB p65 was suppressed via a p38, ERK and SMAD pathway, which provides a novel perspective on the biological function of PS against muscle damage.

3081 AChE-Independent Phosphoprotein Signaling in an Acute Mouse Model of Gulf War Illness
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Roughly 25-30% of veterans from the 1991 Persian Gulf War suffer from a persistent and amplified form of sickness behavior, classified as Gulf War Illness (GWI). Previous studies investigating GWI have suggested that exposure to organophosphates (OP) in theater, as well as the irreversible acetylcholinesterase (AChE) inhibitor and chemical warfare agent, sarin, as well as other pesticides, may have contributed to GWI symptomatology. Additionally, concomitant exposure to high physiological stress in theater has been implicated in the initiation of the GWI phenotype. Traditionally, inhibition of AChE and the subsequent accumulation of acetylcholine (ACh) result in the activation of the cholinergic anti-inflammatory pathway. However, we have shown that the link between GWI and neuroinflammation appears to contradict this effect of AChE inhibitors. Therefore, it is plausible that exposure to OPs both alone and in combination with corticosterone (CORT; used as a physiological stress mimic) may target biomolecules other than AChE to induce the neuroinflammatory effects observed in our validated mouse model of GWI. To further investigate this phenotype, adult male C57BL/6J mice were exposed to CORT in the drinking water for 4 or 7 days. On the 5th or 8th day, mice were exposed to a single dose of a sarin surrogate, disopropyl fluorophosphate (DFP; 4.0mg/kg, i.p.). To fully evaluate the brain-region specific effects of DFP and CORT+DFP on AChE, ACh concentrations were measured in cortex (CTX), hippocampus (HIP) and striatum (STR) using HLIL UPLC-MS/MS. Mice were euthanized using focused micro-wave irradiation to ensure rapid inactivation of AChE, as well as endog-

3082 Geriatric Toxicology: Understanding Human Relevance of Toxicities Observed at Late-Stage of Long-Term Studies
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Toxicity testing in animals is traditionally conducted at fixed doses (i.e., mg/kg/day) without adjustments for the occurrence of dose- and/ or age-dependent non-proportionality. The time-dependent dose non-proportionality is associated with age-dependent changes in physical, physiological and biochemical parameters possibly requiring a change in dosing for geriatric patients. Similar changes occur in animals, leading to higher than anticipated systemic dose resulting in "late occurring" toxicities in chronic studies. Determining the time of departure from dose proportionality is important to either adjust the external dose to maintain the anticipated systemic dose or interpret such toxicities in the context of systemic dose non-proportionality for better human health risk assessment. Pethoxamid, an herbicide, is >90% absorbed from the GI tract of rats and predominantly (~75%) eliminated through bile. In the rat carcinogenicity study, pethoxamid increased thyroid follicular cell hyperplasia (TFCH) and adenoma in male rats at the top dose (1600 ppm), only at termination (105 weeks) without such induction at interim sacrifice (after 27, 53, or 79 weeks of exposure). An increase in TFCH was observed in 13-week study at ~1.6- and ~3-fold higher doses [Pethoxamid: Draft Assessment Report, 16 August 2002]. The effects observed at the end of the carcinogenicity study were likely from geriatric changes (e.g., metabolism, clearance) increasing the sys-

3083 Mouse Model of Intestinal Mucositis Induced by 5-Fluorouracil and Irinotecan
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Chemotherapy-induced mucositis is a common severe side effect experienced by colorectal cancer patients during treatment. We aimed to investigate the gastrointestinal toxicity of 5-fluorouracil and irinotecan in Balb/C mice (n=8/group) were injected with saline, 5-fluorouracil (30 mg/kg, i.p.) for 5 consecutive days (males and females) or irinotecan (75 mg/kg, i.p.) for 4 consecutive days (males only). Body weight and diarrhea were assessed over the test period and inflammatory and histopathological responses were investigated 7 days after the final dose. 5-fluorouracil-induced mucositis was more severe compared to controls (5-fluorouracil: +2.3 and +2.4, p<0.001 for males and females respectively, and irinotecan: +0.9, p<0.001). 5-fluorouracil,
but not irinotecan, tended to increase the myeloperoxidase activity in both males and females. 5-fluorouracil and irinotecan increased ileum IL-1β levels (5-fluorouracil: +36%; 5-fluorouracil: +700% and +800%; p<0.001 for males and females respectively, and irinotecan: +93%; p<0.05) and plasma TNF-α levels (5-fluorouracil: +700% and +800%; p<0.001 for males and females respectively, and irinotecan: +825%; p<0.05) without modifying ileum TNF-α levels. Plasma IL-15 levels were below the limit of quantification in all groups. Both treatments affected the intestinal architecture and decreased the villus/crypt ratio (5-fluorouracil: -45% and -33%; p<0.001 for males and females respectively, and irinotecan: -23%; p<0.01). These findings suggest that 5-fluorouracil- and irinotecan-induced gastrointestinal toxicity in mice shows similarities with the clinical animals mucositis manifestations. Therefore, these mouse experimental mucositis models offer a promising tool for evaluating the side effects of novel chemotherapeutic agents or the efficacy of potential treatments against chemotherapy-induced mucositis.

**3084 Evaluation of Serial Sampling Procedures to Enable Clinical Pathology Evaluations in Mice**


Toxicology studies conducted in CD-1 mice that include clinical pathology assessments are often designed with separate cohorts for hematology and clinical chemistry evaluations in order to yield adequate blood volumes suitable to generating a complete panel of endpoints. Consistent with 3R's and evolving institutional best practices, the objective of this study was to determine the feasibility of utilizing the same subjects, rather than different cohorts, to establish a comprehensive panel of endpoints in two phases. Animals were selected from an in-house naive mouse colony (Phase I) or ordered from a vendor (Phase II) so as to have body weights generally representative of mice which might be assigned to either a 4-week or 13-week toxicology study design. Blood samples for hematology (0.25 mL) and clinical chemistry (1 mL) were collected from all animals via the maxillary vein on Day 1 and a standard hematology panel was evaluated. On Day 2, the animals were submitted to necropsy for terminal blood collections, drawn from the vena cava after CO2 inhalation (maximum obtainable volume), for clinical chemistry testing. A standard clinical chemistry panel was evaluated, except for electrolytes. Samples were provided for individual analyses and stored at -80°C until analysis was completed in two phases. Hematology data were obtained from 42/44 animals, and clinical chemistry data were obtained from 41/44 animals. Data generated were comparable to compiled historical control values; there were no significant or biologically meaningful differences as a function of the modified, single cohort procedure. Therefore, it is feasible to collect both hematology and clinical chemistry samples from the same mouse without using cohorts when serial sampling is used, for CD-1 mice that are 10 weeks or older, provided that the extended range of sampling employed to capture hematology and clinical chemistry data is able to be accommodated from a scientific/experimental design perspective.

**3085 Nitrogen Retention and Protein Quality in Dogs and Cats Fed Commercial Pet Food**

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Protein, as a nitrogen-containing compound, is essential for growth and metabolism. The crude protein content listed on pet food is often misleading when referring to usable protein. A portion of the listed protein may actually be from non-digestible organic nitrogen or potentially toxic inorganic non-protein nitrogen sources, neither of which are retained or used by the animal. To analyze nitrogen retention and screen for non-protein nitrogen, four commercial pet foods for each species were provided to dogs and cats and one lab-made diet for both species were evaluated and coated with a non-digestible marker, chromium oxide. Seven dogs and eight cats were randomly assigned each diet (n=4 for each diet). The dogs were switched to the diets for two days and retention while using was collected over a 48 hour period of feeding chromium coated diet, followed by total marked fecal collection on the subsequent days. Total nitrogen intake did not differ significantly (p>0.05; one-way ANOVA) among diets in both cats and dogs fed the diets, nor did fecal or urinary output. Nitrogen retention was calculated based on nitrogen (%) consumed in feed versus nitrogen lost in feces and urine. The amount of nitrogen retained ranged from 93-96% in dogs and 91-95% in cats, but did not statistically differ among diets in both species. While price did not correlate well with nitrogen retention in dogs, there was an inverse relationship in cats. One reason for the lowest price diet, the lowest protein diet, and vice versa, suggesting that low-priced cat foods had better protein quality. No significant differences in plasma or urine nitrite or nitrate were noted among diets in dogs or cats. Further studies will examine amino acid content in these same diets and percent retention of the amino acids as a more accurate indicator of protein quality and retention. Furthermore, the diets will also be examined for additional toxic nitrogen metabolites. Ultimately, the results of this study show that despite large differences in price, all pet food diets lacked excess levels of toxic nitrogenous compounds and protein quality had no correlation to price.

**3086 Evaluation of Immune-Mediated Idiosyncratic Drug Toxicity Using Chimeric HLA Transgenic Mice**


Immune-mediated idiosyncratic drug toxicity (IDT) is a rare adverse drug reaction, potentially resulting in death. Although genome-wide association studies suggest that the occurrence of immune-mediated IDT is strongly associated with specific human leukocyte antigen (HLA) allo-types, these associations have not yet been prospectively demonstrated. In this study, we focused on HLA-B*57:01 and abacavir (ABC)-induced immune-mediated IDT, and constructed transgenic mice carrying chimeric HLA-B*57:01 (B*57:01-Tg) to determine if this in vivo model may be useful for evaluating immune-mediated IDT. The local lymph node assay (LLNA) using B*57:01-Tg, B*57:03-Tg (negative control), and their littermates (LMs) was conducted by applying 25 μL (50 mg/kg/day) of ABC or vehicle to the dorsum of the ear for 3 consecutive days. After 24h, lymph nodes were drained and weighed, and H&E staining on the ear section was performed. The percentages of bromodeoxyuridine (Brdu)+, IL-2+, and IFN-γ+ in CD8+ T cells were assessed using FACS. ABC-activated CD44+CD26lowCD8+ memory T cells were measured after mice were fed for a week with either a normal diet or rodent chow containing 1% (v/v) ABC. LLNA results demonstrated that percentages of Brdu+, IL-2+, and IFN-γ+ in CD8+ T cells of ABC - applied B*57:01-Tg mice were significantly higher than those in LMs, resulting in the infiltration of inflammatory cells into the ear. These immune responses were not observed in B*57:03-Tg mice. Furthermore, oral administration of 1% (v/v) ABC significantly increased the percentage of CD44+CD26lowCD8+ memory T cells in lymph nodes and spleen derived from B*57:01-Tg mice, but not in those from B*57:03-Tg mice and LMs. These results suggest that B*57:01-Tg mice potentially enable the reproduction and evaluation of HLA-B*57:01 and ABC-induced immune-mediated IDT. Chimeric HLA transgenic mice may be an appropriate tool to prospectively evaluate the detrimental potential of HLA-related IDT compounds in preclinical studies.

**3087 Ambulatory Continuous Intravenous Infusion in Group-Housed Non-Human Primates**


Many new drugs (for example, oncology drugs, biologics) are administered by continuous intravenous infusion, requiring the use of this administration route in non-clinical animal safety studies. When working with nonhuman primates (NHPs), there is increasing awareness of the benefits of group-housing during safety studies and we have been running toxicity studies in under group-housed conditions for several years. For infusion toxicology studies this brings additional technical and organizational challenges. In the work presented here we have evaluated the feasibility of ambulatory infusion in group-housed non-human primates. Cynomolgus monkeys were surgically implanted with a medical grade catheter via the femoral vein, opening in the vena cava. The catheter was connected to an ambulatory infusion line issuing from the infusion pump. Monkeys were then fitted with a protective jacket housing the infusion pump with reservoir. The catheterized monkeys were group-housed in small groups of 2 to 5 animals. Over a period of 5 weeks groups of animals received either 0.9% saline or 5% dextrose for injection by continuous infusion at low (1.0 mL/kg/hr) and high (2.5 mL/kg/hr) rates. Group-housing did not interfere with the continuous administration of the dose formulations of saline or dextrose. The test item dose accountability was within an acceptable range of +/- 10% of theoretical volume over the course of the infusion period. These vehicles were well-tolerated and no adverse effects were observed in terms of clinical signs, body weight or clinical pathology. Macroscopically, tissue changes were comparable to findings in single-housed catheterized animals. In conclusion, the continuous intravenous infusion mode of administration is considered compatible with group-housing of NHPs in...
non-clinical regulatory studies and making these studies compliant with the new European guidelines on animal welfare. Moreover, this model is in line with the 3Rs, since it is a refinement of the standard tethered infusion model and allows the conduct of group-housed toxicology studies enabling freedom of movement in their environment.

3088 Sublingual and Buccal Administration in Minipigs
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The oral cavity is an attractive site for the delivery of drugs. The oral mucosa is well supplied with both vascular and lymphatic drainage and first-pass metabolism in the liver and further systemic elimination in the gastrointestinal tract are avoided. The buccal area is well suited for a retentive device and for mucosal vaccination which offers the advantage that both local immune responses, at the site of pathogen entry as well as systemic immunity can be obtained. The minipig is an attractive model for oral cavity drug and vaccine delivery as the buccal epithelium is nonkeratinized and of comparable thickness to that of humans. Furthermore, the porcine immune system has been well studied and shows comparable structure and function to the human immune system. We have performed studies in the minipig with application of oral patches and devices, including method feasibility studies. Because of the limited mobility and elasticity of the jaw and lips, respectively, application and subsequent inspection of the oral patches are challenging and sedation of the animals is often required. Typically the human administration of these devices is intended to be daily, or even more frequently, meaning that the animal model should be amenable to these regimes. There are different types of device, from those that are intended to dissolve in a short time, often a few minutes to those where an active drug substance is released slowly over a period of hours. The latter type of device is challenging in the context of animal studies. In the study described in this poster, we have therefore evaluated the feasibility of application of different drug delivery systems for oral cavity administration. Different application times and locations within the mouth were tested. The success and ease of application and retention were found to vary depending on the size and shape of the drug delivery system and the location of application. In the poster we present structures to demonstrate the various possibilities. Our conclusion is that both sublingual and buccal administration of drugs and vaccines are feasible in the minipig, and we have subsequently performed further studies involving active drug substances.

3089 A Novel Mouse Model for High-Throughput Small Molecule Screen of Hepatocyte Maturation
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Cultured primary human hepatocytes are the gold standard for drug and toxicity testing. However, good quality cells are limited and they rapidly lose their mature phenotype once isolated. This loss of maturity in culture creates a limited window of usefulness of these cells which creates a hurdle for conducting drug and toxicological assays. Several culturing techniques such as sandwich and 3D cultures have been developed to extend the mature and useful period of hepatocytes. Unfortunately, no system has yet succeeded in maintaining in vivo expression levels of all xenobiotic metabolizing enzymes in long term culture. To improve the functional life of cultured primary hepatocytes we have set up a robotic screening scheme to screen libraries of small molecules to identify candidates that will maintain mature phenotype of hepatocytes ex vivo. First, we developed a mouse model with click beetle red (CBR) luciferase inserted into the albumin (Alb) gene, a marker of mature hepatocytes and click beetle green (CBG) luciferase inserted into the alpha fetoprotein (AFP) gene, a marker of fetal hepatocytes. Next we isolate hepatocytes from young mouse and culture them in 96 well plates for 48 hours in the presence of the molecule of interest. After 48 hours, we lyse the cells and add d-luciferin to measure the levels of luciferase in the lysate. A successful molecule will maintain higher levels of the albumin linked CBR signal compared to untreated wells and will not raise the levels of AFP/CBG. Using this assay, we have identified several potential candidate compounds that maintained high albumin levels and currently we are validating these compounds by Illumina RNA Sequencing. In the future, we shall test the drug metabolizing ability of primary cells treated with these compounds in long term culture. In conclusion, we have developed a novel mouse model for high throughput small molecule library screening to identify candidates for maintaining hepatic maturity in culture. Using our screen, we have also successfully identified potential candidate molecules that are being validated.

3090 Behavioral and Neuroanatomical Outcomes following Early-Life Chlorpyrifos Exposure in a Preclinical Rat Model
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Organophosphorus pesticides (OPs) are among the most widely used pesticides in the world and have been implicated in the etiology of neurodevelopmental disorders including developmental delay and autism spectrum disorder (ASD). Exposure to chlorpyrifos (CPF), one of the most widely used OPs, has been linked to mental and motor delays, as well as increased rates of attention issues, in children. The present study of CPF developmental neurotoxicity examined sophisticated social behaviors of both males and females in a novel rat model of CPF exposure. Newborn Sprague-Dawley rats were administered either coconut oil vehicle or one of two CPF doses (1.0 or 3.0 mg/kg CPF) via daily subcutaneous injections from postnatal day (PND) 1 to 4. Isolation-induced ultrasonic vocalizations (USVs) were collected from pups at the peak day of USV emission and postnatal whole brain anatomical phenotyping via high resolution magnetic resonance imaging (MRI) was applied at PND 7. At the juvenile age, sociability was assessed using the three-chambered social approach and dyadic reciprocal social interaction assays, and motor activity was evaluated via an open field exploration assay. Female pups exposed to 1.0 mg/kg CPF emitted fewer vocalizations than did pups treated with vehicle or 3.0 mg/kg CPF, and MRI results are currently undergoing analysis. CPF exposure did not affect the sociability phase of three-chambered social approach; however, performance in the social novelty paradigm suggests that CPF disrupted recognition of previously acquired social cues. Relative to vehicle controls, CPF altered juvenile social interactions on several key parameters in a sexually dimorphic manner. Female rats exposed to 3.0 mg/kg CPF exhibited reduced levels of pouncing and rough-and-tumble playing, an effect not observed in males. Similar exploratory behavior in a novel open arena was observed across treatment groups, eliminating hypo- and hyperactivity as confounding behaviors. Taken together with existing literature, the impairments discovered here suggest that developmental OP exposures disrupt neurological development and neural correlates that underlie complex social behaviors.

3091 Effects of Iron Deficiency Model in Cynomolgus Monkeys on Serum Phosphorus and FGF23
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It has been suspected that severe nutritional ferropenic anaemia provokes alterations in the metabolism of calcium and phosphorus. The purpose of this study was a) to create a new animal model for iron deficiency evaluation by controlled blood removal and b) to monitor the effect of iron deficiency on clinical signs, hematology parameters and phosphorus in monkeys. For this purpose, 4 female Cynomolgus monkeys (2.5 years old, 2.4kg) were fed with a low iron diet (PMI No. MH241) and supplements (fresh fruits and dietary enrichments). Monkeys had also 15% of their whole body blood volume removed weekly for 4 weeks. Hemoglobin concentration and hematocrit levels were monitored on a weekly basis. Animals were considered in severe anemia when hemoglobin levels dropped below 7 g/dL and hematocrit under 21%. Serum phosphorus and iron concentrations were measured in Weeks 2 and 4. Fibroblast growth factor 23 (FGF23) concentration was measured in Week 2. A terminal blood collection was collected one week after the last blood removal (Week 5) and analyzed for all parameters. Gradual decreases in hemoglobin and hematocrit were observed during the 4 week blood removal period: group mean hemoglobin concentration decreased from 13.1 to 10.0g/dL and hematocrit from 43% to 32.7%, over 5 weeks. Mean group serum iron concentration remained stable between pretreatment and Week 4: 150.3 to 141 µg/dL. There were no clinical signs observed and noticeable changes observed in serum phosphorus and FGF23 concentrations, except for one single animal where a significant decrease in FGF23 was observed at Week 5 compared to baseline (109 to 32pg/mL). This animal was also noted to have the lowest hemoglobin level (8.5g/dL) and hematocrit (28.9%). Based on the
results observed, it is considered that this monkey iron deficiency model is acceptable, as it induces a relevant reduction in hemoglobin and serum iron concentrations without inducing any discomfort or severe clinical signs to the animals. More importantly, there was no significant effect on phosphorus regulation within the period of observation.

Ulcereative dermatitis (UD), is a common skin condition seen in laboratory mice. This condition is characterized by lesions present essentially in the dorsal area frequently accompanied by moderate to severe pruritus leading to excessive scratching behavior. It is considered one of the most important welfare issues in research as it might result in unscheduled euthanasia prior to study termination. The cause of UD is unknown but is considered to be multifactorial with potential genetic, behavioral, environmental, infectious, or exogenous origin. The condition also seems to be affected by sex, age and diet. UD is currently treated with hydrotherapy combined with different topical treatments such as diluted povidone iodine or chlorhexidine solution or antibiotic ointment. Such treatments are not always effective and might interfere with research results. The necessity to euthanize animals early when condition is severe and unresponsive to treatment has an impact on survival rate, a critical factor on carcinogenicity studies. In an attempt to decrease incidence of ulcerative dermatitis, our laboratory gradually introduced curative and preventive nail trimming in rodents on long term studies. The rationale is that the reduction in pruritus is performed by constant licking of the conscious animals. The rodent is restrained with one hand placed around the thorax and nails are a cut with a standard human nail clipper. No restrainer is necessary. Data from twelve 104-week mouse carcinogenicity studies were evaluated to investigate the relationship between nail trimming and incidence of UD as well as to compare the number of unscheduled euthanasia following nail trimming implementation. There is an evident decrease of incidence of UD on all studies following nail trimming implementation (data indicate minimally 1/3 reduction; up to nearly 45 UD prior to nail trimming compared to 0 to 15 once nail trimming was applied). Furthermore, the number and duration of treatment required also decreased and fewer unscheduled euthanasia for welfare reasons were necessary. Nowadays, preventative nail trimming is implemented as a routine during the day to day activities. This method is safely and quickly performed without additional stress of anesthesia and the use of a restrainer. It is a great improvement for rodent health as well as for the outcome of research studies.

Establishing bioequivalence between an innovator and a generic topical ophthalmic product is challenging because of the clinically inaccessibility of the target of the eye. Clinical PK endpoint studies are characterized by significant inter-subject variability and an inability to enroll sufficient participants. A promising alternative is a Physiologically-Based Pharmacokinetic (PBPK) model to predict drug concentrations in ocular tissues. The current study investigated the distribution of dexamethasone in different tissues of the eye at different time points following topical ocular administration of TobraDex ST* (Tobramycin/dexamethasone) suspension to male New Zealand white rabbits. Results of the current study will be used to test the predictive ability of an existing PBPK model. 42 New Zealand White rabbits were divided into 7 time point (0.5, 1, 2, 3. 4, 6, and 8 hours post-treatment) groups of 6 rabbits per group. 30µl of TobraDex ST* was instilled in the right eye of each rabbit in all groups. Samples were obtained from each rabbit of a group at its designated time point. Sampling included tears, cornea, conjunctiva, aqueous humor, vitreous humor, sclera, retina and iris-ciliary body as well as blood. A liquid chromatography mass spectrometry/mass spectrometry method was developed to measure dexamethasone concentrations in all tissues and plasma. Measured concentrations were compared to model predictions to further inform the model development.

This study was performed to evaluate the protective effect of butea monosperma flower (BM) and boerhaavia diffusa root (BD) extract in New Zealand White rabbit glaucoma and Wistar rat cataract experimental models. Glaucoma was induced by ocular instillation into the left eye of a 1% w/v dexamethasone suspension (DMS) at the dose volume of 50µL, 4 times a day with a 2h interval between each instillation for 14 days. The right eye served as the control. Groups G2 and G3 were treated with BM and BD extracts, respectively, and G4 with mixture of BM + BD extract. Groups G5 and G6 were treated with Timolol and Isotine eye drops, respectively. Group G1 received normal saline. Each formulation was instilled in the same manner as DMS, after 15 minutes of DMS instillation. Eye examination by direct ophthalmoscope and intraocular pressure (IOP) measurement by digital tonometer were performed twice weekly. Cataracts were induced in rat pups (10-14 days of age) by a single subcutaneous injection of sodium selenite (25 mmol/kg) to 6 groups (G2-G7). G2 was treated with sodium selenite alone. G3 and G4 were treated with BM and BD extracts, respectively. G5 was treated with BM + BD extracts, G6 group was treated with Isotine eye drops. Each formulation was instilled at dose volume of 20µL, 4 times a day with a 2h interval between each instillation for 14 days in both eyes. Group G1 was injected with normal saline and served as a control. At study termination, lens homogenates and tissues were evaluated for lipid peroxidation and reduced glutathione. Glaucoma model revealed that IOP was increased in rabbits treated with DMS alone (G2). IOP of treated eye was comparable with untreated eye in all other groups (G3 to G6). Eye examination did not reveal any abnormality in treated eyes compared with untreated eyes. The signifcant prevention of IOP increase in BM and BD treated groups even after DMS administration indicated that BM and BD have preventive therapeutic use for controlling IOP in Glaucoma. Cataract model revealed cataract formation on day 4 after sodium selenite injection in all pups without any significant difference of size, shape and density. Lipid peroxidation and glutathione levels were also comparable in all groups. Results indicate that BM and BD lack significant protective effect in cataract model of rat pups.
Many neurodegenerative diseases including Alzheimer’s (AD), Parkinson’s (PD), and Huntington’s diseases are characterized by the accumulation of misfolded protein aggregates; dysfunction in protein degradation may contribute to their pathogenesis. Autophagy, a normal homeostatic process involved in protein degradation, is characterized by the formation of phospholipid containing vesicular structures known as autophagosomes that can contain a variety of cytoplasmic cargos such as damaged organelles or potentially toxic macromolecules such as protein aggregates. Disruption of autophagy in knock out mouse models leads to neurodegeneration and genetic mutations in autophagy-related genes have been implicated in the pathogenesis of AD and PD. To better understand autophagic activity in neurons in vivo, we created a stable transgenic zebrafish line that expresses eGFP-Mapl1lc3b specifically in post-mitotic neurons under the elavl3 promoter. Microtubule associated protein 1 light chain 3 (Mapl1lc3), in its unbound form is commonly called LC3-I. The lipidized form of LC3, LC3-II, then gets incorporated into the membrane of the autophagosome. LC3-II is degraded by lysosomal hydrolyses upon fusion of the autophagosome with the lysosome. Thus, the uptake of LC3-II into autophagocytic vesicles can be used to monitor autophagic activity. As proof of principle, we modulated and quantified the number of autophagic vesicles via treatment with known autophagy inducers (rapamycin) and inhibitors (3-methyladenine, protease inhibitors). Through these experiments, we could also quantify autophagic flux (i.e. rate of autophagosome accumulation) in specific neuron populations in the living zebrafish larvae. We then tested if treatment with diesel exhaust particulate extract (DEPe), an environmental toxin that our lab has shown results in loss of amnestic neurons in zebrafish larvae, affects autophagic activity. Here, we demonstrate that DEPe inhibits autophagic activity in zebrafish neurons. As dysregulation of autophagy has been shown to be an important factor for neurodegenerative disease etiology, this proven transgenic line will be useful in screening for both toxins that inhibit and potential therapeutic compounds that may induce autophagy.

The environmental component plays a definite role in the development of diseases and in particular cancer as their incidence is continuously growing globally. Belfiore et al. (2018) expressed a whole-genome mouse array in zebrafish as a whole animal model to simulate relevant chemical exposures in order to develop preventive measures is our major objective. A heterogeneous population model was used, obtained from crosses and a segregation sequence over 30 generations leading to two genetically modified profiles in lines of mice genetically selected for minimal AIRMAX or minimal AIRMIN acute inflammation reactivity. Two carcinogenic agents were evaluated in the AIRmin model: DMBA [7,12-imethylbenz[a]anthracene] and urethane which are both pro-carcinogenic agents that can be activated via metabolization by cytochrome P450. DMBA was applied topically on theadipocytes which are both pro-carcinogenic agents that can be activated via metabolization by cytochrome P450. DMBA was applied topically on

Seizure liability is a severe adverse effect and it is usually detected as convulsions in toxicology studies. Non-convulsive seizures however induce only subtle behavioral changes and their assessment in animals is challenging. Electroencephalography (EEG) is the only method to correlate animal behavior to seizure activity and video-EEG is the current gold-standard for preclinical seizure liability assessments (Authier et al., 2014). As clinical observations and PK sampling time points in toxicological studies are usually predefined, they are not ideally suited for comprehensive symptom detection and for exact correlation of drug plasma levels to symptoms. Video EEG allows following animals continuously and could improve symptom detection rate and characterization of neurological symptoms. Implanted ports for CSF collection allow sampling from conscious animals and we investigated if these implants can be combined with EEG electrodes in the same animals to enable simultaneous use of two “windows to the brain”. Three beagle dogs that already had CSF ports were implanted with EEG electrodes. To evaluate a possible impact on signal quality, another three dogs without pre-existing CSF ports were also implanted with EEG transmitters. After baseline EEG recordings were comparable between both groups, reference and in-house compounds with different modes of action were tested in these dogs. As again no differences were found in visually and reference and in-house compounds with different modes of action were tested in these dogs. As again no differences were found in visually and

Effective management of joint pain can be challenging in veterinary species, particularly in horses where standard treatment with systemic non-steroidal anti-inflammatory drugs and intra-articular corticosteroids are inconsistently effective and risk adverse effects. Local anesthetics, such as bupivacaine, administered intra-articularly have demonstrated reductions in equine joint pain in vivo, however effects are short lived (hours) and in vitro studies report chondrotoxicity. The recent introduction of bupivacaine in a sustained release liposomal formulation (approved for use in the dog) offers a potential longer acting therapeutic option for treating joint pain in horses. However, potential systemic or local toxicity needs to be assessed before this treatment can be recommended for intra-articular administration. To that end, 16 exercised university owned Thoroughbred horses received a single intra-articular administration (right antebrachialcarpal joint) of either 0.12 mg/kg liposomal bupivacaine (N=12) or an equivalent volume of 0.9% saline (N=4). Synovial fluid was collected from the joint prior to drug administration and daily for up to 5 days post administration. Saline treated joints were used to assess the effect of repeated arthrocentesis on joint parameters. Synovial drug concentrations were measured using liquid chromatography-tandem mass spectrometry. Enzyme linked immunosorbent assays were used to assess cartilage degeneration (C2C, C1C2) and cartilage turnover (CPI, CS846). Analysis to date indicates liposomal bupivacaine persists in the joint for up to 96 hours without lameness, joint effusion, or overt chondrotoxicity. Cartilage turnover was minimally altered in injected and uninjected joints up to 96 hours. These data indicate that normal bupivacaine may provide a non-toxic option for treatment of equine intra-articular pain, and support further studies in diseased joints following chronic administration. Supported by UC Davis Center for Equine Health and the California Horse Racing Board.
3100 Evaluation of Difference between Homosexual and Heterosexual Housing Conditions on Various Toxicologically Important Parameters of Male Wistar Rats

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Rodents are a naturally social species, and numerous lines of evidence shows to have behavioural and physiological changes that occur from solitary housing conditions. For routine toxicity studies, animals are kept under homosexual housing conditions and not under heterosexual conditions. Sexual activity can influence the normal well-being of animals. Hence, it can be assumed that confinement of rat in homosexual conditions for longer duration may affect their health thereby outcome of chronic toxicity studies, where animals live in homosexual atmosphere, may not be factual especially for parameters related to reproduction. To prove this hypothesis, a study was planned to evaluate the effect of homosexual and heterosexual housing on various parameters of male Wistar rats. Rats were divided into two groups; heterosexual living conditions (sexually active) and homosexual living conditions (sexually non-active). Sexually active male were taken from the breeding colony and when retired from the colony they were still continued to be cohabited with females. For sexually inactive group, two male rats of approximately same age as sexually active were kept in a single cage. The effect on hematologic, clinical chemistry, organ weight, gross pathology and histopathology were assessed after 6, 12, and 18 month of housing. There were no significant changes noticed in any of the clinical pathology parameters evaluated except slightly high levels of cholesterol and triglycerides in rats of homosexual housing condition up to 18 month of experiment. A significant decrease in mean weight of reproductive organs was noticed in sexually active rats when compared to sexually active. There were no significant changes were noticed at histopathology in any of the organs. Some of the changes like higher incidences of inflammation in kidneys, atrioventricular changes in spleen and interstitial hyperplasia in testes in sexually active animals cannot be confirmed due to housing conditions as they are quite common at this age. We can conclude that sexual activities may not affect the health of the animals whether animal housed homosexual or heterosexual conditions.

3101 Targeted Deletion of Cyp1b1 in Retinal Vascular Supporting Cells Is Sufficient for Attenuation of Retinal Neovascularization and Trabecular Meshwork Dysgenisis


Cytochrome P450 1B1 (CYP1B1) is expressed in human and murine ocular tissues with important roles during development. Mutations in this gene are reported in patients with congenital glaucoma. Although Cyp1b1 is expressed in retinal vascular cells including endothelial cells (EC), pericytes, and astrocytes of the retina, their cell autonomous contribution to the neovascularization defect observed in global Cyp1b1 deficient mice remains unknown. In addition, mice deficient in Cyp1b1 present developmental abnormalities in their trabecular meshwork cells and their organization. Here we determined whether lack of Cyp1b1 expression in EC, PC or AC impacted retinal neovascularization or trabecular meshwork dysgenesis. We generated Cyp1b1 targeted transgenic mice with vascular cell specific targeted Cre-deletion (Cyp1b1EC, Cyp1b1PC and Cyp1b1AC). Pathologic retinal angiogenesis during oxygen-induced ischemic retinopathy (OIR) was evaluated by collagen IV staining of retinal neovascularization and trabecular meshwork. Structural morphology of the trabecular meshwork was determined by transmission electron microscopy utilizing eyes from 8-week-old Cyp1-deficient mice. The quantitative assessment of retinal neovascularization at P17 during OIR, showed decreased neovascular tufts in retinas of Cyp1b1EC mice compared with control or other targeted transgenic mice. Morphometric analysis data showed that 8-week-old Cyp1b1AC mice exhibited a morphologic and integrity of trabecular meshwork similar to global Cyp1-deficient mice. Thus, Cyp1b1 expression in perivascular supporting cells (PC) plays an essential role during ischemia-mediated retinal neovascularization and development of trabecular meshwork.

3102 Evaluation of Oil Based Oleic Acid in the Formation of a Model of Acute Respiratory Distress Syndrome

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Multiple models of acute respiratory distress syndrome (ARDS) have been utilized in toxicological assessments including pulmonary embo- lization or induction of pulmonary inflammation by direct injection of endotoxin (LPS) or oleic acid. Most publications indicate a volume of oleic acid administered without specification regarding the formulation administered to induce pulmonary dysfunction. This study aimed to assess kinetics and hemodynamics effects in a reliable and reproducible model following administration of oil based oleic acid (OB). As an ancil- lary goal, this study aimed to compare these results to an aqueous based oleic acid (AB) formulation. Alpha-chloroalcohol and fentanyl anesthetized beagle dogs were acutely instrumented for ECG, arterial blood sampling, endotracheal, pulmonary and systemic blood pressures. Following stabilization, OB (0.1 to 0.15 mL/kg; n=8) was administered via injection into the right atrium. Following administration, cardiac output (via thermodilution) and blood gas analysis were performed every 30 minutes for at least 90 minutes. ARDS was achieved when the PaO2/FiO2 ratio was less than 200 mmHg. Under baseline conditions, all animals displayed normal hemodynamic values (HR: 79±5 bpm and SAP: 120±11 mmHg) and oxygenation status (PaO2/FiO2: 478±8 mmHg%), Administration of OB altered hemodynamic values consistent with autonomic activa- tion (HR: 139±12 bpm and SAP: 130±12 mmHg, P<0.05) and impaired oxygenation (PaO2/FiO2: 186±32 mmHg%, P<0.05) at 90 minutes when compared to baseline values. Additionally, OB caused an increase in intratracheal pressure and reduced pulmonary compliance (3.7±0.3 to 6.2±0.2 mmHg at baseline and 90 minutes, respectively; P<0.05) with dogs exhibiting crackles on auscultation. Within 60 minutes following OB administration, 5/8 dogs achieved ARDS criteria and 6/8 had achieved ARDS criteria by 90 minutes. As a comparison, AB (n=2) did not result in any hemodynamic or oxygenation changes through the moni- toring period. Additionally, AB resulted in no changes in peak ventila- tory pressures and no dogs achieved ARDS criteria by 90 minutes. Given these results, OB oleic acid is a reliable and efficient model to produce ARDS and is sufficient to produce a model of ARDS in the anesthetized beagle dog.

3103 Assessing the Impact of Ethanol on Developing and Adult Zebrafish (Danio rerio)

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Zebrafish have been used for many years in developmental and repro- ductive biology, yet little is known about the adult zebrafish liver. Additionally, many of the zebrafish liver cell types have yet to be charac- terized in zebrafish, including Kupffer cells, endothelial cells, and stel- late cells. There is minimal information available regarding zebrafish liver transporters and nuclear receptors. Therefore, it’s important to understand the mechanism of xenobiotic metabolism in zebrafish to support their use as a model organism for studying the biological effects of xenobiotics. The value of zebrafish as a model organism is based on a broad set of conserved gene similarities involved in metabolism and transportation of endogenous and exogenous chemicals. While there are molecular similarities between zebrafish and humans, there are also differences that may impact how compounds are metab- olized. Therefore, it’s absolutely necessary to investigate and validate the assumptions that zebrafish possess conserved genes, enzymes, and processes involved in all stages of metabolism. This study investigates the impact of ethanol exposure on liver toxicity markers in adults and the impact of ethanol on developing larva. For the adult study, rela- tive expression levels for each gene were determined and normalized to β-actin. Of the seventeen genes that were assayed via qPCR, our results demonstrate a significant up-regulation of the following genes following ethanol treatment: abcc4 (m3d), abcc1 (m1p), abcc4 (m4p), abc9 (sur2), abc5, mate1, oatp1 and slc4a2 (ae2). Alcohol metabolism increases a precursor production, a precursor for cholesterol and fatty acid synthesis. Historically, this increase in acetate levels driven by alcohol is a hepatic ion flow during xenobiotic metabolism.
Migration of intra-articular implant wear particulates from the knee joint has been studied in various animal models, as well as in post-mortem samples from patients who received total knee joint replacement. However, there still exists a need for a suitable animal model for tracking the migration of such debris from the knee joint. To fill this need, a proof-of-concept porcine model was developed for particle migration from the knee joint into the draining lymphatic system. Vitreous carbon particles were bilaterally deposited both intra-articularly and extra-articularly in the hind limbs in 6 Yorkshire swine. The regional/draining lymph nodes were qualitatively assessed weekly by a veterinarian via manual palpation to detect any post-operative enlargement or change in consistency. At 6 weeks, the draining lymph nodes were harvested and processed for histology. Microscopic evaluation revealed carbon particle migration from the knee into iliac lymph nodes (12 of 12) and inguinal lymph nodes (6 of 12), but no evidence of particle migration into popliteal lymph nodes. Particle associated inflammation was generally minimal, consisting of neutrophils, histiocytes and multinucleated giant cells. Overall, this study established a needed animal model for evaluating particle migration to the draining lymph nodes from the knee joint.

**Development of a Porcine Lymph Model to Study Particle Migration**

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Sarcoidosis is a debilitating inflammatory condition characterized by granulomatous lesions in the affected organ. We have previously developed a murine model of chronic granulomatous inflammation elicited by multiwall carbon nanotubes (MWCNT). Previous studies have shown the MWCNT model shares several characteristics with pulmonary sarcoidosis, including decreased expression and activity of peroxisome proliferator activated receptor gamma (PPARγ) and elevated expression of CCL2, osteopontin and metalloproteinase-12 (MMP-12), proteins thought to promote granuloma formation. PPARγ is a nuclear transcription factor which acts as a negative regulator of macrophage activation and is essential for pulmonary homeostasis. These observations suggest that PPARγ has a crucial role in the regulation of pulmonary granuloma formation. We hypothesized that administration of rosiglitazone, a PPARγ specific ligand, would limit alveolar macrophage activation in response to MWCNT instillation. Wild-type C57Bl/6 mice were given rosiglitazone laden diet (6mg/kg/day) three days prior and continuing until 10 or 20 days post MWCNT instillation. Alveolar macrophages and whole lung tissue were collected and evaluated for inflammatory gene expression and histological changes in granulomatous lesions using a previously developed scoring system. We found that animals instilled with MWCNT receiving rosiglitazone laden diet for 10 days demonstrated a reduction in CCL2(90%), osteopontin(75%) and MMP12(79%) compared to mice receiving control diet (n=3/group, p<0.001). The frequency and severity of granulomatous lesions were evaluated but no difference was observed 10 days following MWCNT instillation (n=5). Those animals which received rosiglitazone laden diet for 20 days following MWCNT instillation also demonstrated a significant reduction in CCL2(65%), osteopontin(33%) and MMP-12(67%) (n=10, p<0.001). Evaluation of pulmonary granulomas 20 days following MWCNT instillation found that the frequency and severity of granulomatous lesions were significantly less in those animals receiving rosiglitazone laden diet compared to controls (n=23, p<0.001). These data suggest that PPARγ activation can limit alveolar macrophage inflammatory gene expression and may serve as a novel target to limit pulmonary granuloma formation.

**Rosiglitazone Represses Pulmonary Granuloma Formation and Alveolar Macrophage Inflammatory Activation**

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Effective identification of neonatal animals, especially small rodents, is a challenge due to their small body size, making it difficult to use conventional identification methods such as ear tags or ear notch. Methods such as needle marking or permanent markers have their limitations due to the cost or stability of the identification, respectively. Other methods such as paw tattoo system used by some toxicology tests for reproductive studies have limitation of the maximum producible numbers. Traditionally, digital amputation has been used for this purpose, however animal welfare aspects of this method is significant concern. Here we explain a permanent identification by dot-tattoo marking of the digits using small gauge needles. In this study, we used a 31 gauge needle with minimal amount of green tattoo dye and either of front or hind limbs of right or left sides were used as per need. Both dorsal and palmar surface of the digits were evaluated. The method was associated with minimal bleeding and without any complications of using electric tattoo machine. There was no significant effect on neonatal growth or health. Using a guide map, this method provides up to 10,000 identification numbers. Therefore, we suggest that the method to be considered as a simple and cheap yet less painful animal identification method for colony management of studies involving massive neonatal production such as transgenic or knock-out studies as well as large reproductive or general toxicology studies.

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Historical Background Control Data in the RccHan: WIST Rat in Chronic Toxicity Studies

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Historical background control data supports improved interpretation of lesions in chronic toxicity studies. Two chronic toxicity studies were performed to generate historical background control data in the RccHan®:WIST rat model. Both studies utilized the same source of rats but were initiated approximately fifteen months apart. In the first study, 150 male and 150 female RccHan®:WIST rats were pair housed under standard housing and husbandry conditions with ad libitum access to a standard diet (20% protein, 4.5% fat) and water. In the second study, 130 male and 130 female RccHan®:WIST rats were pair housed under standard housing and husbandry conditions with ad libitum access to a lower energy diet (16% protein, 4% fat) and water. Additionally, in the second study, rats were administered tap water (10 mL/kg body weight) by once weekly gavage. Body weight, food consumption, morbidity and mortality were monitored throughout the in-life phase of the studies. Interim necropsies were performed at one, three, and six months in twenty animals per gender for generation of historical control data at common acute or subchronic study time points. At the conclusion of the study period, animals were submitted for necropsy and microscopic evaluation of select organs was performed. Neoplastic and non-neoplastic lesions were characterized and complete blood count/serum chemistry and urinalysis were performed. A detailed analysis of survival, plastic lesions were characterized and complete blood count/serum chemistry and urinalysis were performed. A detailed analysis of survival, plastic outcomes, and is thus often preferable to a 2-year rodent study. Future work will verify these preliminary findings. This work was supported by a fellowship from the SOT to ARH, P20GM103549 and P30GM118247 (MTP, WXD), and AA020518 (WXD).

3110

Oral Gavage Dosing of Neonatal Rats as a Test System for Toxicology and Safety Evaluation of Drugs Designed for Neonates and Children


Oral gavage is a common experimental procedure in adult rat. Although this method is considered a well established approach for most toxicology and drug safety testing in adult rat test system, application of this method for evaluation of drugs designed to be used for children is questionable. Considering basic physiologic differences between neonates and adult, especially in digestive system, establishing a neonatal test system to evaluate the safety of the oral drugs targeting pediatric patients is necessary. We have developed a daily oral gavage system for neonatal rat using conventional plastic mouse gavage canola. Following manual restraining of the pups using their dorsal skin, and pre-measurement of the cannula on the abdominal surface using the pup’s milk-filled stomach as land mark, oral gavage was done using an inert dye (tattoo ink). The neonates especially in earlier post-natal age accepted the procedure with minimal resistance. Successful dosing was confirmed by dis-coloration of white milk-spot into the ink color in 1 to 5 minutes. Necropsy of the neonate after 1 hour showed normal flow of the ink in stomach and intestine. We suggest that oral gavage of neonatal rat from early post natal age could be considered as an ideal system for safety evaluation of foods an nutrients for newborns as well as oral medications in pediatric medicine.

3111

Exploring the Hyaluronan Network in an Animal Model of Alcoholic Liver Disease

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Advanced liver disease, regardless of etiology, remains a pharmacologically intractable condition. Indeed, only liver transplant is in ‘cure’ a cirrhotic liver. Over consumption of ethanol, in both acute and chronic settings, can cause significant liver injury. While many mechanisms of liver injury induced by ethanol are known, these advances have not led to improvements in therapies for patients suffering from alcoholic liver disease. Therefore, we must gain additional insights into what drives liver disease pathogenesis and progression after alcohol exposure in the hope of identifying potential points of therapeutic intervention. Our laboratory is interested in how the extracellular matrix (ECM) is involved in liver injury as well as repair after different hepatotoxin exposures, including alcohol. Specifically, are we interested in a molecule called hyaluronan (HA), a ubiquitous ECM molecule. While we have considerable evidence to suggest that HA and binding proteins and receptors (the HA network) are involved in liver injury and required for liver repair after acute carbon tetrachloride exposure in mice, we do not know if the HA network is altered by exposure to ethanol and if so, we speculate that this study was to characterize the HA network in mice after exposure to ethanol using the ‘acute-on-chronic’ paradigm and compare those data to those collected from mice given a control diet. Ethanol-exposed mice displayed an upward trend in the following: plasma HA (mimicking patients with liver disease), the number of HA-positive cells in liver (minimal amount binding protein), hepatic tumor necrosis factor (TNFα) and TNF-stimulated gene (Tsg6, another HA binding protein) transcript levels. We did not observe differences in expression of the HA synthases or in enzymes responsible for the breakdown in HA between control or ethanol-exposed mice. Finally, we observed a downregulation of the HA receptor (an HA binding protein) in HA binding proteins and this was paralleled by Rhamm levels in human alcoholic liver disease patients. Overall, these data suggest that the HA network may be altered by ethanol exposure and might contribute to liver disease. Future work will verify these preliminary findings. This work was supported by a fellowship from the SOT to ARH, R20GM103549 and P30GM118247 (MTP, WXD), and AA020518 (WXD).

3112

Minimal Degenerative and Regenerative Renal Changes in 26-Week Tg.ras.H2 Mouse Carcinogenicity Study

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The 2-year rodent carcinogenicity assays involving conventional rats and mice have been conducted for over 3 decades. As an alternative to the 2-year rodent carcinogenicity bioassays, 26-week short term carcinogenicity bioassays were approved using transgenic mouse strains, including Tg.ras.H2. The Tg.ras.H2 model, which can be used for both genotoxic and non-genotoxic compounds, has gained popularity and its use has increased over the years. Currently, more than 75% of all mouse carcinogenicity studies are being conducted in Tg.ras.H2 mice. The Tg.ras.H2 model predicts neoplastic findings relevant to human cancer risk assessment, produces fewer non-biologically significant neoplastic outcomes, and is thus often preferable to a 2-year rodent study. We published the largest historical control database for both neoplastic and non-neoplastic lesions in Tg.ras.H2 mice Our current historical control data base has grown sufficiently that we can now begin to look at looks at effects of vehicles or vehicle components on both non-neoplastic and neoplastic findings. We evaluated the incidence of renal lesions in male and female mice in 26-week Tg.ras.H2 oral gavage carcinogenicity studies with methylcellulose as one of the ingredients of the vehicle. Our evaluation indicated that renal degenerative and regenerative tubular basophilia changes were significantly higher in males than in females. The historical control data base shows 350 mice/sex in 14
Diet-related, insulin deficiency and/or resistance, is fast gaining the attention of a global epidemic. Apart from traditional risk factors, recent studies attributed the increased incidence of diabetes to environmental factors. Certain epidemiological studies have associated increased incidence of diabetes with Dichlorvos (DDVP, an organophosphorus pesticide) and Atrazine (Herbicide) exposure. However, exposure to xenobiotics other than those identified with diabetes is unusual. Therefore, we evaluated the diabetogenic potential of these chemicals by exploiting the conserved Insulin/Insulin growth factor-like signaling (IIS) as well as glucose homeostasis in Drosophila melanogaster. Flies reared on food with DDVP (1.5 ng/ml of food) or Atrazine (20 µg/ml) during development contained significantly elevated glucose, trehalose levels and reduced Akt and Forkhead box O (Foxo) phosphorylation. DDVP exposed flies had significantly reduced transcript/protein levels of Drosophila insulin-like peptides (dilps), GLUT1 (receptor for glucose assimilation) and increased transcript levels of insulin receptor (InR) suggesting insulin deficiency typical of type 1 diabetes. In contrast, flies exposed to Atrazine had significantly elevated transcript/protein levels of dilps, InR and GLUT1, pointing to insulin resistance typical of type 2 diabetes in response to Atrazine exposure. Together, these findings reflect not only the diabetogenic potential of DDVP and Atrazine but also their differential modulation of insulin signaling pathways. Further, the study provides experimental evidence to the epidemiological propositions on the diabetogenic potential of DDVP and Atrazine. Finally, our study projects the utility of Drosophila as a potential model for deci- phering and understanding the influence of xenobiotics on the onset of diabetes.

Niclosamide is a salicylanilide anthelmintic drug used worldwide for the treatment of tapeworm infections due to low oral bioavailability. Recent drug repurposing screens have revealed that niclosamide has the potential to treat cancer, osteoporosis, and endometriosis through interaction with multiple signaling pathways. Based on a LOPAC2060 library screen within zebrafish embryos, we previously identified niclosamide as a potent developmental toxicant resulting in static exposure from 5 h post-fertilization (hf, ~50%–epiboly) to 25 hpf (prim-6 stage). Given that niclosamide delivery for new applications may require non-oral routes of exposure, the potential developmental toxicity of niclosamide should be re-evaluated, as these exposure routes may result in the embryo's exposure in utero during pregnancy. Therefore, using zebrafish as a model, the objective of this study was to further investigate the toxicity of niclosamide during early development. Zebrafish embryos were exposed to vehicle (0.1% DMSO) or niclosamide (0.078-0.625 µM) from (1) 2 to 6 hpf as a baseline exposure; (2) 2-3, 2-4, 2-5, or 2-6 hpf to assess the potential for recovery (0.156-0.625 µM); or (3) 2-6, 3-6, 4-6, and 5-6 hpf to identify sensitive windows of exposure (0.156-0.625 µM). For all exposures, epiboly progression was assessed by quantifying percent epiboly and cell height above the yolk at 6 hpf. Collectively, these studies revealed that (1) niclosamide induced a concentration-dependent delay in epiboly initiation and progression; (2) recovery following niclosamide exposure was dependent on the exposure window and concentration; and (3) the sensitive window of niclosamide exposure was between 3-4 hpf, a mid-blastula transition characterized by yolk sac second gradient activation and initiation of cell motility. To identify the sensitive period of epiboly delay, embryos were treated with nicotine for 3 hpf, followed by solvent (0.1% DMSO) or niclosamide (0.156 or 0.3125 µM) from 2-6 hpf, transferrred to clean media, and reared until 24 hpf (prim-5 stage). Surprisingly, niclosamide-induced epiboly delay resulted in a distribution of outcomes at 24 hpf, including embryonic lethality, developmental delays, and normal development. Using a multi-pronged strategy, we are currently investigating the mechanism of niclosamide-induced epiboly delay as well as rate of niclosamide uptake and depuration within zebrafish embryos.

Tris(1,3-Dichloro-2-Propyl) Phosphate Disrupts Dorsoventral Patterning in Zebrafish Embryos

Tris(1,3-dichloro-2-propyl) phosphate (TDCIPP) is a high-production volume organophosphate flame retardant widely used within the United States. Within zebrafish, initiation of TDCIPP exposure at 0.75 h post-fertilization (hf) results in genome-wide alterations in methylation during cleavage (2 hpf) as well as epiboly delay or arrest (at higher concentrations) during late-blastula and early-gastrula (4-6 hpf). To determine whether these TDCIPP-induced effects were associated with impacts on the transcriptome, embryos were exposed to vehicle (0.1% DMSO) or 2 µM TDCIPP from 0.75 hpf to 6 hpf, and total RNA was extracted from triplicate embryo pools per treatment and hybridized onto duplicate Affymetrix Zebrafish Gene 1.0 ST Arrays per RNA sample. Based on transcriptome-wide profiling, TDCIPP resulted in a significant impact on biological pathways involved in dorsoventral patterning and bone morphogenetic protein (BMP) signaling. Consistent with pathway-level responses, TDCIPP exposure also resulted in strongly doralized embryos by 24 hpf - a phenotype that mimicked the effects of dorso- morphin, a potent and selective BMP inhibitor. Moreover, the majority of doralized embryos were preceded by epiboly arrest at 6 hpf. Our microarray data also revealed that the expression of sizzled (szl) - a gene encoding a secreted Frizzled-related protein that limits BMP signaling - was significantly decreased by nearly 4-fold at 6 hpf. Therefore, we used a splice-blocking morpholino to test the hypothesis that knockdown of szl phenocopies TDCIPP-induced delays in epiboly progres- sion. Interestingly, contrary to our hypothesis, injection of szl MOs did not affect epiboly progression but, similar to chordin (chd) morphants, resulted in ventralized embryos by 24 hpf. Overall, our findings suggest that TDCIPP-induced epiboly delay may be independent of szl expres- sion and function, and that TDCIPP-induced dorsalinization may - similar to dorso- morphin - be due to interference with BMP signaling during early zebrafish development. Therefore, our ongoing work is focused on investigating the mechanism of TDCIPP-induced epiboly delay and dor- salization using a combination of mRNA sequencing, reverse genetics, immunohistochemistry, in situ hybridization, and cell-based assays.

Evaluation of Embryonic Zebrafish as an Alternative Whole-Animal Model for Nephrotoxicity Screening

Due to an increasing demand for testing of new and existing chemi- cals and legal restrictions for use of animals, there is a strong need for alternative approaches to assess systemic toxicity. Embryonic zebrafish (Danio rerio) are increasingly recognized as a promising alternative whole-animal model that may be able to overcome limitations of cell-based in vitro assays and bridge the gap between high-throughput in vitro screening and low-throughput in vivo tests in animals. The aim of the present study was to investigate if zebrafish embryos might be a suitable high-throughput amenable model for nephrotoxicity testing. In this study a range of well characterised compounds that are known nephrotoxins in humans and rodents (aristolochic acid, cadmium chlo- ride, potassium bromate, ochratoxin A) were investigated for their nephrotoxic effects in embryonic zebrafish. Fertilized zebrafish eggs of the AB/AB strain were distributed into 96-well plates for treatment with compounds for 48 h (3 - 5 days post fertilization) with nephrotoxic compounds. H&E staining revealed marked proximal tubule injury as evidenced by dilated tubules, tubule cell necrosis and tubule cell disorganisation following treatment with aristolochic acid and ochratoxin A. No damage was seen in control tubules. Embryos treated with cadmium chloride showed a slightly dilated proximal tubules compared to the control embryos, whereas no kidney injury was evident in embryos treated with potas- sium bromate. Consistent with the morphological changes in the pro- nephrone in response to compound exposure, WISH analysis revealed marked upregulation of cadh17 expression at sites representing the proximal tubule in embryos treated with aristolochic acid, ochratoxin A and cadmium chloride, but not after treatment with potassium bromate. qRT-PCR analysis of genes identified as sensitive kidney injury markers based on rodent studies (ctu, ctf1a, havcr1, hmx1a, ssp1) showed inconsistent results in treated zebrafish. Taken together, our results suggest that embryonic zebrafish may be a suitable model for nephrotoxicity screening and that cadh17 expression may be a good marker for xenobiotic induced injury to the pronephros.
3117 Does the Zebrafish Embryotoxicity Assay Predict Variations or Malformations in Rat Prenatal Developmental Toxicity Studies?

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The zebrafish model is predicted to potentially detect the presence of compounds capable of causing prenatally developmental toxicity. The common prediction models are based on teratogenic indices, the relation of effective concentration of lethality and of embryotoxic findings. Thereby, it is only possible to distinguish between positive or negative outcome in vivo. But in the in vivo prenatal developmental toxicity studies one is classifying the findings as variations and malformations which provides the opportunity to assess a chemical as negative, developmental toxic, or teratogenic with different regulatory consequences. We were aiming to assess if the differentiation of endpoints in the scoring system of the zebrafish embryotoxicity as general embryotoxic and specific embryotoxic endpoints could improve the accuracy of prediction. We used a data set based on 31 public known as well as in house chemicals of the company with balanced different potentials of prenatal developmental toxicity. In each test group 12 embryos with intact chorion were cultured from 1.5 to 120 hours post fertilization. A morphological score system with 38 parameters was used to describe the development of zebrafish embryos in the concentration range from the no observed adverse effect concentration up to the concentration causing mortality. These results were used to assess which classification of morphological zebrafish endpoints are indicators for general embryotoxicity (e.g. hatching, cell viability, yolk) to predict variations in vivo or indicators for specific embryotoxicity (e.g. head, trunk, body shape, cardiovascular system, and fins) to predict malformations in vivo. The common prediction model (PM) resulted in an accuracy of 79%, a sensitivity of 86%, and specificity of 73%. None of the so far analyzed combination parameters for general or specific embryotoxicity resulted in a good predictivity to distinguish between a negative, developmental toxic and teratogenic results in vivo. To improve the prediction model for zebrafish embryotoxicity a definition of the concern of morphological alterations and an international harmonized terminology is needed, like it is given for the in vivo prenatal developmental toxicity studies.

3118 Zebrafish and Medaka as Alternative Models for Developmental Toxicity Assessment of Chemicals, Including Behavior as an End Point

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There is a worldwide trend to move away from animal testing for the human and environmental safety assessment of cosmetics and personal care products. Fish embryo models (zebrafish (Danio rerio) and Medaka (Oryzias latipes)) are highly popular in toxicology, both in research required potential regulatory applications. In compliance with international animal welfare regulations (e.g. Dir 2010/63/EU), the fish embryo models provide an ethically acceptable small-scale analysis system with the complexity of a complete organism. The objective of this study was to compare the potential of both models, zebrafish and medaka, for the teratogenic, developmental and behavioral assessment of chemicals. The study was initiated with 5 hydrophilic reference compounds: 3 known to be teratogenic in both rodents and humans (Amanadine, 5-Fluorouracil, Lithium Chloride), and 2 non teratogenic compounds (Ascorbic acid and Ceftriaxone). Embryos were exposed to these compounds from 2-4 hpf (hatching and fertilization) to 4 dpf (days post fertilization) for zebrafish and 9 dpf for medaka. At the end of the test the evaluation of survival, morphological alterations and locomotor activity was carried out in them. While 3 out of 5 compounds (Amanadine, Ascorbic acid and ceftriaxone) were properly classified with both assays, 5-Fluorouracil was only detected as teratogenic for medaka embryos. Surprisingly, Lithium Chloride did not induce morphological alterations in zebrafish nor medaka. However, locomotor activity was clearly affected in zebrafish embryos treated with this compound. These results indicate that medaka embryos could be more sensitive than zebrafish embryos to certain hydrophilic teratogenic compounds (such as 5-Fluorouracil). It also suggests a potential low uptake of hydrophilic compounds by zebrafish embryos and highlight the importance of conducting internal dosing assays for proper test item classification. Besides this, including behavior endpoints would likely increase the sensitivity of both models to properly detect teratogenic compounds.

3119 Development of an Oxidative Stress In Vitro Assay in Zebrafish (Danio rerio) Cell Lines


Oxidative stress is a common mechanism for different toxicological endpoints of relevance both for aquatic and human toxicology. The nuclear factor erythroid 2-related factor 2 (Nrf2) is a key regulator of cellular defense against oxidative stress and Nrf2 has been shown to be correlated with classical toxicological endpoints. In vitro methods using fish cell lines for the assessment of aquatic toxicity is highly needed for mechanistic studies and as an alternative to vivo studies. We now describe an in vitro assay to study oxidative stress using zebrafish (Danio rerio) cell lines. Transfection efficiency of twelve commercially available transfection reagents were tested in the zebrafish cell lines ZFL, ZF4, and Pac2. The most efficient reagent for each cell line was selected for further experiments. Cells were transiently transfected with an Nrf2-responsive luciferase plasmid. The assay was tested using known oxidative stress inducing chemicals (tertiary butyl hydroquinone, hydrogen peroxide, and sulfurophane) in increasing concentrations (0.1, 1, 10, and 100 μM) for 24 h. Of the investigated cell lines, ZF4 and ZFL showed higher sensitivity to known inducers and were used to study oxidative stress of pesticides (diazinon, deltamethrin, Aarazine, metazachlor, tertiary butylazine, diuron). Cells were incubated with increasing concentrations (6.25, 12.5, 25, 50, 100 μM) of each pesticide for 24 h. Diazinon, deltamethrin, metazachlor, and diuron statistically significantly increased the Nrf2 expression. The described assay could be a valuable tool for future research in aquatic toxicology to study the toxicity of both pure chemicals and environmental water samples.

3120 Utilization of 21st-Century Predictive Tools for Characterizing the Acute Toxicity Potential and Human Risk of Exposure to Chemical Weapon Precursor Compounds

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Traditional methods for evaluating chemical toxicity are time-consuming, expensive, and require the exposure and sacrifice of live animals. New methods must be developed to meet the future challenges facing the Threat Agent Science (TAS) program, due to an increasing number of acutely hazardous chemicals requiring more stringent, mechanistic characterization. To support this, we are developing a toxicity characterization framework to support rapid assessment of new substances. This toxicity characterization framework describes an integrated approach to toxicity assessment by incorporating computational and in vitro testing strategies with existing toxicity data to provide faster and more comprehensive risk assessments compared to traditional methods. As a proof-of-concept, we show the evaluation of 54 chemical weapons precursor compounds (CWPCs) using this framework. Chemicals were first grouped according structural similarities for group-based hazard assessments and physiochemical property predictions. Initial LD50 predictions were made using internal QSAR models built on the RTECS/Leadscope database. Alternative test systems were used to fill data gaps and assess relative potency for downstream risk assessments. These test systems included high-content imaging of HepG2 cells, cardiomyocyte functionality and zebrafish (Danio rerio) embryonic behavior assays. Lastly, in vitro to in vivo extrapolation (IVIVE) was used to inform the risk assessment and predict hazardous exposure scenarios for a subset of the CWPC list. Overall, our toxicity characterization framework provides a potential solution to the future challenges of the TAS program. A draft version for public release; distribution unlimited. Disclaimer: Research was conducted in compliance all Federal requirements. The views expressed are those of the authors and do not constitute endorsement by the US Army.
LD50 values, e.g., rat oral toxicity for sodium nitrite (200 mg/kg) and caf-

5, 6, 7 or 14, and observed the eggs for 48 hours for viability. LD50

or the albumen of embryonated chicken eggs on either Incubation Day

and allowed to interact naturally

in situ

that cardiovascular, nervous, endocrine, digestive and excretory

models human biologic responses to exogenous chemicals such

assays for assessment of oral toxicity are limited both in number and

eye irritation, skin irritation, skin sensitization). However, alternative

in vitro

studies in rats. Several

Correctly identifying a chemical’s acute systemic toxicity hazard level

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Valle,

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For genotoxic carcinogens, the existence of thresholds, i.e. doses below

which a chemical does not exhibit adverse effects, remains controvers-

ial, while for epigenetic carcinogens the presence of no-adverse-eff-

fects (NOAELs) has been widely accepted. The assumption that exposure to genotoxic carcinogens at low level leads to tumor forma-

tion, results in strict regulations in human health risk assessments that

the exposure to carcinogens should be as low as reasonably achievable

(ALARA). The current experiment investigated the dose-response effect

and the possibility of NOAELs for formation of DNA adducts, a crucial

event in the carcinogenicity of DNA-reactive chemicals, by a group of

naturally occurring alkenylbenzenes with previously reported genotoxic

potential: methyl eugenol, estragole, myristicin and a mixture, nutmeg oil.

Previously, in Turkey Egg Genotoxicity Assay (TEGA) methyl eugenol,

estragole and myristicin produced DNA adducts at the highest tested
doses. In the current study, a wide dose range of the compounds was

evaluated in TEGA using \(^{32}\)P-nucleotide postlabeling (NPL) assay which

detects the formation of DNA adducts. Methyl eugenol (0.005-4 mg/

egg), estragole (0.025-40 mg/egg), myristicin (0.08-50 mg/egg) and nutmeg oil (0.6-100 mg/egg) were injected daily into the air sac of turkey

eggs on days 22-24 of incubation. Control groups received vehicle, 20\%

aqueous solution of Kolliphor HS15. Liver samples for the NPL assay were

collected three hours after the last dose. While tested alkenylbenzenes

produced DNA adducts in a dose-related fashion, for each compound

a NOAEL below which no DNA adducts were formed was detected. For

methyl eugenol and estragole, the NOAEL was 0.025 mg/egg ( \(-0.67\) mg/

kg bw), followed by myristicin at 0.33 mg/egg ( \(-9.3\) mg/kg bw) and

nutmeg oil at 1 mg/egg ( \(-27.8\) mg/kg bw). These findings suggest that

thresholds for the effects of genotoxic carcinogens exist. Importantly,

the NOAELs for alkenylbenzenes were above the available estimated
daily intake in humans, suggesting negligible hazard to human health.

Detection of No-Adverse-Effect-Levels (NOAELs) for Formation of DNA Adducts by Alkenylbenzenes in the Alternative Model Turkey Egg Genotoxicity Assay (TEGA)

3122 The In Ovo Oral Toxicity Assay: A Rat Acute Oral Systemic Toxicity Replacement

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Correctly identifying a chemical’s acute systemic toxicity hazard level is critical for labeling the material with the proper safety precautions needed for handling and transportation. Acute toxicity categories are assigned, in part, by an oral toxicity estimate, as determined by lethality studies in rats. Several in vitro and alternative assays have been developed and codified for assessment of other acute toxicity hazards (i.e., eye irritation, skin irritation, skin sensitization). However, alternative assays for assessment of oral toxicity are limited both in number and accuracy. We aim to develop a metabolically competent assay system that models human biologic responses to exogenous chemicals such that cardiovascular, nervous, endocrine, digestive and excretory systems as well as general homeostatic mechanisms are represented and allowed to interact naturally in situ. We administered seven reference chemicals (i.e., strychnine, caffeine, sodium nitrite, acetylsalicylic acid, ethanol, sodium chloride and sucrose) directly into the yolk and/or the albumen of embryonated chicken eggs on either Incubation Day (ID) 5, 6, 7 or 14, and observed the eggs for 48 hours for viability. LD\(_{50}\) values for each experimental condition were calculated following Log-Probit methodology. We demonstrate that reference chemicals elicit dose-responsive toxicity in embryonated chicken eggs, and we are able to stratify lethality responses based on the relative toxicity of agents used in our experiments. Egg LD\(_{50}\) values were generally similar to rat LD\(_{50}\) values, e.g., rat oral toxicity for sodium nitrite (200 mg/kg) and ca-

feine (190 mg/kg) had average egg LD\(_{50}\) values of 136 and 237 mg/kg, respectively. A robust validation would qualify or disqualify IOOTA as not only an inexpensive alternative assay to mammalian hazard identi-

fication, but could serve as a stop-gap assay until sufficiently accurate in

vitro and computer models are developed.

In Ovo Oral Toxicity Assay: A Rat Acute Oral Systemic Toxicity Replacement

3124 Dopaminergic Neurotoxicity of Polybrominated Diphenyl Ether Flame Retardants in Caenorhabditis Elegans

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Polybrominated diphenyl ethers (PBDEs) were utilized for four decades as a flame retardants in infant products, polyurethane foam, and carpet padding. PBDEs were sprayed onto the products and tend to migrate and accumulate in air, soil, and water after time. Epidemiological evidence suggests adverse behavioral and cognitive effects can occur when exposed to high levels during key points in development. Penta-BDE is a mixture of congener units with brominated diphenyl ether-47 (BDE-47) being the most commonly detected in human tissues. Increased blood levels of BDE-47 are proportional to the development of adverse motor and cognitive abilities in children. Specific behaviors including hyperactivity and attention deficits are prominent and similar to those observed in attention deficit/hyperactivity disorder (ADHD). The target of BDE-47 induced neurotoxicity remains unknown, but could be linked to modulation of dopaminergic (DAergic) homeostasis. Hermaphroditic Caenorhabditis elegans have 6 anterior DA neurons, (2 pairs of CEP, 1 pair of ADE). Like humans, DA in the nematodes is responsible for attention, movement, and the recognition of reward. To determine if dopamine homeostasis is altered by BDE-47, this study exposed Larval 1-staged (L1) C. elegans (BZ55-strain and N2, wildtype) to BDE-47 at 3 concentra-

tions (2.5µm, 5µm, 10µm, and control 0.1% dimethyl sulfide). To achieve chronic exposure, treated L1 worms were directly transferred to nema-

tode growth media plates. Worms were permitted to develop to Larval stage 4 (L4) before analyzed using fluorescent microscopy (200X magn-

ification). Green pixel values of ADE and CEP cell bodies in L4-stage (BZ553) were analyzed using ImageJ software. Mean area classifications were also scored. Pixel mean values and mean area were proportional to adverse neurodevelopment and analyzed by ANOVA and bonferroni posthoc testing. The BDE-47 concentrations utilized did not significantly alter the size of the DA neurons. Further, protein analysis continues of the DOP-3, receptor concentration to determine if BDE-47 can alter DA homeostasis.

Genome-Wide Identification of Mutational Signatures Related to the Toxicity of AFB\(_1\) and FB\(_1\) Mixtures Using Caenorhabditis elegans Model

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Mutational signatures hold promise in molecular cancer epidemiology because they bear witness to mutagenic exposures, as well as illuminate endogenous mutagenic processes and mechanisms of DNA damage and repair. Aflatoxin B\(_1\) (AFB\(_1\)) and Fumonisin B\(_1\) (FB\(_1\)) are the most common food contaminants whose co-existence was found in food-stuffs all over the world, particularly in high liver cancer risk areas. AFB\(_1\) is a known human carcinogen and FB\(_1\) is a class 2B carcinogen (IARC) with strong tumor promotion activity in animal models. Studies in animal models have shown that the co-exposure to AFB\(_1\) and FB\(_1\) elicits considerably stronger toxic and carcinogenic effect than when exposed to either of the toxins alone. C. elegans has been a valuable model organism, with its simplicity, completely mapped genome, as well as several homolo-
gies to mammalian systems. Specifically, C. elegans possess the CYP450 homologues capable of metabolizing AFB\(_1\), as well as Cys homologues which are tied into the mechanism of action for FB\(_1\). In the present study, C. elegans was used as a model to evaluate combative toxic effects of mycotoxin mixtures. Combined exposure to AFB\(_1\) and FB\(_1\) elicited stronger toxic response in terms of growth inhibition, brood size reduc-
tion, as well as lifespan reduction, in the wildtype N2 strain C. elegans, than either AFB\(_1\) or FB\(_1\) alone, with combination index (CI) lower than 1.0 for all tested dose combinations. AFB\(_1\) exposure induced a dose-re-
sponse in detectable DNA lesions while FB\(_1\) did not show an induction of DNA lesion; however, an additional 40 µM of FB\(_1\) combined with various concentrations of AFB\(_1\) resulted in a higher level of DNA lesion than that of AFB\(_1\) alone. Furthermore, whole-genome sequencing of model muta-
tional signatures, via analyzing worm populations exposed to AFB\(_1\), only and AFB\(_1\)+FB\(_1\), mixtures, showed that AFB\(_1\) induced substitutions of gua-
nines in a GpG context, and was similar to the mutational signatures of AFB\(_1\) and FB\(_1\) mixtures; however, their mutational frequency increased with addition of FB\(_1\). Thus, this model system, combined with genome sequencing data, can provide new insights into the contribution of mutagenic exposure to the process of carcinogenesis.
Cannabinoid agonists and antagonists have known inhibitory effects on mammalian tumor cell proliferation in vitro. Previous work has shown that cannabinoids also inhibit regeneration in the planarian model species, _Girardia dorotocephala_ (Woodworth, 1897). During cephalic regeneration, ocelli (eyespots) appear to be reduced or absent when exposed to cannabinoids. Due to these observed effects, the hypothesis is that cannabinoids may inhibit the expression of important genes in the planarian visual system was tested. _Sine oculis_ (sixi), a constitutive gene required for proper development and maintenance of ocelli was selected. Specimens of _Girardia dorotocephala_ were maintained under standard lab conditions and fasted at least 7 days before conducting studies. Decapitated and intact planarians were used to determine if cannabinoids had any effect on _sine oculis_ expression during development and normal functioning. Animals were placed into one of the following treatment groups: media control, DMSO solvent control, 8 micromolar WIN 55212.2 (cannabinoid agonist), and 10 micromolar rimonabant (cannabinoid antagonist). Total RNA was extracted on an individual basis at 24 hours. Using reverse transcriptase PCR and gene-specific endpoint PCR, _sine oculis_ gene expression was determined. No differences were detected in gene expression between control and experimental groups. These data indicate that exposure to synthetic cannabinoids do not affect the expression of the _sine oculis_ gene in either intact or decapitated planarians and the lack of ocelli development may be related to other visual system-related genes or genes involved in other aspects of tissue regeneration. Future work will focus on those genes having a much more global role in planarian regeneration. Supported by NSF/HBCU-UP grants 1622811 and 1531014.

### Chemical Structure Plays an Important Role in the Ability of Cannabinoids to Inhibit Planarian Regeneration


Cannabinoids, both agonists and antagonists, inhibit normal tissue and organ regeneration in the planarian, _Girardia dorotocephala_ (Woodworth, 1897). While the mechanism underlying this inhibition has yet to be determined, the chemical structure of the cannabinoid may play an important role, leading to the following hypothesis: the chemical structure of a cannabinoid is an important factor in its ability to inhibit planarian regeneration. Two cannabinoid antagonists of very different structures were used. Rimonabant, a 1,5 diarylpyrazole, is a well-established cannabinoid antagonist and has served as prototype for the development of other cannabinoid antagonists. The second compound, PSNCBAM-1, is based on the urea molecule. Both drugs are antagonists of the type 1 mammalian cannabinoid receptor. Specimens of _Girardia dorotocephala_ were maintained under standard laboratory conditions and fasted at least 7 days before conducting studies. Decapitated animals were placed into one of the following treatment groups: media control, DMSO solvent control, 3 micromolar rimonabant and 5 micromolar PSNCBAM-1, and allowed to regenerate over a period of 5 days. Planarians were euthanized with 1M HCl and stained with aceticarmine to observe ocelli development. There was no significant difference in head and ocelli development between control animals and those treated with PSNCBAM-1. In contrast, regeneration of the head and ocelli was markedly reduced in animals treated with rimonabant. Two other compounds based on rimonabant, AM251 and NIDA-41020 (an iodine atom and a methoxy group respectively replacing the chlorine atom at the 5-phenyl position), also produced significant inhibition of planarian regeneration similar to rimonabant. These data indicate that the structure of a cannabinoid antagonist plays a major factor in its ability to inhibit planarian regeneration and specifically that the rimonabant-type structure has a greater effect on regeneration than a urea-based structure. Future studies will examine other cannabinoid antagonists of varying chemical structures to determine if these findings are consistent. Supported by NSF/HBCU-UP grants 1622811 and 1531014.

### Comparison of the Expression of Sine oculis in Decapitated and Intact Planarians Exposed to a Cannabinoid Agonist and Antagonist


### Evaluation of Metabolic Response during Lipid Starvation of a Model Parasite Perkinsus Marinus

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Our laboratory is interested in understanding the response of _Perkinsus marinus_, a protozoan parasite of oysters, to lipid starvation. Previous work has shown that this parasite is capable of synthesizing its own fatty acids, as well as acquiring them from their host. We seek to understand whether the capacity to synthesize fatty acids is a means to generate membrane components during lipid starvation, or if alternatively if this biosynthetic capacity is supplemental to growth in an infected host. As citrate can serve as a source of Acetyl CoA, we are also investigating the role of cytosolic aconitase as a potential regulator in this process, as there is evidence for phosphorylation of this enzyme to favor citrate production. No one, to our knowledge, has delineated the role of cytosolic aconitase in fatty acid biosynthesis. First, we were interested in the impact of cell growth under a known fatty acid inhibitor, Triclosan. We saw a time dose dependent shift when cells were exposed to higher concentrations at longer time intervals. Following this, we were interested in what would happen when the cells underwent lipid starvation. We have performed differential mRNA gene expression in _P. marinus_, in which cells were starved of lipids for 11 days, as compared to lipid replete cells. There was not a noticeable upregulation of FAI-pathway enzymes for saturated fatty acid synthesis, although citrate synthase was upregulated approximately 3-fold, consistent with allisteric activation of fatty acyl CoA oxidation. Polyketide synthase delta 5 and 6 fatty acid synthase and delta 5/6 fatty acid desaturase were upregulated by approximately 2-fold. Surprisingly, a number of enzymes involved in beta-oxidation were upregulated, which contrasted with the expectation that free fatty acids from triglycerides would be directed towards synthesis of membrane. Consistent with this, ACC-2 was down regulated 2-fold. Interestingly, maltose acetyl transferase was also upregulated 2-fold, implying that acetyl units from beta-oxidation may be re-directed to sugar acetylation. The implications of this complex interplay will be discussed, along with future experiments to address lipid and sugar metabolites present in this parasite under lipid starvation conditions. Finally, the activity and phosphorylation status of aconitase-1 will be assessed under these conditions.

### Chemical Structure Plays an Important Role in the Ability of Cannabinoids to Inhibit Planarian Regeneration


Cannabinoids, both agonists and antagonists, inhibit normal tissue and organ regeneration in the planarian, _Girardia dorotocephala_ (Woodworth, 1897). While the mechanism underlying this inhibition has yet to be determined, the chemical structure of the cannabinoid may play an important role, leading to the following hypothesis: the chemical structure of a cannabinoid is an important factor in its ability to inhibit planarian regeneration. Two cannabinoid antagonists of very different structures were used. Rimonabant, a 1,5 diarylpyrazole, is a well-established cannabinoid antagonist and has served as prototype for the development of other cannabinoid antagonists. The second compound, PSNCBAM-1, is based on the urea molecule. Both drugs are antagonists of the type 1 mammalian cannabinoid receptor. Specimens of _Girardia dorotocephala_ were maintained under standard laboratory conditions and fasted at least 7 days before conducting studies. Decapitated animals were placed into one of the following treatment groups: media control, DMSO solvent control, 3 micromolar rimonabant and 5 micromolar PSNCBAM-1, and allowed to regenerate over a period of 5 days. Planarians were euthanized with 1M HCl and stained with aceticarmine to observe ocelli development. There was no significant difference in head and ocelli development between control animals and those treated with PSNCBAM-1. In contrast, regeneration of the head and ocelli was markedly reduced in animals treated with rimonabant. Two other compounds based on rimonabant, AM251 and NIDA-41020 (an iodine atom and a methoxy group respectively replacing the chlorine atom at the 5-phenyl position), also produced significant inhibition of planarian regeneration similar to rimonabant. These data indicate that the structure of a cannabinoid antagonist plays a major factor in its ability to inhibit planarian regeneration and specifically that the rimonabant-type structure has a greater effect on regeneration than a urea-based structure. Future studies will examine other cannabinoid antagonists of varying chemical structures to determine if these findings are consistent. Supported by NSF/HBCU-UP grants 1622811 and 1531014.

### Profiling Environmental Chemicals Which Modulate the TGFβ/SMAD Signaling Pathway

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The transforming growth factor beta (TGFβ) superfamily regulates cell growth and development. Disruption of the TGFβ pathway in early development significantly affects organogenesis and cytodifferentiation. Dysregulation of TGFβ signaling in adults impairs tissue repair and may lead to fibrosis. Despite the complexity of downstream effects for the TGFβ signaling pathway, SMAD-dependent TGFβ signaling acts conservatively through SMAD 2/3/4. In the present study, the SMAD Binding Element (SBE)-beta lactamase (BLA) HEK293T cell line responds specifically to the activation of the SMAD 2/3/4 complex. The SBE-bla assay was first validated by screening the Library of Pharmacologically Active Compounds (LOPAC) library which contains 1280 compounds plus 88 Tox21 compounds at 7 concentrations ranging from 2.4 nM to 28 μM in three independent runs. The signal to background ratio was 2.37 ± 0.07 and 2.19 ± 0.10 in the agonist and antagonist mode, respectively. After online validation, this assay was used to screen the Tox21 compounds at 7 concentrations ranging from 2.4 nM to 28 μM in three independent runs. The signal to background ratio was 2.37 ± 0.07 and 2.19 ± 0.10 in the agonist and antagonist mode, respectively. The Z’ factor was 0.68 ± 0.05 and 0.56 ± 0.06 in the agonist and antagonist mode, respectively. After online validation, this assay was used to screen the Tox21 10K compound library. From the primary screening, we identified a group of compounds that are known to modulate the TGFβ/SMAD pathway (flavopiridol and colchicine) as well as a number of novel compounds (dipentaerythritol pentaacrylate). In addition, the activity of these TGFβ/SMAD-active compounds in other developmental pathways are examined in comparison, including the retinol signaling pathway (RSP) and the sonic hedgehog (SHH) pathway. Some hits show specific patterns in modulating TGFβ/SMAD, RSP and SHH pathways. These results will be used to prioritize potential developmental toxicants for further investigation.
Recent studies suggest that human derived intestinal epithelial cells (IEC) cultured as polarized monolayers on permeable Transwell® filters are effective at differentiating between hazardous and non-hazardous proteins. This experimental platform is based on apical exposure of IEC monolayers to a test protein for 24 hr followed by assessment of whether monolayers display evidence of loss of barrier integrity or cell viability. In this study, Caco-2 and T84 IEC polarized monolayers were evaluated for barrier integrity and cytotoxicity following exposure to hazardous and innocuous proteins for 24, 48 and 72 hr. Hazardous proteins included Cladostrium difficile toxin A (ToxA), Streptolysin O (SLO), Wheat Germ Agglutinin (WGA), and Phaseolus vulgaris haemagglutinin-E (PHA-E). Non-hazardous proteins included bovine serum albumin (BSA), porcine serum albumin (PSA), and fibronectin (Fn). The objective was to determine whether extended duration (48 and 72 hr) altered responses observed at 24 hr of exposure. Results from the 24 hr duration were similar to those previously reported. In general, evidence of diminished barrier integrity or cell viability observed following exposure to hazardous proteins for 24 hr was more prominent at 48 and 72 hr for both cell lines. Non-hazardous proteins exhibiting no impact following 24 hr of exposure, also failed to elicit any effects at 48 hr. Results were not as clear at 72 hr as control values (no protein) exhibited slightly higher background, possibly because of the prolonged period without serum in the culture medium. Results from these studies further support the utility of using cultured human IEC polarized monolayers to differentiate between hazardous and non-hazardous proteins and suggest that an exposure interval of 48 hours may be optimal for this purpose.

**3130 Validation and Comparison of the Neutral Red Uptake Assay in BALB/c 3T3 and CHO-WBL Cells**


The neutral red uptake (NRU) assay is used to evaluate the cytotoxicity of a variety of chemicals and agents, including tobacco products. We previously validated the NRU assay in BALB/c (3T3) cells according to OECD Guidance Document 129, and report here validation of the NRU assay in CHO (WBL) cells according to Health Canada Official Method T-502. CHO and BALB/c cells are exposed to test or control articles, in a 96-well plate format, for 24 hr with newborn calf serum or 48 hr without serum, respectively. After removing the treatment media, fresh media with neutral red (NR) is added for an additional 3-hr incubation. Only viable cells incorporate and bind NR, and cytotoxicity is detected by a concentration-dependent decrease in NR staining as quantitated by the optical density at 540 nm. Analysis of our current historical control population doublings during the 24- or 48-hour exposure times. However, our standard concentration ranges for SLS were 6.8 to 100 µg/mL for BALB/c and 13.6 to 200 µg/mL for CHO, which produced IC50 values of 19.99 ± 3.35 µg/mL and 89.15 ± 3.38 µg/mL, respectively. Based upon these results, BALB/c cells treated for 48 hr in serum-free media are less sensitive to the cytotoxic effects of SLS and may be more appropriate to evaluate e-liquids formulations and e-cigarette condensates, which generally have proved to be relatively innocuous. In contrast, CHO cells treated for 48 hr with serum are less sensitive to the cytotoxic effects of SLS and may be more appropriate to evaluate whole smoke from combusted cigarettes and their smoke condensates, which are relatively more cytotoxic. Additional experiments to evaluate the toxicity of SLS in BALB/c cells with and without serum using a 48-hr treatment, and in CHO cells using 24- and 48-hour treatments in the presence of serum, concurrently are in progress. Other studies with combusted cigarettes and their smoke condensates, as well as with e-liquids formulations and e-cigarette condensates, are planned.

**3131 An Integrated Multi-Organ Culture System to Evaluate Tobacco-Extract Systemic Responses**

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There is growing interest in the use of organ-on-a-chip technology for pre-clinical toxicity testing. In this pilot study, the local and systemic effects of cigarette total particulate matter (TPM) was measured in human lung and liver tissues. A three-organ dynamic multi-organ plate (Hu-DMOP®) was constructed in triplicate, in the following format: lung tissue (MucilAir™), lung-1, sandwich cultured human hepatocytes (SCHH) and a second lung tissue (lung-2). Communication between compartments was via a ‘simulated blood flow system’ consisting of a one-way fluidics/dialysis network, which allows for exchange of test article and metabolites. TPM (165 µg/ml in 0.02 mL) or vehicle control was applied apically to lung-1 for 24 h. Movement of TPM was assessed by tracking nicotine using LC/MS/MS. Lactate dehydrogenase (LDH) release was used to monitor tissue health. TPM mediated changes in the expression of key genes (IGFBP2, G6PD, GPX2, AKB810, NQO1, CYP1A2, TALDO1 and HMOX1) was determined by qRT-PCR at 24 h. Nicotine was first detected in lung-1 basolateral space at 1 h and reached a nadir (10 ng/mL) by 3 h. Nicotine in liver and lung-2 (4 ng/mL) was present after 6 h post TPM exposure. Cell viability remained high with LDH release below 10%. TPM exposure increased G6PD 4-fold and GPX2 3-fold in lung-1. These genes were only among 2-fold in lung-2 but AKB810 was induced 12-fold. In liver, AKB810 was increased 6-fold and NQO1 8-fold. These data demonstrate organ-organ interactions on compound effects. In conclusion, this study has demonstrated that the Hu-DMOP® can be used to measure the localized and systemic responses of a tobacco product in vitro.
To efficiently predict dose-dependent in vivo toxicity by alternative methods, the development of generic physiologically based kinetic (PBK) modelling-based reverse dosimetry approaches for large numbers of chemicals is required. Our previous study showed that PBK modelling-based reverse dosimetry of in vitro uterotropic in vivo dose-response data obtained from the uterotrophic assay to evaluate the model predictions. The current study indicates the feasibility of using a combination of in vivo toxicity data and a generic PBK model to predict in vivo uterotropic response for groups of estrogenic chemicals. Further studies can expand the current approach for other in vivo endpoints.

Using Python Coding to Automate and Improve Acute Toxicity Estimate Calculations for Agrochemical Formulations

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Agrochemical formulations are complex mixtures containing a variety of components including active ingredients, solvents, adjuvants, stabilisers and preservatives. LD/LC50 values for systemic effects (acute oral, dermal and inhalation) historically derived from animal tests can be determined from available acute toxicity data for individual ingredients within a formulation using the Acute Toxicity Estimate (ATE) calculation. The calculated values are classified into categories for transport and regulatory requirements. An automated computational protocol would alleviate the burden and several hour time commitment of manual calculations, minimise the possibility of error, and ensure consistency. To this end, we have developed a Python-based reverse dosimetry tool, PyATEmix, to automate ATE calculation composition and ingredient toxicity as inputs to provide estimates of acute toxicity and hazard classification estimates. Integrating the raw data retrieval process within Syngenta by connecting to relevant internal databases with the PyATEmix tool significantly reduced individual calculation time to a few minutes, and enabled calculations from number of formulations at one time. PyATEmix was validated using an expert database which simultaneously ensured data quality. With the ability to generate ATE calculations for a large number of formulations quickly, PyATEmix was used to assess the predictive power of ATE calculations. A retrospective comparison was conducted on a large dataset of agrochemical formulations with corresponding in vivo data. Trends could be identified with specific active ingredients and formulation types. Various limitations were identified including: impact of in vivo study design; overestimation due to high acute toxicity ingredients; and limited available acute toxicity data particularly in the inhalation area. Although this reduced the accuracy of the ATE tool generally a good comparison was achieved. PyATEmix is a useful tool to quickly and consistently derive ATE calculations and to assess the utility and applicability of ATE calculations as an alternative, non-animal, method to evaluate human safety of agrochemical formulations.
The increased scrutiny for health effects of bisphenol A (BPA), a chemical used in producing plastics, paper, and food packaging, led to its replacement with substitutes, such as BPS and BPF. Due to their massive production scale, BPA and its substitutes can be widely detected in a multitude of environmental samples and human biologics. The high degree of structural similarity between BPA and its substitutes suggests that they may possess similar bioactivity and toxicity. Therefore, removing them from the environment and reducing their human exposure may be important from a public health standpoint. Here, we propose to use a bioinformatics method based on manganese peroxidase (MnP), an enzyme produced by almost all wood-decomposing fungi, to degrade bisphenol compounds. In this study, MnP was immobilized into vault nanoparticles to enhance its thermal stability without losing its enzymatic activity. The degradation efficiency of vault-packaged MnP (vMnP) was evaluated and compared with its unpacked native form (nMnP). The result showed that vMnP was able to mediate a markedly faster and more sustained degradation than nMnP. The reproductive toxicity of bisphenols and their degradation products were also examined in Caenorhabditis elegans to evaluate the detoxification efficiency of vMnP. Our results showed that vMnP catalyzed reaction could eliminate the reproductive toxicity of bisphenols as neither fertility damage nor increased germline apoptosis could be observed from the worms exposed to the degradation products. These results thus suggest that packaging of the enzymes in vaults not only enhanced enzymatic kinetics but also mitigated the toxicity of degradation products.
racy of lipophilic chemicals and pre/pro-hapten was 84% (56/67) and 92% (34/37), respectively. These results suggested that the EpiSens®A could have high predictive performance in evaluating a broad set of chemicals including lipophilic chemicals and pre/pro-hapten, which are difficult to evaluate using existing test methods.

3141 Troubleshooting during In-House Validation of 3T3 NRU Phototoxicity Assay

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The 3T3 Neutral Red Uptake (NRU) Phototoxicity assay is an established in vitro assay used to evaluate the potential phototoxicity of a test article. The assay methods and prediction model are described in OECD Test Guideline (TG) 432 and the IVETITTO Protocol No. 78. Since the introduction of the EMA and ICH guidance, the 3T3 NRU-PT has been used quite extensively within the pharmaceutical industry. This abstract introduces some of the technical problems that was encountered during validation in our test facility and how they were overcome. In this assay, problems such as lot variability of serum and positive control, cell loss, neutral red solution preparation and even occasional room temperature variation were encountered all of which can interfere with measurements. 1) Due to lot variability of new born calf serum (NBCS), the NBCS was screened to ensure the doubling time of 3T3 cells were within 20-25 hours; 2) Cell washing should be performed with caution as the cells are easily detached from the wells during washing especially after test article treatment and irradiation; 3) Plates without irradiation should be kept in dark box and should not be disturbed, and the room temperature should be monitored; 4) The procedures to prepare the neutral red solution was refined to avoid commonly seen crystallization of neutral red (NR), which will disturb the OD reading; double filters were used - NR stock solution was filtered through a 0.22 micron filter and NR medium was also filtered before use through a 0.45 micron filter; 5) The red dye was also excluded to be transferred from medium to the cells. The lot variability has been observed and the improper selection of CPZ may disqualified the assay. After addressing these technical issues, the validation was completed with 7 studies and tested 8 compounds (Amiodarone HCl, Chlorpromazine HCl, Norfloxacin, Anthracene, Protoporphyrin IX, Disopyramide, L-Histidine, Hexachlorophene and Sodium Lauryl Sulfate). PIF and LIF were calculated based on the relative mean tissue viability for all four petroleum streams, a class of UVCB substances. In an effort to reduce animal testing, validation studies were conducted with the following in vitro systems: EpiSkin-SMTM involving reconstituted human epidermis and OECD 492 -EpiOcular TM involving reconstituted human corneal epithelium. Over the past few years, several in vitro and in chemico assays have been developed to predict the skin sensitisation potential of chemicals. The Direct Peptide Reactivity Assay (DPPA) is an in chemico assay that can be used to discriminate between sensitizers and non-sensitizers. This assay measures peptide reactivity of test chemicals by quantifying the depletion of synthetic heptapeptides containing either lysine or cysteine. Haptenation, i.e., the covalent binding of low-molecular weight substances (hapten) to proteins in the skin is considered a prominent mechanism through which chemicals or their metabolites become antigenic. Therefore, information from peptide reactivity assays such as the DPPA is considered relevant for the assessment of the skin sensitisation potential of chemicals (OECD N° 442C). Although DPPA is used to detect sensitizers, it has the limitation of a lack of ability to detect photosensitizers. To overcome this limitation, JRF has introduced an additional photoinactivation parameter into the DPPA assay. A group of 14 chemicals having various industrial and medical applications were selected for this study: 5 chemicals were food additives, 5 chemicals had industrial applications and other 4 chemicals were drugs. Each chemical was irradiated with dose of 5 J/cm2 UVA, in presence of Cysteine and Lysine heptapeptides. After 24 hours post incubation in the dark, peptide depletion was measured by a UV-HPLC method at 220 nm. The difference in peptide depletion (Δ) between UV treated and untreated samples was used to determine the effect of photosensitisation. Out of 14 non UV-irradiated chemicals, 11 were classified as non sensitizers

3143 Cosmetics Europe Evaluation of Five In Silico Skin Penetration Models

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The ADME TF aims to evaluate and develop in silico skin penetration models using relevant measured values e.g. partition (K) and diffusion (D) coefficients and water solubility. Since there are widely differing opinions on different in silico models, we have evaluated 3 open source and 2 commercial models in order to identify 1-2 that will be investigated further. Models varied in complexity but were primarily based on physical chemistry of diffusion, with different degrees of physiological relevance built in. Simulations of the cutaneous distribution of 25 chemicals were run and compared with measured in vivo human skin penetration data. None of the models adequately predicted the amount of chemical that evaporated. This was shown to be important since the prediction of dermal delivery (DD) was improved when the evaporated amount was accounted for in the simulations. The ability to predict the amount in epidermis and dermis varied between models; whereas, the amounts in the receptor fluid were generally over-predicted by 3 models and under-predicted by 1 model. The over-prediction of DD by 3 models is considered to be conservative in terms of human safety assessment. Interestingly, measured K/D values improved the prediction of DD by 1 model, while DD was predicted well by models using Q SAR values. The general effect of using ethanol instead of PBS as the solvent on DD was indicated by 4 models, although they all over-predicted the DD after application in ethanol. The DD for some chemicals were less well predicted by more than one model e.g. 4-chlorobutyric acid and triosolan, although the latter was applied in ethanol, which could account for this issue. In conclusion, our evaluation highlighted important differences in 5 models. The 4 more complex in silico models could predict the DD of 25 chemicals relatively well, especially if the fraction evaporated was considered. Amounts of chemical in the epidermis and dermis was less well predicted, as was the amount evaporated. Future work will investigate how measured data can be used to improve the models further.

3142 Validation of In Vitro Skin and Eye Irritation Tests for Petroleum Streams, a Class of UVCB Substances

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Currently few data are available to assess the utility of in vitro skin and eye irritation alternatives for determining the irritation potential of petroleum process streams, a class of UVCB substances. In an effort to reduce animal testing, validation studies were conducted with the following four NBCS was screened to ensure the doubling time of 3T3 cells were within 20-25 hours; cell washing should be performed with caution as the cells are easily detached from the wells during washing especially after test article treatment and irradiation; 3) Plates without irradiation should be kept in dark box and should not be disturbed, and the room temperature should be monitored; 4) The procedures to prepare the neutral red solution was refined to avoid commonly seen crystallization of neutral red (NR), which will disturb the OD reading; double filters were used - NR stock solution was filtered through a 0.22 micron filter and NR medium was also filtered before use through a 0.45 micron filter; 5) The red dye was also excluded to be transferred from medium to the cells. The lot variability has been observed and the improper selection of CPZ may disqualified the assay. After addressing these technical issues, the validation was completed with 7 studies and tested 8 compounds (Amiodarone HCl, Chlorpromazine HCl, Norfloxacin, Anthracene, Protoporphyrin IX, Disopyramide, L-Histidine, Hexachlorophene and Sodium Lauryl Sulfate). PIF and LIF were calculated based on the relative mean tissue viability for all four petroleum streams, a class of UVCB substances. In an effort to reduce animal testing, validation studies were conducted with the following in vitro systems: EpiSkin-SMTM involving reconstituted human epidermis and OECD 492 -EpiOcular TM involving reconstituted human corneal epithelium. All four streams were not classified as eye irritants in the in vivo studies according to the criteria of the Globally Harmonized System of classification and Labelling of Chemicals (GHS). The toxic results correlated with the EpiOcular™ in vitro results indicating that the relative mean tissue viability for all four petroleum streams was more than 60% compared to the mean tissue viability of the negative controls thus resulting in no GHS eye irritation classification. Results from the in vivo studies indicated that all four petroleum streams met the classification criteria for a GHS Category 2 skin irritant. These in vivo results did not correlate with the in vitro Episkin-SMT™ results. All four petroleum streams tested resulted in mean tissue viabilities of more than 50% compared to the mean tissue viability of the negative controls thus resulting in no GHS skin irritation classification. These results suggest that additional work is needed to understand the lack of correlation for the skin irritation tests which could improve the applicability of the in vitro tests for assessing petroleum derived substances. Results from EpiOcular™ show that it may be possible in vitro alternative model for assessing eye irritation potential based on the observed correlation of in vivo and in vitro results for nonirritating petroleum streams, however no validation data are available to evaluate for known eye irritation potential.

3144 Assessment of Potential Photosensitization Via In Chemico Direct Peptide Reactivity Assay


Over the past few years, several in vitro and in chemico assays have been developed to predict the skin sensitisation potential of chemicals. The Direct Peptide Reactivity Assay (DPPA) is an in chemico assay that can be used to discriminate between sensitizers and non sensitizers. This assay measures peptide reactivity of test chemicals by quantifying the depletion of synthetic heptapeptides containing either lysine or cysteine. Haptenation, i.e., the covalent binding of low-molecular weight substances (hapten) to proteins in the skin is considered a prominent mechanism through which chemicals or their metabolites become antigenic. Therefore, information from peptide reactivity assays such as the DPPA is considered relevant for the assessment of the skin sensitisation potential of chemicals (OECD N° 442C). Although DPPA is used to detect sensitizers, it has the limitation of a lack of ability to detect photosensitizers. To overcome this limitation, JRF has introduced an additional photoinactivation parameter into the DPPA assay. A group of 14 chemicals having various industrial and medical applications were selected for this study: 5 chemicals were food additives, 5 chemicals had industrial applications and other 4 chemicals were drugs. Each chemical was irradiated with dose of 5 J/cm2 UVA, in presence of Cysteine and Lysine heptapeptides. After 24 hours post incubation in the dark, peptide depletion was measured by a UV-HPLC method at 220 nm. The difference in peptide depletion (Δ) between UV treated and untreated samples was used to determine the effect of photosensitisation. Out of 14 non UV-irradiated chemicals, 11 were classified as non sensitizers
**3145** Non-Animal Skin Sensitization Safety Assessments for Cosmetic Ingredients: What Is Possible Today?


A key part of the safety assessment for local effects of cosmetic ingredients is the evaluation of skin sensitization, i.e. their potency to induce an immune response in the skin involving the innate and the adaptive immune system. For decades the murine local lymph node assay (LLNA) has been key for assessing the threshold dose per exposed skin area at or below which contact allergy induction does not occur in humans. Nowadays cosmetic regulation requires industry to perform safety assessments without the use of animals. Correspondingly, we introduce the use of an integrated testing strategy - based on a publicly available online tool for predicting skin sensitization potency - using examples of some hair dye and fragrance ingredients. We describe that already today a combination of Q(SAR) predictions and in vitro data on peptide reactivity, keratinocyte and dendritic cell activation allow for predictions of skin sensitization potency. Furthermore, testing of suitable structural analogues and sets of data for skin sensitization along with the chemical of interest can be applied in a weight-of-evidence approach to support the estimation of an allergy induction threshold by read-across.

We conclude that, depending on the testing strategy and the quality of the available data, predictions of skin sensitization potency can be made with a substantial degree of confidence.

**3146** Prediction of Skin Sensitization Potential in Compounds of Military Interest by In Vitro Methodologies


Environmental safety and occupational health (ESOH) assessments are critical to the U.S. Army in protecting Soldiers, civilians, and the surrounding communities. The Army Public Health Center (APHC) evaluates ESOH for Army materiel and chemicals of concern (CoC). Basic toxicological test data (“six-pack” including acute oral/dermal toxicity, ocular/dermal irritancy, skin sensitization (SS), and subchronic toxicity) are often not available for CoC. SS is an important occupational endpoint where data are required. In silico modeling, and in vitro (IVT) and/or in vivo (IVO) tests are employed in a phased approach to fill these data gaps. When QSAR modeling predicts a CoC may be a skin sensitiser, a set of IVT assays are used as an initial screen. This panel includes: Direct Peptide Reactivity Assay (DPRA), human cell line activation test (h-CLAT), and LuSens ARE-Nrf2 activation assay. Each assay addresses different aspects of the SS adverse outcome pathway and is used in a weight of evidence approach to predict SS. Five potential replacements for RDX (2,4-dinitroxyrazole (DNR), 2,4,6-trinitro-3-bromonitrophenyl (TNB), 1,3-dimethyl-hexahydropyrimidine (DHP), and methyl trinitropyrazol (MTPNP)) were evaluated using this panel (TOPKAT modeling predicted potential SS for all 5 compounds) along with 4 CoC with existing IVO data for comparison purposes (acrolein (AC), monocrotophos (MCP), parathion (PA) and phosphamidon (PM)). The listed chemicals were tested by SS for h-CLAT, and S were tested by DPRA. For the h-CLAT, 8 of the 9 chemicals were positive. AC, DNP, TNBPA, LLM-105, and MTPNP were positive for both CD54 and CD86 expression, while PA, MCP, PM, and DNP were positive using the DPRA. The remaining 4 chemicals will be tested by DPRA and all 9 chemicals will be tested in the LuSens assay. Preliminary data indicates that AC, MCP, PM, PA, and DNP are likely sensitizers. The results for AC and MCP agree with IVO data suggesting they are sensitizers. However, PM and PA were not observed to cause S5 IVO, but were positive using the IVT method, indicating a need for further study. For the RDX replacements, anecdotal reports indicate DNP and MTPNP may be sensitizers (in agreement with data produced by the IVT test battery). The use of IVT tests permits the rapid assessment of CoC and may help to preemptively screen for, and/or corroborate occupational reports of skin sensitization.

**3147** The Assessment of Phototoxicity Using the 3T3 Neutral Red Uptake (NRU) Phototoxicity Assay and a Modified Photo-Direct Peptide Reactivity Assay (DPRA)


Phototoxicity may be broadly defined as the ability of a compound to become toxic, or more toxic, in the presence of UV-exposure, and encompasses photorrnattirity, photoallergenicity, and photogenotoxicity. The validated 3T3 Neutral Red Uptake (NRU) Phototoxicity assay (OECD TG 432) is a recommended first tier test to address this endpoint, although the exact mechanism is not determined. Seven compounds, identified as photoirritants and photoallergens (chlorpromazine (chlor), 6-methylcoumarin (6-MC) and amiodarone), photol慧gen (hexachlorophene), photoirritant (anthrache), allergen (cinnamic aldehyde (CAI), and non-allergen and non-photoirritant (lactic acid (LA))), were evaluated. Chlor, 6-MC, anthrache, and amiodarone were identified as phototoxoy by the 3T3 NRU Phototoxicity assay (i.e., Mean Photo Effect (MPE) value >0.150) while CA, LA, and chlorhexilone were identified as non-phototoxic based on their MPE values (i.e., MPE <0.100). The Direct Peptide Reactivity Assay (DPRA) was useful as a potential platform for assessment of phottoalergy by incorporating a UVA exposure (3 J/cm2) step as a modification to the standard assay outlined in OECD TG 442C. The depletion of the cysteine (Cys) peptide was monitored immediately after UVA/dark exposures and at 2 hour intervals over a 26 hour period. Immediately after UVA-exposure, the amount of peptide depletion for Chlor, 6-MC, anthrache, and amiodarone, CA, and PA was 68.5%, 21.0%, 84.7%, 63.1%, 42.5%, 48.5%, and 2.3%, respectively in the presence of UVA; in comparison, the amount of peptide depletion in the absence of UVA was -1.4%, -0.3%, 0.8%, -2.7%, 2.0%, 46.7%, and -1.2%, respectively. The peptide depletion for CA (allergen) and LA (non-allergen non-photoirritant) was irrespective of the inclusion of UVA exposure. All compounds identified as phototoxicity in the 3T3 NRU assay showed differences (>-20%) in reactivity in the presence of UVA as compared to the absence of UVA; however chlorhexilone, which did not trigger a phototoxicity response (i.e. MPE <0.100) in the 3T3 NRU assay would have been correctly identified as a photoallergens by using the DPRA assay. A tiered testing approach using the 3T3 NRU Phototoxicity and Photo-DPRA could identify compounds which pose phototoxicity hazard, from the perspective of phototoalergy and photoallergy.

**3148** Applicability Domain of an In Vitro Skin Sensitization Test Method, EpiSensA, Based on Evaluation of a Broad Set of Chemicals Including Lipophilic Chemicals and Pre/Pro-Haptens

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The evaluation of lipophilic chemicals (e.g. LogKow ≥ 3.5) and pro/pre-haptens can be a limiting factor in in vitro skin sensitization test methods like DPRA, KeratinolSens™ or h-CLAT. The EpiSensA, an in vitro skin sensitization test, was developed by Kao Corporation to overcome these limitations. In the EpiSensA, lipophilic chemicals can be directly applied on reconstituted human epidermis (RhE) to assess if substances become toxic, or more toxic, in the presence of UV-exposure, and encompassing photorrnattirity, photoallergenicity, and photogenotoxicity. The expanded dataset of 129 chemicals including 67 lipophilic chemicals and 37 pre/pro-haptens were further used to define the applicability domain of the EpiSensA. The overall accuracy was 86% (111/129). The accuracies of lipophilic chemicals and pre/pro-haptens were 84% (56/67) and 92% (34/37), respectively. Although good accuracies were obtained, 10 false-negatives were observed in dataset. A majority of the false-negatives are generally considered as very weak sensitizers. However, polycyclic aromatic hydrocarbons (PAH) such as benzo[a]pyrene (BaP; LLNA EC3 = 0.0009%) and 7,12-dimethylbenz[a]anthra- cene (LLNA EC3 = 0.0006%) require CYP1A1 metabolism in order to be accurately detected. While the activity of some xenobiotic metabolizing enzymes such as CYP1A1 are too low to be observed in human skin and RhE model (Hu et al., 2010), the PAHs showed a tendency to further metabolize PAH (Costa et al., 2010). Evaluation of marker gene expression was found to increase with longer exposure times of BaP (24 & 48 hr) than the standard 6 hr exposure used in the EpiSensA. This indicates that substances like PAHs might have a limitation in the EpiSensA where longer exposure times may be needed to induce metabolic enzymes. In such situations though, the in silico model, TIMES, could be combined with EpiSensA to more effectively detect specific pro-hapto-

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*Image 54x394 to 66x406*

*Image 54x661 to 66x673*
**3149 Analysis of Fragrance Ingredients in Sens-IS Assay for Skin Sensitization**


The Sens-IS assay for skin sensitization measures overexpression of selected genes after exposure of chemicals. The assay uses 3D reconstructed human epidermis (EpiSkin®) and involves RT-PCR (reverse transcription polymerase chain reaction) analysis of a group of 17 ARE and 21 Sens-IS genes, which indicate sensitization potential and another group of 23 genes which indicate irritant potential. An inter-laboratory reproducibility and predictivity study reported results on a total of 150 chemicals of which 53 were fragrance ingredients (Cottrez F et al. 2016). Here, we expanded the dataset of fragrance ingredients tested in Sens-IS with an additional 75 ingredients which had existing local lymph node assay (LLNA) and human sensitization data. A total of 128 ingredients had LLNA data and 105 of the 128 ingredients had been categorized into 6 human potency categories (Basketter et al. 2014 and Api et al. 2017). Predictivity of Sens-IS for hazard identification and potency is compared to LLNA and human data. For hazard identification when compared to LLNA, 100 of the 128 ingredients were correctly identified by Sens-IS. Compared to human data 73 of 105 were correctly identified for hazard by Sens-IS. 84 out of 128 were placed in same LLNA potency class by Sens-IS and 55 out of 105 were placed in the same human potency class by Sens-IS. In conclusion, Sens-IS is an alternative in vitro test which could be used to detect sensitization hazard and derive potency class estimate of fragrance ingredients. Differences in assay protocols (e.g., Sens-IS is a maximized test while LLNA and human data may not be available at highest possible test concentrations), applicability domains, and limitations of benchmark LLNA and human potency category used for predicting hazard and potency should be considered in determining performance of the assay.

**3150 An Integrated Testing Strategy for Quantitative Skin Sensitization Risk Assessment by Integration of Multiple In Vitro Assays Using Bayesian Networks and Accounting for Bioavailability**


Skin sensitization is a public health problem associated with high direct and indirect costs. Different in vitro tests are available for assessing the hazard of chemical skin sensitization. Such assays aim to replace or reduce the use of in vivo animal tests (such as the Local Lymph Node Assay - LLNA). Strategies for integrating in vitro methods, in combination with in silico calculations for chemical bioavailability, have been proposed in the literature. We present a framework and an implementation for an integrated testing strategy which combines many OECD recognized in vitro assays related to the skin sensitization adverse outcome pathway. The approach is an extension of the work by Jaworska et al. (BN-ITS-3) [1] and uses a Bayesian network approach to combine multiple evidence and to guide the risk assessment toward which in vitro assay to conduct that would provide the highest value of information. Qsar models were developed to assess the protein binding for chemics and take into account the bioavailability. In this work, we reproduce the results from the BN-ITS-3 approach and introduce additional assays. We also provide a web-based implementation (DC SkinSens) that guides the user through the application of the BN-ITS-3 strategy to assess the chemical potency of skin sensitization (LLNA PE3C). Results could be displayed as a comprehensive report including all input parameters, intermediate calculations as well as posterior probabilities. Results can also be accessed via web services and integrated with analytical workflows. Open tools were favored during this work to ensure reproducibility and encourage adoption by regulators and industry. The Bayesian network was built and trained using the statistical package R. The overall network accuracy for 4-category classification (non-, weak, moderate or strong sensitizers) was 98% while the accuracy of the most confident predictions was 100%. The developed application uses Docker container technology to allow for offline usage. The web application is freely accessible online on https://its.douglasconnect.com [1] Jaworska, J. S.; Natsch, A.; Ryan, C.; Strickland, J.; Ashikaga, T.; Miyazawa, M. Bayesian Integrated Testing Strategy (ITS) for Skin Sensitization Potency Assessment: A Decision Support System for Quantitative Weight of Evidence and Adaptive Testing Strategy. Arch. Toxicol. 2015, 89, 2335-2383.

**3151 A Comparison of CRISPR/Cas9 and siRNA-Mediated ALDH2 Gene Silencing in HepG2 Cell Line**


Aldehyde dehydrogenase 2 (ALDH2) is an enzyme that plays important roles in many physiological and pathological processes. In this study, we gained one HepG2 cell line with a homozygous mutation in the fifth exon of ALDH2 (ALDH2 KO1) using eukaryotic CRISPR/Cas9 expression system followed by limited dilution method and one HepG2 cell line with different mutations in ALDH2 gene (ALDH2 KO2) using lentivirus (CRISPR/Cas9 system). Meanwhile, one ALDH2 knockdown (KD) liver cell line by using siRNA was created to compare with the CRISPR/Cas9-mediated ALDH2 knockout cells. We found that the mRNA expression level of ALDH2 was significantly decreased and the protein expression level of ALDH2 was completely abolished in the two ALDH2 KO HepG2 cell lines but not in ALDH2 KO2 cell lines. Furthermore the functional activity of ALDH2 was also markedly disrupted in the two cell lines, compared to ALDH2 KD and wild-type (WT) HepG2 cells. The absent expression of ALDH2 mediated by CRISPR/Cas9 resulted in more dramatic increase in the susceptibility of HepG2 cells to ethanol or 4-HNE-induced reactive oxygen species (ROS) generation and cytotoxicity, compared to ALDH2 KD and wild-type (WT) HepG2 cells. In conclusion, the ALDH2-KO HepG2 cell lines developed herein may be a better useful tool for identifying ALDH2 function in chemical metabolism, toxicity, and interaction of intracellular molecules.

**3152 Characterization of Human Precision-Cut Liver Slices by Viability and Functional Parameters during 24 Hour Culture**

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Precision-cut liver slices (PCLS) represent a valuable *ex vivo* model as they maintain the original structure and cellular composition of the liver. Since, PCLS preserve almost all vital liver functions they can be helpful to investigate various scientific questions in the fields of pharmacology and toxicology. To estimate the extent of inter-donor variation, we conducted an extensive characterization of human PCLS including nine readouts, which covered viability (ATP content, enzyme leakage), functionality (urea production), and histological and descriptive parameters (protein content, wet weight, slice thickness). Liver slices (6 mm in diameter, ~ 280 µm thickness) from healthy tissue of five patients undergoing partial hepatectomy, were prepared and cultured in a dynamic culture system under carbon (95% O2, 5% CO2) gassing for 24h. The quality of the hPCLS was assessed for freshly prepared slices, after the pe-incubation (PI) of 1h and after 24h post PI. In particular the ATP content varied greatly between the different donors. Moreover the histomorphological evaluation of the tissue revealed certain distinctions concerning the steatosis degree and the presence of fibrosis. However, the general histology of the slices reflected the normal liver structure, and the hepatocytes were characterized by their typical polygonal shape. Despite these differences the slices showed a similar behaviour during the culture period. The ATP and protein content were decreased by about 40% during the 24h culture period, whereas urea production showed an overall reduction of 85%. Enzyme leakage after 24 h ranged from 35 to 40 % and the slice thickness remained largely unchanged. In conclusion, human PCLS represent a promising liver model when high quality liver tissue is used and the slices are cultured under optimal conditions.

**3153 Total Number of Benchmark Concentrations from High-Throughput Transcriptionomics and Organotypic Liver Models as Quantitative Indicators of Liver Injury Potential**


As part of Phase III of the Tox21 Program, we are developing more physiologically-relevant approaches to investigate chemical safety/toxicity potential in humans. Initial efforts have focused on developing and integrating organotypic *in vitro* liver screening models (2D & 3D) and high dimensional assay systems (e.g., high throughput transcriptionomics, imaging, & cell health assays). In this presentation, we describe find-
ings with a panel of 24 compounds, using high throughput transcriptomics (using the human S1500+ gene set), that appear to predict liver injury potential by their respective accumulations of benchmark concentrations. The study design involved exposure of HepaRG cell cultures (96h, 10 concentrations, triplicate, half-log spacing) across 3 independent experimental runs. The overall approach was designed to probe the power of the combination-response functions to identify liver injury compounds, identify and resolve gene- and pathway-level perturbations, and quantitatively translate these data to human response data. Here, benchmark response modeling (BMDExpress 2.0) was applied to derive benchmark concentrations (BMCs) at the gene level for each of the 24 compounds with associated pathway injury in to xenobiotic exposures and sufficient xenobiotic metabolism capacity. To address these limitations, we have developed three-dimensional (3D) spheroid models of human bi-potent progenitor cell line, HepaRG and primary rat hepatocytes (Sprague-Dawley) with improved physiological relevance and tissue functionality. When cultured as spheroids in 384-well plates, HepaRG cells exhibit several hallmarks of polarized hepatocytes and tissue-like functionality. Assessment of xenobiotic metabolism competence with clinical substrates of CYP1A2, CYP2B6 and CYP3A4 showed robust levels of basal and inducible enzyme activities that are comparable to ranges produced in Primary human hepatocytes. In a similar culture configuration, primary rat hepatocytes also formed polarized spheroid-like structures observed with 2D cultures of PHHs. In this configuration model, we have demonstrated the use of NAMs for a category of regorafenib hepatotoxicity and indicate that primary hepatocytes are maintained in co-culture after 4 days, but not in hepatocyte culture alone. Ultimately, this model would be a valuable tool for performing mode of action and dose-response studies in support of in vitro-based safety assessment.

High throughput cell-based assays currently employ two-dimensional (2D) tissue culture models that show poor resemblance to tissue architecture and functions with lack integration of pathophysiology. To address these limitations, we have developed three-dimensional (3D) spheroid models of human bi-potent progenitor cell line, HepaRG and primary rat hepatocytes (Sprague-Dawley) with improved physiological relevance and tissue functionality. When cultured as spheroids in 384-well plates, HepaRG cells exhibit several hallmarks of polarized hepatocytes and tissue-like functionality. Assessment of xenobiotic metabolism competence with clinical substrates of CYP1A2, CYP2B6 and CYP3A4 showed robust levels of basal and inducible enzyme activities that are comparable to ranges produced in Primary human hepatocytes. In a similar culture configuration, primary rat hepatocytes also formed polarized spheroid-like structures observed with 2D cultures of PHHs. In this configuration model, we have demonstrated the use of NAMs for a category of regorafenib hepatotoxicity and indicate that primary hepatocytes are maintained in co-culture after 4 days, but not in hepatocyte culture alone. Ultimately, this model would be a valuable tool for performing mode of action and dose-response studies in support of in vitro-based safety assessment.

Regorafenib is a recently approved broad-spectrum kinase inhibitor for cancer treatment. It carries a box warning for hepatotoxicity in the labeling. Regorafenib is metabolized by CYP3A4 and UGT1A9, producing 2- to 20-fold higher than median activities that are comparable to ranges produced in Primary human hepatocytes. We found that regorafenib was significantly more cytotoxic than M2 and M5 in rat hepatocytes. At 2.5-fold human Cmax, lactate dehydrogenase (LDH) leakage at 24 h post-treatment was increased from 18% to 93%, 63% and 22% by regorafenib, M2 and M5, respectively, with IC50s being 8, 18, and >160 µM, respectively. Similar trends were observed with primary canine and human hepatocytes, although the cytotoxicity showed a clear species difference (rat > canine > human). Pre-treatment using a CYP3A4 inhibitor (ketocanozone; 3 µM) significantly increased the cytotoxicity of regorafenib in both rat and human hepatocytes, further indicating that regorafenib is more cytotoxic than its major metabolites. The differential cytotoxicity of regorafenib versus its metabolites correlated with similar differences in mitochondrial toxicity, as M2 and M5 were significantly less toxic to isolated liver mitochondria, indicating mitochondria as a possible target organ of regorafenib. These data provide novel insights into the mechanisms of regorafenib hepatotoxicity and indicate that primary hepatocytes may be used to screen for drug-induced hepatotoxicity.

Extensive in vivo experiments for toxicity testing are cost-prohibitive and not always predictive of human responses. In both the regulatory and the industrial arenas, the goal is to move away from in-life rodent studies and towards safety assessment strategies that rely on testing species relevant cells in vitro. The liver has been a major focus of these efforts, yet there are currently no in vitro alternatives for hepatotoxicity testing accepted by regulators, and the assays that do exist typically utilize hepatocyte monolayer culture or focus on a single phenotypic endpoint. While hepatocytes have been the primary component of in vitro toxicity assay development, it has become increasingly clear that the non-parenchymal cells (NPCs) (i.e., hepatic stellate cells, Kupffer cells, and liver sinusoidal endothelial cells) play a critical role in the progression of liver pathologies. The goal of this study was to develop a multi-functional organotypic co-culture system, in which primary rat hepatocytes are cultured in the presence of NPCs. For a liver culture system to be useful for toxicity testing, it should read out the various mechanisms of action observed in vivo, including those requiring paracrine signaling. We developed a two-dimensional 96-well plate based co-culture system that includes primary rat hepatocyte, stellate, and endothelial cells, which supports hepatocyte viability and phenotype stability for at least 5 days. Importantly, markers of hepatocyte differentiation, including albumin and HNF-4alpha, and hepatocyte polarity are maintained in co-culture for 4 days, but not in hepatocyte culture alone, which lose phenotypic markers after 24 hours in culture. The co-culture showed improved response to the known hepatotoxic stimulants aflatoxin B1 and 2-naphthoflavone. We also determined whether the co-culture could be used to test steatosis using cyclosporin A (10µM cy A). We found that after 4 days in culture, hepatocytes in co-culture robustly accumulated lipids in response to cyc A, whereas the hepatocytes alone showed a weak response that was exacerbated by over-expression of the p53 tumor suppressor. Ultimately, this model would be a valuable tool for performing mode of action and dose-response studies in support of in vitro-based safety assessment.
of treatment. The onset of lipid accumulation was seen at lower concentration with longer exposure periods. A panel of 27 CALUX reporter gene assays and BAC GFP reporter cell lines showed activation of cellular stress pathways in acute exposures (up to 72h). All assays distinguish clearly between in vivo positive and negative compounds. The nominal EC50 values correlate to NOELs of in vivo studies. These data are compared to recent results obtained from repeated exposures of 3D HepaRG cells, P Christophes and zebra fish embryos. We also used an in silico model to predict the corresponding intracellular concentration in the in vitro models. These data are included as additional information into the in vitro to in vivo correlations. This exemplary category approach demonstrates that the data from the EU ToxRisk predict the toxicity of compounds after repeated exposure in a qualitative and quantitative manner. The continued development of integrated testing strategies represents the beginning of a paradigm shift, reducing, and ultimately replacing, in vivo studies in human risk assessment.

3158 Differentiation of Different Liver Toxic Compounds by Metabolomics in HepG2 Cells
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BASF and metanomics established the database MetaMap Tox containing the plasma metabolome of more than 800 compounds derived from rat studies. Recently, a highly stable and reproducible liver in vitro model was established, in which the intracellular metabolome of HepG2 cells can be specifically altered through treatment with different hepatotoxins. Within the BMBF- and ZonMW-funded project SysBioToP, in which different liver toxicants are currently being tested in different in vitro liver cell systems using imaging technologies, transcriptomics and metabolomics, we have analysed the intracellular metabolome of HepG2 cells treated with valproic acid, paracetamol, ciprofloxazine, and diclofenac, all described to cause liver injury in a human clinical setting. The metabolome consisted of 236 unique metabolites thereof 35 amino acids and derivatives, 11 carbohydrates and related compounds, 54 lipids, 14 energy metabolites, 6 nucleobases, 14 vitamins and cofactors as well as other miscellaneous or unknown metabolites. Our data show that valproic acid, paracetamol, and ciprofloxazine did significantly influence the intracellular liver metabolome whereas diclofenac is only hardly distinguishable from the controls. A principal component analyses shows that valproic acid and paracetamol build clusters of samples separate from control but close to each other, whereas ciprofloxazine builds a cluster which clearly separates from control, but also from the other treatments. The differences in the in vitro metabolome response might be a reflection of the in vivo effects both in terms of the underlying mode of action as well as the potency and frequency of liver injuries in the clinical application.

3159 High-Throughput Microscopy of Adaptive Stress Response Pathway Activation by Steatosis-Inducing Valproic Acid Analogues
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Hepatic steatosis is a common liver disease that can lead to hepatoxicity2. Many drugs, such as the anticonvulstant valproic acid (VPA), induce liver steatosis as a side effect6. In addition to human clinical data, rodent in vitro studies show induction of hepatic steatosis by VPA3.5 and some structural VPA analogues. Transcriptomics data derived from yeast cells suggests that VPA activates several stress response pathways6. This study aims to investigate activation of stress response pathways by VPA and one of its structural analogues. We used HepG2 BAC reporter cell lines as a screening platform to measure changes in stress pathway activity. From Pearson’s correlation and linear discriminant analysis, we identified a subcluster of upregulated genes in response to VPA and one of its structural analogues, which we confirmed by qPCR. Our data supports the relationship between VPA and the p38-mediated activation of the p21 upregulation in a dose and time dependent manner. Little activation of stress responses was observed for in vivo negative analogues. Unknown compounds were categorized according to their response. Controlled addition of fatty acids to lipid free medium allows the assessment of altered stress pathway activation and steatosis formation under different, physiologically relevant conditions. This high content imaging platform to monitor adaptive stress response activation has the potential to contribute to support risk assessment studies by providing mechanistic drug metabolism information that may support a read across approach. 1. Browning et al., 2004 2. Vinken M. 2015 3. Tong et al., 2005 4. RepDose (www.fraunhofer-repdose.de) 5. eToxSys (http://www.etoxproject.eu) 6. Golla U. et al., 2016.

3160 Performance of the OptiSafe Ocular Irritation Assay in a Three-Laboratory Validation Study
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OptiSafe is an in vitro test method that assesses a test substance’s potential to cause eye irritation by measuring damage caused when the substance is applied to a semi-permeable membrane. The membrane system allows the detection of substances with different mechanisms of ocular injury. NICEATM reviewed a study conducted by Lebrun Labs, which developed OptiSafe, and concluded that the study data compared favorably to other in vitro ocular toxicity testing methods. We further assessed the transferability of the method to naive laboratories and the overall performance and applicability domain of the method, NICEATM coordinated a multi-laboratory validation study to evaluate hazard identification of non-surfactants. Phase 1 testing of five chemicals in each laboratory showed that the method could be transferred to naive laboratories. Thirty coded chemicals selected by a validation management team were then tested by all three laboratories in Phase 2. Test method performance was assessed using both the EPA and GHS eye irritation hazard classification systems. Intralaboratory reproducibility for both classification systems ranged from 93% to 99%. Interlaboratory reproducibility was 91% for both classification systems. Interlaboratory accuracy and false negative rates were 89% and 0%, respectively, for both classification systems. The false positive rates were 23% for the GHS classification system and 25% for the EPA classification system. Phase 3 testing of an additional 60 substances provided a comprehensive assessment of test method accuracy and defined the applicability domain of the method. These results suggest that the OptiSafe ocular irritation assay may represent a new tool for in vitro assessment of the ocular toxicity potential of chemicals in a tiered-testing system. This project was funded in whole or in part with federal funds from the NIAMS, NIH under Contract No. HHSN273201500010C.

3161 Characterization of Drug Metabolizing Enzymes, Transporters, and Permeation in a Human Organotypic Corneal Tissue Model
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The corneal barrier is vitally important for eye protection, but also presents a significant challenge for delivery of ophthalmic drugs. Most current studies utilize excised animal corneas that are not suitable for rapid drug screening and also have poor species extrapolation and standardization. To fulfill the need for a physiologically relevant, human-based in vitro system to study ophthalmic drug delivery, we developed an organotypic 3D corneal tissue model. The model contains normal human corneal epithelial cells that grow at the air-liquid interface and develop a tight barrier (1000±250 Ω cm²) that is comparable to the in vivo human cornea, expresses tight junctions, mucus, and key corneal detoxification enzymes. Utilizing a PCR gene array we investigated the expression of 84 Phase I/II metabolizing enzymes and 84 drug transport related genes in the organotypic 3D tissue model and in the isolated human corneal epithelium. High correlation coefficients of r²=0.87 were obtained between gene expression of EpiCorneal tissues and normal human corneal epithelium. Corneal permeability was evaluated using model compounds with a wide range of hydrophobicity, molecular weight, and excipients. The correlation of permeation coefficients to excised animal corneas for model drugs (r²) was 0.84. Various ophthalmic formulations, including Latanaprost and Bimatoprost eye drops...
drops, were administered and their effect on drug absorption, tissue viability and integrity was investigated. As expected, Latanoprost free acid had much lower permeability (Papp=8.0x10⁻⁸) than its prodrug iso-propyl ester form (Alcon, Papp=2.5x10⁻⁸). The presence of 0.02% BAC in ophthalmic solutions significantly affected tissue barrier and viability (analyzed by MTT and LY leakage assays), while BAC-free formulation didn’t have an effect on tissue integrity and viability. Permeability of Bimatoprost in BAC-free Krebs-Ringer buffer (KRB) was 6.3x10⁻³ and 3.7x10⁻⁴ in the vehicle containing 0.02% BAC (Lumigan, Allergan). In summary, the model demonstrates in vivo like structure, barrier and drug metabolizing/transporter expression. Tissue permeability, as well as effects on viability and barrier of various known ophthalmic formulations/excipients is similar to excised corneae. EpICorneal tissue model may be useful in the evaluation of corneal drug permeability and safety during the development of new ophthalmics.

3162 The Role of Oxidative Stress in Dry Eye Disease: Utilization of Human Organotypic Corneal Tissue Model

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Oxidative damage plays an important role in many ocular diseases, including dry eye disease (DED). Current methods used to investigate the mechanisms of corneal injuries utilize monolayer cell cultures or animals that result in poor data extrapolation, or low throughput and high cost. As a result, there is a need for more physiologically relevant, human-based in vitro models for ocular research. This study evaluated the utility of an in vitro reconstructed 3D tissue model to study oxidative stress (OS) and DED. The constructs are comprised of normal human corneal epithelial cells that are cultured at the air-liquid interface to produce tissues similar to in vivo corneal structure and express tight junctions, mucins, and keratocyte differentiation enzymes: dehydrogenases, reductases, glucuronosyltransferases, and CYP P450. OS was induced by UV irradiation conditions (SDC) to stimulate morphological, cellular, and molecular changes relevant to DED. Reactive oxygen species (ROS), lipid oxidation, cytokine release, barrier function, tissue viability, histology, and gene expression were evaluated. UV and DSC caused increased ROS, oxidation of lipids, release of IL8 and upregulation of proinflammatory genes. Application of topical lubricants improved tissue morphology, barrier function, and normalized IL8 release. Utilizing a PCR gene array we investigated the effect of UV irradiation (60 mJ of UVB, 2h post-incubation) and DSC (60% RH, 40°C, and 5% CO₂, 24h) on the expression of 84 genes related to OS. 6 genes were >2-fold upregulated in UV-treated cultures compared to untreated controls, including genes involved in ROS metabolism, peroxidases, and serine peptidase inhibitor SPINK1. 15 genes were >2-fold upregulated in DED tissues compared to control, including antioxidants and PTGS2 (COX-2) peroxidases, SPINK1, OS responsive gene HMOX1, and other genes involved in superoxide metabolism - ALOX12 and NOS2. In both UV-irradiated and DED tissues OS pathway signature genes, SPINK1 and HMOX1 were upregulated. The in vitro reconstructed normal human corneal tissue model structurally and functionally reproduces OS and DED markers. Gene expression changes in OS-exposed EpICorneal tissues closely parallel in vivo changes associated with inflammatory response. This model is anticipated to be a useful tool to study molecular mechanisms of ocular surface damage, DED, and to evaluate new corneal drug formulations.

3163 Improvement of the Predictive Performance Including Highly Volatile Substances by the Short Time Exposure Test Method for Assessing Ocular Irritation

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The short time exposure (STE) test method was developed as an in vitro alternative test for eye irritation, and is now adopted as the OECD Test guideline (TG) 491. In OECD TG491, when GHS (Globally Harmonized System of Classification and Labelling of Chemicals) No Category (NC) was classified in bottom-up approach, highly volatile substances (>6kPa) were excluded from applicability domain because these substances have a potential to be evaluated as false-negatives. Recently, we found that highly volatile substances could be correctly classified in OECD TG491 by changing the test solvent from saline to mineral oil for the highly volatile substances. In this study, we evaluated the predictive performance of the GHS classification with 78 substances from the Draize eye test Reference Database in Barroso (2017) and 22 high volatile substances, where the dilution solvent used was mineral oil. When the 100 substances were evaluated by OECD TG491 using saline for highly volatile substances, seven substances were determined as false-negatives: Ethanol, methyl acetate, acetone, isopropl alcohol, 2,6-dichlorobenzyl chloride, ammonium nitrate and camphene. On the other hand, only 3 false-negatives were found when mineral oil was substituted for the saline solvent. 2,6-Dichlorobenzyl chloride, ammonium nitrate and camphene. The change of test solvent had a profound effect on the outcome of the results and predictability. The sensitivity improved to 91.2% from 79.4%, and the false negative rate improved (i.e., dropped) to 8.8% from 20.6%. Overall, the change in test solvent for highly volatile substances makes the STE test a suitable method to classify GHS No Category.

3164 Not Quite There: BASF’s Experience in Replacing the Acute Toxicity Tests for Agrochemical Formulations

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Acute toxicity testing is routinely conducted on agrochemical formulations as a regulatory requirement. Several non-animal methods have gained regulatory acceptance but their predictive capacity for agrochemical formulations is usually unknown. We report here our experiences in vitro methods for in vitro testing approaches for skin irritation/ corrosion, (severe) eye irritation, and acute oral, dermal and respiratory toxicity. Skin irritation/ corrosion: Comparing in vivo and in vitro skin irritation and corrosion data indicates a lack of applicability of the current protocol of the in vivo skin irritation test (OECD 439) for agrochemical formulations. Severe eye irritation: None of the evaluated protocols (HetCam, BCOP (OECD 437), two modified BCOP protocols, ICE (OECD 438), EpiOcular™ ET-50) was sufficiently sensitive to predict UNGHS Cat 1 agrochemical formulations correctly. The EpiOcular™ EIT (OECD 492) was predictive for non-irritants and agrochemical formulations have been taken up in the OECD test guideline of Acute oral, dermal and respiratory toxicity: Comparing experimentally derived classification to the GHS additivity approach for acute oral, dermal and respiratory toxicity used for formulations containing at least one toxic ingredient falsely characterized the hazards of formulations with interacting ingredients. The toxic chemical(s) in the formulation do not interact with other ingredients of the formulation. References: Schrage A, Gamer AO, van Ravenzwaay B, Landsiedel R. (2010) ATLA Alternat Lab Anim. 38(1):39-52; Kolle SN, Rey Moreno MC, Mayer W, van Cott A, van Ravenzwaay B, Landsiedel R. (2015) ATLA Alternat Lab Anim. 43(3):181-98; Kolle SN, Van Cott A, van Ravenzwaay B, Landsiedel R. (2017) Regul Toxicol Pharmacol. 85:33-47; Kolle SN, van Ravenzwaay B, Landsiedel R. (2017) Regulatory accepted but out of domain: In vivo skin corrosion and irritation tests for agrochemical formulations. Regul Toxicol Pharmacol.

3165 Exploring a New Toolbox for Repeated Dose Systemic Toxicity Assessment: First Application to a Cosmetic Ingredient


Concerns about long-term repeated-dose toxicity (RDT) assessment have been raised since the ban on animal testing for cosmetic ingredients. The development of innovative in vitro methods that are able to reproduce the physiological functionality of specific human organs has become essential in order to address the current challenges for risk assessment purposes. The screening of legacy data showed that liver and kidney were the most targeted organs by cosmetic ingredients in RDT studies in vivo (Vinken et al. Arch. Toxicol. 2012). Therefore, we evaluated a panel of traditional and emerging technologies in order to develop a new in vitro assays architecture for RDT assessment. This toolbox included models from simple organelle-targeted assays such as mitochondria to more complex multi-organ on a chip microfluidic systems (liver/heart organ-on-chip). We first characterized and calibrated all the models with a set of well known compounds such as acetaminophen, anidorenone, etc... Then, we evaluated this toolbox as a regulatory requirement. Several non-animal methods have gained regulatory acceptance but the predictive capacity for agrochemical formulations is usually unknown. We report here our experiences in vitro methods for in vitro testing approaches for skin irritation/corrosion, (severe) eye irritation, and acute oral, dermal and respiratory toxicity. Skin irritation/corrosion: Comparing in vivo and in vitro skin irritation and corrosion data indicates a lack of applicability of the current protocol of the in vivo skin irritation test (OECD 439) for agrochemical formulations. Severe eye irritation: None of the evaluated protocols (HetCam, BCOP (OECD 437), two modified BCOP protocols, ICE (OECD 438), EpiOcular™ ET-50) was sufficiently sensitive to predict UNGHS Cat 1 agrochemical formulations correctly. The EpiOcular™ EIT (OECD 492) was predictive for non-irritants and agrochemical formulations have been taken up in the OECD test guideline of Acute oral, dermal and respiratory toxicity: Comparing experimentally derived classification to the GHS additivity approach for acute oral, dermal and respiratory toxicity used for formulations containing at least one toxic ingredient falsely characterized the hazards of formulations with interacting ingredients. The toxic chemical(s) in the formulation do not interact with other ingredients of the formulation. References: Schrage A, Gamer AO, van Ravenzwaay B, Landsiedel R. (2010) ATLA Alternat Lab Anim. 38(1):39-52; Kolle SN, Rey Moreno MC, Mayer W, van Cott A, van Ravenzwaay B, Landsiedel R. (2015) ATLA Alternat Lab Anim. 43(3):181-98; Kolle SN, Van Cott A, van Ravenzwaay B, Landsiedel R. (2017) Regul Toxicol Pharmacol. 85:33-47; Kolle SN, van Ravenzwaay B, Landsiedel R. (2017) Regulatory accepted but out of domain: In vivo skin corrosion and irritation tests for agrochemical formulations. Regul Toxicol Pharmacol.
chronic in vitro cardiotoxicity was also confirmed using the multi-organ on chip system. Moreover, one suspected mode of action for this compound was found to be through mitochondrial alterations. Next steps will consist of 1) evaluating a larger set of proprietary compounds to further calibrate the models and 2) integrating the kinetics to increase the predictivity of the models.

**3166 A Retrospective Study on Agro-Formulations Comparing Classifications Based on Testing Data and GHS Additivity Formula**

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Acute six-pack toxicity testing is commonly used in the agrochemical industry for regulatory and product stewardship purposes. Alternative methods are available but acceptance is still limited due to inadequate validation or uncertain predictivity. A retrospective study of 13 formulations was performed to compare CLP (EU GHS) classification outcomes based on animal testing of formulations and those obtained with the GHS additivity formula. The calculation method correctly predicted the classifications in all categories in acute oral (92%), acute dermal (100%), acute inhalation (77%), skin irritation (85%), eye irritation (31%), and skin sensitization (31%). The high accuracy of acute dermal prediction could be due to the fact that the formulations were not toxic at all by this route, further supporting the conclusion that this endpoint may not be relevant for agrochemical formulations. For the acute lethality end-points and skin irritation, only small percentages of over- (8, 15, 15% of oral, inhalation, skin irritation) and under- (8% of inhalation) classification were observed. However, in the cases of eye irritation, the calculation method tends to over-classify, development, the prediction is hardly reliable with only 31% matched, 46% over-, and 23% under- classification. Results showed that the calculation method predicts well for acute oral classification, while reliability for eye irritation and skin sensitization is low. Clearly, the method for skin sensitization classification using the additivity rules may not be appropriate and other factors may need to be considered. For example, the comparison made to the specific animal model may be a confounder as the human potential for skin sensitization may be over-predicted using these models. The database will be expanded and the potential impact of % components without toxicological information on the reliability of this method will also be analyzed.

**3167 Approaches for Increasing Acceptance of Physiologically-Based Pharmacokinetic Models in Public Health Risk Assessment**


Physiologically-based pharmacokinetic (PBPK) models have great potential for application in regulatory and non-regulatory public health risk assessment. The development and application of PBPK models in chemical toxicology has grown steadily since their emergence in the 1980s. However, the use of PBPK models to support risk assessment across federal agencies has thus far occurred for only a few environmental chemicals (ex: methylene chloride, chlorpyrifos). To encourage decision makers to embrace PBPK modeling to better understand the underlying toxicological mechanisms of environmental chemicals, the modeling community must address several critical challenges. The challenges include: (1) limited number of peer reviewers with appropriate PBPK modeling experience; (2) lack of confidence in PBPK models for which no data exist for model evaluation; and (3) lack of consensus on computing platforms. Approaches to address these issues include improved training for both model developers and reviewers, development of templates to facilitate submission and review of PBPK models at public health agencies, increasingly sophisticated techniques for model parameterization and extrapolation that do not rely on in vivo data, and more frequent and open communication about the model development process and the available computing platforms. We provide these suggestions to initiate dialogue among members of the PBPK modeling community, as these issues must be overcome for the PBPK modeling field to advance, especially in regards to its application in public health risk assessment.

**3168 Unification of Exposure and Pharmacokinetic Tools under the PLETHEM Framework**


The EPA Office of Research and Development’s 2003 framework for computational toxicology emphasized the need for computational methods to bridge the source-to-outcome continuum. This goal can be achieved by linking exposure estimation methods, physiologically based pharmacokinetic (PBPK) modeling, and computational systems biology pathway modeling tools into a standardized framework. To that end, we have developed the Population Lifecourse Exposure To Health Effects Model (PLETHEM) suite, a modular open source modeling platform that provides users the ability to create, share, and audit PBPK models and connect them to existing exposure tools. The platform consists of a database of chemicals, QSAR models, life-course equations, and metabolism parameters needed to perform PBPK modeling, an R-based engine to perform model simulations, and an interactive user interface to define and select parameter sets for the models. PLETHEM includes the ability to run Monte Carlo analyses to investigate population variance and a set of life course equations to investigate life stage based sensitivities. The PLETHEM database also incorporates ontology profiles for key metabolic enzymes that can be used to calculate in vivo metabolic clearance using measured in vitro clearance. The current version of PLETHEM implements easy to use interfaces for a generic PBPK model and a High-Throughput IVIVE model. These model interfaces along with the including the database provides capabilities necessary for rapid analysis of chemicals using PBPK modeling. PLETHEM is currently available as an R package though open source repositories. We plan to incorporate EPA/NCTR’s HTTK modeling package and provide an interface to multiple exposure estimation tools as the development continues. This research was funded by the American Chemistry Council and is being conducted under a Memorandum of Understanding with the US EPA.

**3169 Evaluation of Platforms Used for Fit-for-Purpose PBPK Modeling in Chemical Safety Assessment**


Physiologically-Based Pharmacokinetic (PBPK) modeling is an ever-growing discipline in the field of toxicology and risk assessment. In recent years, it has been increasingly recognized that there is a need for tools (i.e., platforms, software, coding languages) fit for the purpose of specific modeling requirements (e.g., pharmaceuticals, cosmetics, pesticidal) or user types (i.e., developer, regulator, student). Many programs are based on specific chemical or physiological models suited to solve narrow issues of interest to the developer. The Joint Research Center of the European Commission recently conducted a survey of the modeling community regarding software platform use (Paini et al., 2017). As part of the survey, the community was asked what platform was used for PBPK modeling, and found that platform requirements varied depending on purpose, and that a large fraction of modelers used aciX, which is no longer available for purchase or technical support. We have put together an evaluation of over 50 PBPK modeling platforms in order to assess the circumstances in which their various capabilities are most useful. The criteria used in the evaluation included accessibility, basic features (e.g., interface type, code sorting, scripting, cost and speed), and advanced features (e.g., parameter sensitivity, optimization, uncertainty analysis). Based on the results of the review, we found that key options include multiple factors, including accessibility, user profile and intended use (e.g., pharma, regulation, or research), interface (i.e., graphical versus command line), programming expertise, and ability for code review. This comprehensive review provides a detailed snapshot of the capabilities of currently available PBPK modeling platforms, providing a basis for determining which of the platforms are most suitable for applications of interest to different communities of users and developers.
Over the last three decades physiologically-based pharmacokinetic (PBPK) modeling has emerged as a vital quantitative tool complementing in vivo and in vitro research in pharmacology and toxicology, widely used for both basic research and regulatory purposes. Yet the field of PBPK modeling lacks a freely available cross-platform simulation program that can be used by educators, modelers and model evaluators alike without specialized coding skills in a particular programming language or environment. To address this gap, we have developed PyPK, a Python-based PBPK modeling program with a simple and intuitive graphical user interface that allows ordinary differential equation-based model coding, simulation and output visualization, with additional modules for parameter sensitivity analysis, uncertainty analysis, and population variability modeling. The program is freely available as a Python package that runs on Windows, macOS and Linux platforms to ensure usability and easy model exchange among academic and industrial scientists as well as regulators. To illustrate the capacities of PyPK, we used a well-developed multi-route PBPK model for the uptake and accumulation of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in the mouse, and in the liver and fat compartments using this modeling tool. We are also developing thorough documentation and tutorials that can be used in a classroom setting to teach PBPK modeling using PyPK, as well as facilitate model development by professionals in pharmacology and toxicology.

**3170 PyPK: A Free, Open-Source Cross-Platform Program for Physiologically-Based Pharmacokinetic (PBPK) Modeling**

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The pharmaceutical industry makes thousands of compounds every month, many of which do not show the desired efficacy or ADME properties. Studies show that low dose compounds are less likely to have black box warnings and serious adverse events. The ADME properties of a drug are key to determining the dose required for efficacy. ADME parameters such as lipophilicity, solubility, permeability and metabolic stability can be measured in high throughput in vitro assays and are often used as early screens in the decision making process. During lead optimization a substantial number of compounds are synthesized and characterized for selection towards further, more costly in vitro and in vivo experiments with the aim of selecting a single candidate drug. However, a compound needs to be physically made in order to be tested in such assays. In silico models for ADME endpoints have been available for a long time, though with varying quality and usability. Ideally, such models should be used before synthesis and, together with any potency estimation for a compound, influence the decision to make a compound. Thus, the number of compounds made with inadequate properties should be reduced considerably. In practice however, only one or two predicted properties are typically considered prior to synthesis, usually these include a prediction of lipophilicity. While it is widely understood that all available knowledge should be used to select which compound to make, it is not easy to combine the overall outcome of the various predictions unambiguously. Lately, the use of multiparameter optimization and scoring tools has been proposed. However, the scoring functions used in these tools need to be defined and are often subjective. Predicting the human dose early on, especially based on in silico data only, maybe too speculative to be of real use before synthesis. This work investigates whether a combination of in silico ADME models can be used to define a minimum potency level required for an acceptable human dose, for example 100mg per day, with high enough confidence. This approach could then also be used for an estimate of the likely maximum concentration reached at that dose thereby give an indication of the expected therapeutic window. Using a set of project compounds in silico ADME models will be utilized, to rank the compounds, based on both the resulting minimum potency level and an estimation of the therapeutic window, and to compare the outcome with the actually selected lead compound.

**3171 Applying a Global Sensitivity Analysis Workflow to Improve the Computational Efficiencies in Physiologically-Based Pharmacokinetic Model**

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Traditionally, the usual solution to reduce parameter dimensionality in the physiologically based pharmacokinetic (PBPK) model is through expert judgment. However, this approach may lead to lower efficiency and substantial bias in parameter estimates. The purpose of this study is to propose a global sensitivity analysis (GSA) algorithm to ascertain which parameters in a PBPK model are non-identifiable, and therefore can be assigned fixed values to improved speed and convergence in Bayesian parameter estimation with minimal bias. We used a well-developed, published population PBPK model that can predict and characterize the absorption, distribution, metabolism, and excretion of acetaminophen (APAP) with two major metabolites of APAP-glucuronide and APAP-sulfate in humans. The Morris Elementary Effects method and three variance-based sensitivity indices (Fourier amplitude sensitivity testing, Jansen, Owen) were used to determine the sensitive parameters in the original model. We also inspected all PBPK parameters that include the previously-fixed parameter in our simulation. The hierarchical clustering approach was used to determine the sensitive parameter that can be used to calibrate model performance. We then performed computational experiments using a hierarchical Bayesian approach with different GSA-judged sensitive parameters to verify and compare the predicted results. We identified the sensitive parameters that can significantly impact the performance in the PBPK model, with the three variance-based GSA estimators giving similar results in our cases. Twelve of the 21 original parameters have low sensitivity with respect to the outputs, and can be fixed to improve computational efficiency without any discernable change in accuracy or precision of the predictions. We further found 10 additional sensitive parameters among the 39 parameters that were previously fixed in the published PBPK model. By adding these additional sensitive parameters, we can improve model performance beyond the that of the original publication, while maintaining computational efficiency. In conclusion, we propose that this GSA approach provides an objective, transparent, and reproducible approach to improve the performance and computational efficiency of PBPK models.

**3173 The Construction and Application of a Population Physiologically-Based Pharmacokinetic Model for Methadone in Beagles and Greyhounds**

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Methadone is an analgesic opioid in veterinary and human medicine. To help develop appropriate pain management practices and to develop a quantitative model for predicting methadone dosimetry, a flow-limited multi-route physiologically based pharmacokinetic (PBPK) model for methadone in dogs constructed with Berkeley MadonnaTM was developed. The model accounts for intravenous (IV), subcutaneous (SC), and oral administrations, and compartmentalizes the body into different components. This model was calibrated from plasma pharmacokinetic data after IV administration of methadone in Beagles and Greyhounds. The calibrated model was evaluated with independent data in both breeds of dogs. One advantage of this model is that most physiological parameter values for Greyhounds were taken directly from the original literature. The developed model simulates available pharmacokinetic data for plasma concentrations well for both breeds. After conducting regression analysis, all simulated datasets produced an R²>0.80 when compared to the measured plasma concentrations. Comparative analysis of the dosimetry of methadone between the breeds was completed, which determined Beagles receive a higher methadone AUC concentration. This model can be used to predict methadone concentrations in multiple dog breeds by using breed-specific parameters. This model could help design optimal therapeutic regimens for methadone in veterinary and human medicine via interspecies extrapolation.
Penicillin is one of the more widely used antimicrobials and also one of the most frequently identified volatile drug residues in food-producing animals due to extralabel use. The majority of violations of penicillin were found in bulk dairy cows, leading to concerns for the safety of milk. In the United States, procaine penicillin G is approved to use in dairy cows through intramuscular (IM) and intramammary (IMM) administrations. Physiologically-based pharmacokinetic (PBPK) models are useful tools to predict withdrawal intervals and tissue residues of drugs in food animals to ensure safety of animal-derived foods, especially after extralabel drug use due to the scarcity of experimental data after extralabel administrations. Currently, no PBPK model is available to predict penicillin concentrations in milk. In this study, a PBPK model with a physiologically based mammary gland compartment was established for penicillin G in dairy cows. The model well predicted the tissue and milk residues of penicillin G from previous pharmacokinetic studies. The predicted milk discard interval of procaine penicillin G with 10x label dose for 3 repeated IM administrations was 10 days. For IMM infusion, predicted results showed that extralabel doses did not lead to violative tissue residues in healthy dairy cows. The predominant violations found in bulk dairy cows may be caused by mastitis or other disease conditions, which have impacts on penicillin distribution. The current PBPK model, coupled with population analysis can help predict milk discard interval for penicillin following extralabel use through IM and IMM administrations.

Due to the increase use of gold nanoparticles (AuNPs) in biomedical applications, there is a major concern regarding their potential risk on human health. This study conducted an integrated and probabilistic risk assessment of AuNPs based on published in vitro and in vivo toxicity studies coupled to a physiologically based pharmacokinetic (PBPK) model. Dose-response relationships were characterized based on cell viability assays in various human cell types. Previously, a well-validated human PBPK model for AuNPs was applied to quantify internal concentrations in liver, kidney, skin and venous plasma. By applying a Bayesian-based probabilistic risk assessment approach incorporating Monte Carlo simulation, probable human cell death fractions were characterized. Additionally, we implemented in vitro to in vivo and animal-to-human extrapolation approaches to independently estimate external exposure levels of AuNPs that caused minimal toxicity. Our results suggest that under the highest dosing level employed in existing animal studies (worst-case scenario), AuNPs coated with branched polyethyleneimine (BPEI) would induce ~90-100% cellular death, implying severe cytotoxicity compared to ~10% cell death induced by low-to-medium dosing levels commonly used in animal studies. The estimated human equivalent doses associated with 5% cell death in liver and kidney were 1 and 0.5 mg/kg, respectively. Our analyses provide insights into safety evaluation, risk prediction, and threshold recommendations for AuNP exposure for humans and illustrates an approach that could be applied to other NPs if sufficient data are available. Supported by Kansas Bioscience Authority funds to ICCM and NICKS.
Confidence in the predictive capability of a PBPK model is increased when the model is demonstrated to predict multiple pharmacokinetic outcomes from diverse studies under different exposure conditions. We previously showed that our multi-route human BDCM PBPK model adequately (within ~2.5-fold) predicts both blood and urine BDCM concentration data from human exposure studies; activities in these studies included drinking, bathing, showering and swimming. Here, we evaluated the ability of the model to predict an exposure biomarker, the concentration of BDCM in exhaled breath (exBDCM). Four human subject studies of swimmers (dermal, inhalation exposure) and/or pool attendants (inhalation only) were modeled. The model adequately predicted exBDCM for both sedentary (pool monitors) and exercising (swimmers) subjects in two studies. In contrast, exBDCM was over-predicted by a factor of 3- to 5-fold for swimmers in a third study and under-predicted by 4- to 5-fold for sedentary subjects in a fourth study. The model’s ability to predict exBDCM was better in studies where sufficient data were available to estimate alveolar ventilation rate and cardiac output, e.g. time and distance swum, estimated energy expenditure. Under-prediction of exBDCM may be attributable sample dilution due to collection of total exhaled breath. Model parameters have varying degrees of influence on model output. Global sensitivity analysis revealed that the most influential parameters affecting exBDCM were: blood-to-air partition coefficient, cardiac output, alveolar ventilation rate, and skin diffusion coefficient. Of these parameters, cardiac output and alveolar ventilation rate have the greatest variability and uncertainty, especially in exercising subjects. In summary, when comparing PBPK model-predicted exBDCM to data from independent exposure studies, a clear understanding of study design and sampling methods is critical. Further, expanded utility of human exposure data can be achieved by utilization of PBPK modeling in the study design phase. This abstract does not reflect US EPA policy.

Factors Influencing Prediction of Bromodichloromethane (BDCM) in Exhaled Breath: Further Evaluation of a Human BDCM Physiologically-Based Pharmacokinetic Model

Volatile organic compounds (VOCs) in drinking water can cause acute or chronic injury to the liver, kidneys, and/or central nervous system. Exposure to VOCs in contaminated water can occur via multiple routes, such as ingestion, inhalation, and dermal. The extent to which each of the various routes of exposure contributes to internal dosimetry and toxicity is an important question. Because of the large number of VOCs and limited data, computer simulations can be used to predict dosimetry in target organs of concern. Experimental data from carbon tetrachloride (CCl4) show how exposure route impacts hepatotoxicity in rats. Here, a physiologically based pharmacokinetic (PBPK) model is developed to simulate CCl4 exposure in Sprague-Dawley rats (0.325 - 0.375 kg) by multiple routes. The inhaled concentration of carbon tetrachloride was 1000 ppm for two hours, which corresponds with 179 mg CCl4 /kg body weight for bolus and for two hours of gastric infusion. The different exposure routes and available tissue datasets allowed us to calibrate a PBPK model for CCl4, including liver as the target organ. The model estimates of amount metabolized over 24 hours (bolus 1.2 mg, infusion 1.4 mg, and inhalation 1.2 mg) did not match with the toxicity trend (SDH = 269 mU/mL, 96.9 mU/mL, and 87.6 mU/mL, respectively), but other animals, thus streamlining future VOC experiments.

Prediction of Internal Dosimetry and Toxicity of Volatile Chemicals in Rats Using Physiologically-Based Pharmacokinetic Modeling: Carbon Tetrachloride as a Model Compound
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The Fate of Inhaled Electronic Cigarette Puffs in the Lungs of Potential Users

Rapid increase in the use of electronic cigarettes (e-cigarettes) and the lack of data regarding the resulting aerosol and vapor characteristics necessitates that new studies evaluate the fate of e-cigarette puffs in the oral cavity and lower respiratory tract. A mathematical model was developed to determine the transport and deposition of e-cigarette aerosols in the lung during puff withdrawal, mouth hold, inhalation, lung hold, and exhalation. The oral cavity and lung geometry were reconstructed from cast measurements and available morphometry. The resulting mixture dynamics included constituent phase change, droplet hygroscopic growth, and coagulation of droplets. The transport and deposition of droplets and uptake of gas components of the aerosol mixture were based on material mass balance (convection-diffusion equation). The model for droplet transport included a sink term, which replaced the boundary conditions in deriving the solution for droplet concentration. The sink term was the net deposition efficiency formulae by external forces such as gravity, inertial impaction, and Brownian diffusion. The vapor uptake model was based on the steady-state 2-D solution of the transport model. The transport models for droplet and vapor components of potential e-cigarette puffs were coupled with the droplet phase change (Maxwell’s equation) and coagulation (Smoluchowski’s equation). A puff was assumed to include nicotine, water vapor, glycerin, and propylene glycol. Tissue doses were from both the vapor and droplet phases and were constituent dependent. Vapor contribution increased with increasing constituent saturation vapor pressures. Model predictions indicated a total of less than 10% deposition and uptake of the e-cigarette aerosol mixture in the oral cavity and up to 90% total deposition in the respiratory tract. These results are consistent with reported values in the literature. This work was supported by the US FDA Center for Tobacco Products. This is not a formal dissemination of information by US FDA and does not represent Agency position or policy.

Prediction of Internal Dosimetry and Toxicity of Volatile Chemicals in Rats Using Physiologically-Based Pharmacokinetic Modeling: Carbon Tetrachloride as a Model Compound
D. N. Williams, J. E. Simmons, J. V. Bruckner, and M. V. Evans. 1Oak Ridge Institute for Science and Education, Oak Ridge, TN; 2US EPA, Research Triangle Park, NC; and 3University of Georgia, Athens, GA.
Pilots of high-performance aircraft (HPA) may be exposed to various chemical irritants passing through the onboard oxygen generation system. Work was previously conducted using physiologically-based pharmacokinetic (PBPK) modeling and Monte Carlo analysis to estimate distributions of exposures that could result in the target exhaled breath measurements from a High-Performance Aircraft Respiratory Study (HPARS). These reconstructions allowed for the determination of possible exposure distributions across a range of exposure lengths and times, or scenarios, that might be experienced by pilots during flight, but did not account for differences in pharmacokinetics due to flight conditions such as altitude and G forces. The next step, therefore, in this ongoing work is to incorporate descriptions of physiological changes occurring as a result of flight into the existing PBPK model. The end goal of this work is to develop a PBPK model for a virtual pilot to better assess potential in-flight chemical exposures and produce aircraft cockpit exposure guidelines that will ensure limited probability of contaminated cockpit spaces that might contribute to coughing/respiratory symptoms or can be considered a contributing factor in reported symptomology. The work presented here utilizes an updated PBPK model with descriptions for changes in ventilation and cardiac output due to altitude and breathing air oxygen levels as well as changes in tissue blood flows due to G forces. The new PBPK model was applied to reconstruct doses for isopropanol, acetone and toluene based on the HPARS in a manner similar to the previous work augmented by the incorporation of physiological changes due to flight. These reconstructions allowed for the determination of possible exposure ranges experienced during flight, which were then compared to established short-term exposure limits (STELs). Some modeled in-flight concentration isocones for isopropanol exceeded post-sortie breath samples by more than a factor of 1000 and established STELs by more than a factor of 100. By expanding the current PBPK model paradigm to the physiological changes of HPA flight, a capability has been developed to assess true pilot physiology in a “virtual” context.

**3184 Pharmacokinetic (PK) Modeling to Evaluate the Effect of Serum Protein Binding on Bone Marrow Toxicity from Chloramphenicol Eye Drops**

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Chloramphenicol (CAP) is an antibiotic produced by soil bacteria and has been used for decades in systemic treatment of bacterial infections and for topical treatment of eye infections. Natural occurrence in soil results in CAP uptake by crops as a source of low level dietary exposure. CAP intake has also been associated with veterinary and aquaculture use to prevent microbial impacts; however, dietary exposure generally corresponds to lower daily doses than CAP-containing eye drops. Concerns about the risk of rare (1 in 20,000 treated cases) but often fatal aplastic anemia following systemic antibiotic doses of CAP led to banning or strict limitations on clinical, veterinary, and agricultural use. CAP-containing eye drops have not been shown to cause aplastic anemia, which is likely due to lower systemic exposure and high serum protein binding that reduces absorption.

Bone marrow (BM) toxicity requires a sufficient dose and steady-state concentration of unbound CAP in the target tissue. At high doses and/or in cases where serum protein binding of CAP is severely limited, a greater amount of unbound CAP may reach BM and induce cytotoxicity. Amination of metabolites was used to simulate the concentration of BM CAP in blood and BM resulting from a systemic therapeutic dose, a systemic BM toxic dose, from food exposure, and from use of CAP eye drops. Simulations were performed assuming normal (50-60%) and severely depressed serum protein binding (20-30%). Steady state serum concentrations of unbound CAP resulting from eye drop use (0.0055 mg/kg) were 2300- to 4050-fold lower than those resulting from a systemic therapeutic dose (12.5 mg/kg) or a BM toxic dose (22 mg/kg), respectively. Unbound CAP concentrations from dietary exposure were 6.5 to 12.7 million-fold lower than those resulting from systemic therapeutic or BM toxic doses, respectively. These findings suggest that BM toxicity from CAP eye drops and low level dietary CAP exposure is likely negligible. Lower fractions of bound CAP in serum resulted in a higher concentration of unbound CAP reaching BM in all dosing scenarios. The PK model predicts that severely depressed serum protein binding allows for more unbound CAP to reach BM and could be an explanation for the rare outcome of aplastic anemia following systemic dosing regimens.

**3185 The Impact of Genetics on the Pharmacokinetics of Chemicals in a United States Air Force Population**

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Genetic variability continues to be a component of interest in assessing the expected impact of chemical exposures on human health. Until the advent of modern “omics” rapid assessment technologies, broad assessment of genetic variation on individualized outcomes was limited to single gene variants. Here, we explore the potential contribution from the DMET™ Plus array, measured on ~2000 personnel in the Air Force Medical Service (AFMS) Patient-Centered Precision Care (PC2-Z) Program as implemented by the Corell Personalized Medicine Collaborative (CPMC). We assessed the expected impact of genetic variation in metabolic and transporters on the exposure related to commonly encountered in the U.S. Air Force environment. Specifically, 10 putatively functional variants in CYP2E1 (Cytochrome P450, Family 2, Subfamily E, Polypeptide 1) were chosen for initial consideration given the role of this locus in processing volatile organic chemicals (VOCs) such as isopropyl alcohol, cyclohexane and alphatic hydrocarbons. Measured CYP2E1 variation and its expected impact were then incorporated into analyses via PK-Sim® (Bayer) Open Systems Pharmacology Suite to assess the likely influence of genetic variants on blood time course of the aforementioned chemicals. Simulated area under the curve measures for the different chemicals examined varied by as much as one order of magnitude depending on individual CYP2E1 genetic status.
indicating the need to expand assessment of the genetic architecture of detoxification downstream of this key enzyme step. Application of these genome-informed insights will allow a refined estimate of expected exposure response and potentially the prediction of personalized health outcomes.

3186 Modeling the Dynamics and Dosimetry of Electronic Nicotine Delivery Systems (ENDS) Aerosols in the Oral Cavity


Electronic nicotine delivery systems (ENDS) heat liquids containing nicotine, water, propylene glycol, and glycerin and deliver an aerosol mixture that is inhaled by the user. ENDS aerosol deposition is difficult to quantify in the respiratory tract due to thermodynamic changes and variable breathing maneuvers during ENDS usage. An anatomically realistic computational fluid dynamics (CFD) model of the oropharyngeal airway was used to simulate ENDS aerosol deposition during puffing maneuvers. A transient moving boundary procedure was implemented by manipulating the geometry of the anterior oral airway to simulate the predicted anatomic changes of the oral cavity during ENDS use. ENDS aerosols were assumed to be initially composed of droplets containing 1% water, 5% nicotine, 30% propylene glycol, and 65% glycerin. A droplet size at a constant volume of 10^10 #/ml. Aerosol dynamics in the CFD simulations consisted of droplet coagulation, constituent evaporation, and water vapor condensation, along with droplet deposition and vapor uptake on the oral airway boundary. CFD simulations of the inhaled airflow and ENDS aerosol transport and deposition were conducted. The boundary expansion procedure created a negative pressure inside the mouth, driving the ENDS aerosol into the oral cavity. CFD simulation results predicted that the aerosol experienced significant changes while in the mouth cavity. Droplet coagulation increased the aerosol size by approximately 50%, condensation of water vapor onto the droplets also increased the droplet size, affecting the droplet deposition profile throughout the oral airway. An accurate assessment of the dynamics and deposition of ENDS aerosols in the oral cavity was found to be essential to quantify the amounts of each constituent reaching the lung for consideration of the potential health impacts due to ENDS usage. This work was supported by the FDA Center for Tobacco Products. This is not a formal dissemination of information by the FDA and does not represent Agency position or policy.

3187 Estimating Children’s Postnatal Exposure to DDT and DDE Using a Physiologically-Based Pharmacokinetic (PBPK) Model

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Dichlorodiphenyltrichloroethane (DDT) and its metabolite dichlorodiphenylchloroethylene (DDE) are persistent organic pollutants that are widespread in the environment. Although DDT has been banned decades ago in most countries, DDT and DDE are still found in the environment and in breast milk samples. Studies have shown that prenatal exposure can be detrimental to children’s development, but there is a lack of knowledge on the effects of postnatal exposure. Children’s main exposure pathway is mother-to-child transfers through placenta, cord blood and breast milk, which occur during important developmental periods. The objective of this study was to estimate DDT and DDE levels in children participating in the Tohoku University Study of Child Development study (J) using a PBPK model. We used data from 536 Japanese mother-child pairs to estimate children’s monthly DDT and DDE concentrations from birth to 12 months of life, and every six months from 12 months to 36 months of age. We also estimated the maximum child concentration (Cmax) reached between birth and 36 months of age. Median (95th percentile) breast milk levels were 6.60 ng/g-lipid (16.47 ng/g-lipid) for DDT and 136.92 ng/g-lipid (408.88 ng/g-lipid) for DDE. The estimated median (95th percentile) of DDT concentrations at birth in infants was 6.89 ng/g-lipid (17 ng/g-lipid), and the median (95th percentile) of the Cmax was 21.95 ng/g-lipid (21.95 ng/g-lipid). As for DDE, the estimated median (95th percentile) of the concentrations at birth was 142.56 ng/g-lipid (411.31 ng/g-lipid), and the median (95th percentile) of the Cmax was 471.52 ng/g-lipid (1379.14 ng/g-lipid). Children’s levels estimated herein will be used to evaluate associations between exposure during critical windows of development and excessive weight gain during childhood.

3188 Toward Incorporation of the Metabolite Exposure in High-Throughput In Vitro to In Vivo Extrapolation (HT-IVIVE)


Margin of exposure analysis using the HT-IVIVE-predicted steady state exposure and HT-screening bioactivity data demonstrated the importance of considering dosimetry in risk-based prioritization. The current approach however, is limited only to parent compounds leading to low confidence in prioritization. To include the exposure to potentially bioactive metabolites in the current HT-IVIVE equation, efficient tools for identifying potentially active metabolites and predicting their formation and clearance rates are required. In this study, we evaluated the domains of applicability of the current in vitro and in silico prediction tools that are developed for pharmaceuticals to determine their applicability to environmental chemicals and to prioritize the data collection to fill the data gaps for chemical metabolism prediction. The goal is to build a database to facilitate the incorporation of the metabolite exposure in HT-IVIVE to support chemical safety assessment. First, we evaluated the domains of the applicability of the current tools based on structure information. The results of principal component analysis for drug-like and environmental chemicals, represented by the 200 most-prescribed pharmaceuticals in 2016 and ~1600 compounds from Tox Cast, respectively, showed that flexibility and lipophilicity distinguish between the two chemical spaces with drug-like compounds being confined in a narrower space compared to chemicals. The other analysis was performed with a rate of metabolism tool for farm animals, AMMET Predictor** (verr 7.1, SimulationsPlus). In vivo hepatic intrinsic clearance (Clint) values for a subset of Tox Cast chemicals were predicted, results of which revealed that the major data gaps exist for enzymes other than the 5 major CYPs for drug metabolism including, but not limited to, environmental chemicals. Substances were ranked as potential inducers or inhibitors enzymes. In addition, the need for an improved in vitro assay to measure Clin for slowly metabolized chemicals was evident as conventional tools cannot measure those with accuracy. Along with the results from our data collection effort guided by these evaluations, we demonstrate the incorporation of metabolite exposure in the current HT-IVIVE addressing the key limitation in current prioritization scheme in toxicity testing.

3189 Reverse Dosimetry Approach for Potential Endocrine Disruptors: Comparison of a Simple Kinetic Model versus PBTK

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In animal-free risk assessments, effect concentrations are obtained in vitro and are translated to external, e.g. oral, doses by kinetic modeling (reversidosimetry). For this approach, we examined in vitro assays for potential endocrine disruptors quantitatively lowest effect concentrations (LOEC) from in vitro assays to lowest observed effect levels (LOEL). For modeling, we applied 1) a simple kinetic 1 compartment model versus 2) an 8 compartment PBTK model. In approach 1), the calculated, dose dependent maximum plasma concentration was used to bridge in vivo to the in vitro situation. Substance specific input parameters for 1) are molecular weight, plasma protein binding (PPB) and hepatic clearance whereas for more complex PBTK modeling logP and apparent hepatic clearance through Caco-2 cells (Papp) are required. In approach 2), the simple 1 compartment model yielded results closer to the measured in vivo LOELs than the PBTK model for 6 out of the 10 modeled substances (FEN, APAP, CAF, FLU, MITT, TRE). In conclusion, our results demonstrate that for reverse dosimetry, also the application of a simple kinetic model may be possible in principle.
of arsenic in food and water to total exposure and demonstrate the model's value in reconstruction of exposures to IAs in humans, particularly in individuals who are exposed to relatively low levels of arsenic in water or food. Disclaimer: The views expressed are those of the authors and do not necessarily represent the views or policies of the US EPA.

**3190 Quantitative Bias Analysis of the Association of 2,2',4,4',5,5'-Hexachlorobiphenyl (PCB 153) and Type 2 Diabetes Mellitus**


The concentration of persistent organic pollutants such as polychlorinated biphenyls (PCBs) in serum tends to be higher among those with type 2 diabetes mellitus (T2DM). Whether this association is causal is unclear. We hypothesized that this relationship might be caused by interindividual variation in adiposity, which acts as a shared factor in both the rate of clearance of lipophilic chemicals such as PCB 153 and the risk of T2DM. This variance, along with a trend of decreasing exposure over time, might explain the association even in studies where the association had been adjusted for body mass index (BMI) and waist circumference (WC). To test this hypothesis, we conducted a quantitative bias analysis (QBA) on a specific epidemiologic study of diabetes and PCB 153 that was based on subjects in the National Health and Nutrition Examination Survey (NHANES). The QBA used a simulation based on a physiologically-based pharmacokinetic (PBPK) model of PCB 153 that we developed. The exposure model was a stochastic version of a published deterministic, fugacity-based model and described exposure over time for the simulated population. A model of T2DM incidence as a function of age, BMI, and WC was developed, using realistic rate ratios. The distributions of age, BMI, WC, and serum PCB 153 in the simulated population (n = 100,000) closely matched those in the observed population, and a similar proportion of subjects developed diabetes. Using the same statistical approach as the target study, we calculated the odds ratio (ORs) for diabetes in relation to category of serum PCB 153 concentration with adjustment for age, BMI, and WC. In our analysis to date, the ORs in the highest category of PCB 153, compared with the lowest, in the published data was 1.0 (95% CI 0.9-1.1), as compared with the observed value of 6.8 (3.0-15.5). The next steps include refining the model to have a more detailed description of adipose tissue, including visceral and subcutaneous compartments. Visceral fat is a better indicator of T2DM risk than BMI or WC, and some data suggest that there may be differential distributions in PCB concentration between fat types. The results will be used to evaluate the role of pharmacokinetics in the association of PCB 153 with T2DM.

**3191 Evaluation of a Physiologically-Based Pharmacokinetic (PBPK) Model for Inorganic Arsenic Exposure Using Data from Two Diverse Human Populations**

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Physiologically-based pharmacokinetic (PBPK) models are computational frameworks that quantitatively describe physiological and biochemical processes related to absorption, distribution, metabolism, and excretion (ADME) of xenobiotics. PBPK models can be used to interpret results of biomonitoring data to better inform estimates of external exposures and daily intake. These models can be used to report data in multiple studies to a common metric of exposure or dose for purposes of meta-analysis. Predicted dose-response relationships for health effects of chronic inorganic arsenic (iAs) exposures, the magnitude, pattern, and duration of exposure to iAs must be determined. Because information on iAs exposure may be obtained from populations with different sources and temporal patterns of exposure, a common exposure metric is needed to allow comparisons across studies. In this study, a previously published PBPK model was evaluated using data sets for arsenic-exposed populations from Bangladesh and the United States. The human PBPK model consisted of sub-models describing the ADME of iAs and its metabolites. The model was used to estimate total arsenic levels in urine in response to ingestion of iAs. Both arsenic water and dietary intakes were estimated and used to generate the associated arsenic urine concentrations. When arsenic intake from water alone was considered, the results of the PBPK model under-predicted urinary arsenic concentrations for individuals with low levels of arsenic in drinking water and slightly over-predicted urinary arsenic concentrations for individuals with high levels of arsenic in drinking water. When population-specific estimates of dietary intakes of arsenic were included in exposures, the predictive value of the PBPK model was markedly improved, particularly at lower levels of arsenic intake. These results illustrate the PBPK model’s utility in evaluating the contribution of arsenic to total exposures.
4-Hydroxyphenylpyruvate Dioxigenase (HPPD) Inhibition: Associated Tyrosine Elevation in Rats, Mice, and Dogs Shows Significant Species Differences

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Single and repeat dose toxicity studies were conducted in several experimental species. The test item was a HPPD-inhibiting standard drug compound. The single dose studies were conducted in male and female rats and mice by oral-gavage. The repeat dose studies were conducted in male and female rats, mice, and dogs for approximately 28 consecutive days through either continuous dietary administration (rats and mice) or oral capsule administration (dogs). Serum tyrosine concentrations were measured by a LC-MS/MS method at selected junctures in all the studies. A normal physiological baseline level of serum tyrosine was established from the concurrent control groups in each study. Serum tyrosine levels were shown to be comparable among all tested species, including between males and females. Serum tyrosine levels were elevated following treatment with the HPPD inhibiting compound in all species, and the magnitude of the elevation demonstrated marked species differences. The species differences were most apparent in the repeat dose studies. In the single dose studies, serum tyrosine levels increased in both rats and mice. In the repeat dose studies, the tyrosine levels in mice were only slightly increased with values falling within the normal baseline range even at the highest dose level. In the rats, the tyrosine levels increased markedly with even greater tyrosine elevation in males. At the same administered dose level, the serum tyrosine levels were about 10-fold higher in the rats than that in the mice. For the dogs, the serum tyrosine levels also increased markedly but not as high as in the rats. A major physiological basis for both the species and gender difference in response to HPPD inhibition is the existence of an alternate pathway through tyrosine aminotransferase (TAT) for tyrosine catabolism. TAT enzyme is more abundant and effective in mice and humans (males) as compared to rats and dogs. This explains the difference in tyrosine elevation observed in the single and repeat dose studies. Having an understanding of the key physiological differences in TAT capacity can inform experimental species selection and then ensure human relevance considerations for purposes of human health risk assessment with respect to HPPD-inhibiting chemicals.

Application of Probability Boxes to Characterize Mode-of-Action Uncertainties: Thiocyanate as Case Study

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Thiocyanate, which is found in the diet and tobacco smoke, has multiple modes of action for thyroid disruption. Thiocyanate inhibits the active thyroidal uptake of iodide, increases the passive permeability of iodide across the thyroid, and inhibits the organification of iodide for thyroid hormone synthesis. A cumulative dose-response model for thyroid-active chemical mixtures needs to account for the multiple modes of action of thiocyanate on thyroid function. In most cases, not all modes of action for chemicals evaluated are well characterized leaving sparse data for cumulative dose-response analysis. As such, unknown parameters need to be estimated based on the available information. Monte Carlo (MC) simulation has been widely used to randomly simulate a plausible set of unknown parameters from a relevant parameter range but it requires precise assumptions about initial parameter values and the parameter range and its distribution shape. We have examined the use of probability bound analysis and its application to physiologically based pharmacokinetic (PBPK) and dose-response models by generating a probability box (p-box) for the input parameters. Unlike a simple MC simulation, a p-box allows for the comprehensive propagation of variability and uncertainty in the face of uncertain input for any parameter by evaluating all possible distribution shapes within a physiological bound. We generated p-boxes for three of the five sensitive model input parameters based on their minimum, maximum, mean, and standard deviation; bounded values but without any distribution information, and compared the dose-response curves obtained by p-box to MC simulation. We observed that the predicted dose response curves obtained by p-boxes were wider than the curves obtained by MC simulation yet informative about dose-response effects of thiocyanate. We are continuing our efforts towards the implementation of a cumulative dose-response curve for thiocyanate and ultimately the thyroid-active chemical mixtures. Our work allows for quantifying the total uncertainty in the dose-response effects using p-box analysis when existing data are limited and multiple modes of action are involved. Such analysis provides risk assessors with valuable information, such as a well-quantified degree of uncertainty associated with the dose-response relationships, when only sparse mechanistic data are available.

Prediction of the Liver Toxicity of the Endothelin Receptor Antagonists Sitaxsentan and Ambrisentan for the Treatment of Pulmonary Arterial Hypertension with a Quantitative Systems Toxicology Tool (DILIsym)


Sitaxsentan and ambrisentan are highly selective endothelin-1 type A receptor antagonists which were developed for the treatment of pulmonary arterial hypertension. Sitaxsentan was voluntarily withdrawn from the market due to concerns about liver toxicity, whereas ambrisentan is currently on the market. DILIsym, a mathematical framework of drug-induced liver toxicity, was used for quantitative system toxicology studies of the compounds. In vitro assay data has revealed that Sitaxsentan is a potent inhibitor of BSEP, moderate inhibitor of MRP3/4, and moderate bilirubin transporter inhibitor, whereas ambrisentan exhibits mitochondrial electron transport chain (ETC) inhibition and moderate bilirubin transporter inhibition. Hepatic exposure of each compound was estimated by employing the DILIsym physiologically-based pharmacokinetic (PBPK) sub-model; predictions of toxicity risk were subsequently made in simulated humans within DILIsym (SimPops; n=285). Depending on the mode of transporter inhibition and PK variability, 0-11.2% of the SimPops were predicted to have liver toxicity (plasma ALT > 3x ULN) after 18 weeks of 100 mg QD oral dosing of sitaxsentan in DILIsym. On the other hand, irrespective of the mode of transporter inhibition, 0% of the SimPops showed liver toxicity after 10 mg of ambrisentan (QD oral dosing). These simulation results are comparable with the clinical data where 0-7% and 0% of patients experienced liver toxicity for sitaxsentan and ambrisentan, respectively. Further mechanistic simulations showed that synergy between mitochondrial dysfunction and bile acid transporter inhibition was primarily responsible for sitaxsentan induced liver toxicity. Although in vitro data for ambrisentan and sitaxsentan showed potential DILI signals, predicted DILI risk was also dependent upon compound exposure. DILIsym was able to help elucidate the clinical relevance of the in vitro signals.

Oral Irritation Assessment of Electronic Liquids Using an In Vitro Oral Testing Model

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While data are still being collected and analyzed, there were at least 1,300 incident electronic liquid (e-liquid) exposures reported as of May 5th, 2016. This study investigated the oral irritation of 3 different formulations of e-liquid using an in vitro time course assay in the reconstructed tissue model EpiOral™ (MatTek Corporation, Ashland, MA, USA). All products were from the same manufacturer, contained 1.2% nicotine and differed only in their flavorings. The e-liquids were tested neat in duplicate tissues. Tissue viability was measured using the vital dye MTT at 15 minutes, 30 minutes, 1 hour, 2 hours, and 16 hours. The ET50 values (representing the exposure time which reduces the tissue viability by 50%) were calculated and used to rank-order the irritation potential of the products. The ET50 values were 4.9h, 6.0h, and >16h, respectively, for the 3 products tested. The results of this study highlight the applicability of the EpiOral™ model in evaluating the oral irritation potential of a variety of e-liquid formulations. Further work will be performed with the culture medium to analyze the inflammatory profile of e-liquids via cytokine analysis.
The adoption of a new technology into industrial and clinical settings requires rigorous testing to determine reproducibility, reliability, robustness, and transferability. Tissue chips have the potential to be a valuable tool for studying biological function, pharmaceutical development and toxicity testing. Independent testing will increase confidence in the robustness of the tissue chips. As part of NIH funded Tissue Chip Testing Centers, the goal of this study was to test the proximal tubule chip from the University of Washington at Texas A&M. The proximal tubule tissue chip system was obtained from the developers and Nortis, Inc. (Seattle, WA). Human renal proximal tubule epithelial cells from two sources were grown over 28 days in 3D culture under a flow rate of 0.5uL/min. The performance of this system was examined in three rounds of testing: non-specific binding of drugs to the chips, functionality (long-term cell viability, protein and gene expression, ammonia-gene, and drug metabolism), and toxicological responses to nephrotoxic agents (polyoxymyxin B, cisplatin, gentamicin, and cadmium chloride). Parallel studies were performed with the same cells and study designs in traditional 2D culture. Investigations into the non-specific binding of compounds within the 3D platforms showed minimal absorbance to chips (<10%). Fluidic cultures self-organized into tubular structures, and indicated greater metabolic competence through increased 24-hydroxylation of Vitamin D3 and gene expression of CYP24A1 (3D: 49±12 vs 2D: 0.9±0.07-fold change). Additionally, cells demonstrated more sensitive responses to nephrotoxants through increases in KIM-1 release, and significant differences in gene expression (HAVCR1, HMOX1, LCN2, and FN1) compared to traditional static 2D culture. Cross-site comparisons indicated high agreement and demonstrated the transferability of the proximal tubule tissue chip.

Physiologically-based toxicokinetic (PBTK) models that are tailored to the chemicals of interest are generally considered to be more accurate than simpler toxicokinetic (TK) models parameterized with minimal in vitro and in silico data. However, within the context of high-throughput risk evaluation, a slightly lower level of accuracy may perform equally well in estimating relative risk. In this study, we compared fitted human PBTK models for bisphenol A (BPA) to the R package “httk,” which includes a 1-compartment TK model and a generic PBTK model. We also tested a 2-compartment model, consisting of clearance and rest-of-body compartments with physiologically based volumes, connected by the hepatic blood flow. The models from httk assumed a first-order, non-restrictive clearance and were parameterized with logp, pka, and in vitro intrinsic clearance, protein binding and blood-to-plasma partitioning. The fitted PBTK model from Yang et al. (2015) and the httk models yielded similar time-plasma concentration curves in the prediction of human in vivo data from Thayer et al. (2015). The 2-compartment and generic PBTK models made very similar predictions, with root-mean-square error (RMSE) the square root of the average squared difference in predicted concentrations) of 2.9 and 5.1 nM and average-fold error (AFE) (the geometric mean of the quotient of measured and predicted concentrations) of 2.3 and 3.0, respectively. The fitted model (Yang et al., 2015) performed the best (RMSE of 0.33 nM and AFE of 1.3), and the 1-compartment model performed the worst (RMSE of 43 nM and AFE of 20). In this case study, the 2-compartment model performed best among the simplified models in predicting BPA TK, serving as a proof of concept for further investigation of TK models based on high-throughput data. This abstract does not necessarily reflect US EPA policy, Thayer, K. A., et al. “Pharmacokinetics of bisphenol A in humans following a single oral administration.” Environ. Int. 83 (2015): 107-115. Yang, X., et al. “Development of a physiologically based pharmacokinetic model for assessment of human exposure to bisphenol A.” TAAP 289.3 (2015): 442-456.
3202 Development and Evaluation of a Physiologically-Based Pharmacokinetic Model for Cimetidine


Cimetidine is commonly used in the treatment of heartburn and peptic ulcers. Cimetidine has been shown to inhibit multiple CYP450 enzymes including CYP1A2, CYP2D6, and CYP3A4. It was also found to inhibit multiple transporters including MATE1, MATE2-K, OCT1 and OCT2. The purpose of this work was to develop a physiologically-based pharmacokinetic (PBPK) model for cimetidine using GastroPlus™ (Simulations Plus, Inc.) and PBPKPlus™ (Simulations Plus, Inc.) for the simulation of cimetidine after IV and oral administration. The simulated PK profiles of cimetidine. The physiochemical parameters not available in literature were predicted using the ADMET Predictor™ (Simulations Plus, Inc.) and PBPKPlus™ (Simulations Plus, Inc.). The Advanced Compartmental Absorption and Transit (ACAT™, Simulations Plus, Inc.) and PBPKPlus™ (Simulations Plus, Inc.) were used for the simulation of cimetidine after IV and oral administration. The simulated PK profiles of cimetidine were compared with observed profiles for evaluation of the model performance. The fold error values (predicted/observed) ranged from 0.91 to 1.02 for the area under the curve (AUC) values, indicating that simulated results were in agreement with observed data. The developed cimetidine PBPK model was then used to estimate the impact of cimetidine (as perpetrator) on other drugs. Two CYP3A4 substrates were selected including triazolam and midazolam which have built-in PBPK models in GastroPlus®. Using cimetidine as the perpetrator, drug-drug interaction of cimetidine with triazolam or midazolam showed increased in both victim drugs, and the increases were generally in good agreement with observed data reported in literature. This model could be used for evaluating the impact of cimetidine on the exposure of other drugs when coadministered.

3203 Determination of Complex Drug Bioequivalence Using Stable Isotope Tracers


An analytical challenge for complex drug bioequivalence is accurate measurement of encapsulated and unencapsulated drug. To overcome this analytical challenge, a novel ultrafiltration drug release method utilizing stable isotope tracers has been developed. Stable isotopically-labeled active pharmaceutical ingredient (API) is spiked into plasma containing the isotope-labeled API. The isotope-labeled API equilibrates with plasma protein and formulation components identical to the normoisotopic API released from the complex formulation. Therefore, the ultrafilterable fraction of the isotope-labeled API represents a reliable measure of free normoisotopic API fraction in plasma, and can be used to calculate encapsulated and unencapsulated API fractions. To demonstrate the utility of the stable isotope tracer method, we performed an in vitro drug release study in rat and human plasma, and in vivo bioequivalence study in rats, comparing Janssen’s Doxil® and Sun Pharma’s doxorubicin HCl liposome generic. Using the stable isotopically-labeled API as a tracer, the isotope-labeled API was spiked into plasma and doxorubicin preparations were released in human and rat plasma over a 6h period. A parallel design bioequivalence study in rats demonstrated similar encapsulated and unencapsulated drug pharmacokinetic (PK) profiles for both liposomal preparations. Statistical analysis determined the formulations to be bioequivalent on the basis of T<sub>max</sub>, encapsulated C<sub>max</sub>, AUC<sub>0-24</sub>, AUC<sub>0-24</sub> and AUC<sub>0-24</sub>, but not unencapsulated AUC<sub>0-24</sub>. Notably, the estimated unencapsulated drug profiles in this study differed greatly from previously published rat bioequivalence studies utilizing solid phase extraction methods, with regard to unencapsulated drug concentrations, encapsulated to unencapsulated drug concentration ratio, and unencapsulated drug terminal half-life. Funded by NCI-FDA IAA, and NCI Contract No. HHSN26120080001E.

3204 Comparative Disposition of Bisphenol S and Bisphenol AF following Gavage Administration in Harlan Sprague Dawley Rats and B6C3F1/N Mice and Hepatocytes In Vitro


Bisphenol AF (BPAF) and bisphenol S (BPS) are used as cross linking agents in polymers and are structurally similar to bisphenol A (BPA). BPAF and BPS were selected by the National Toxicology Program for toxicological evaluation based on their structural similarity to BPA and lack of adequate toxicity data. Disposition and toxicokinetics (TK) data are essential to put toxicological findings into context. We investigated the disposition and TK of BPAF (3.4-340mg/kg) and BPS (50-500mg/kg) in rats and mice following a single gavage administration. In both species, [14C]BPAF was excreted mainly in feces (33-77%) with some in urine (females, 9-24%; males, 1-6%) by 72h. [14C]BPS was excreted primarily in urine (48-72%); the % excreted in urine decreased while that in feces (16-30%) increased with the dose. In rats, although ~50% of BPAF or BPS dose was excreted in bile in cannulated animals within 24h, ~uncannulated animals, ≤13% of the dose was excreted in feces. Following an intravenous dose of BPAF or BPS, the pattern of excretion was similar to gavage. These data suggests that both were well absorbed following gavage administration, underwent biliary excretion and extensive enterohepatic recirculation and were excreted primarily in feces (BPAF) and urine (BPS). BPAF and BPS were distributed to tissues with <2% of the dose remaining at 72h. The observed plasma BPAF C<sub>max</sub> was similar in mice and rats with elimination half-life <3h; the bioavailability was low and was similar across species and sexes (<1% - 4%). BPS C<sub>max</sub> was ~5-times higher in mice than in rats; however, half-life of elimination was shorter in mice (2.5-3h) than in rats (7-10h) resulting in similar AUC values for rats and mice. The bioavailability of BPS (~0.10 - 18%) was higher than for BPAF and was similar across species and sexes. For BPAF, glucuronide and sulfate conjugates were identified in bile and urine of exposed rats with no parent detected. In addition to glucuronide and sulfate conjugates, parent was also present in urine of BPS-exposed rats. In rodent and human hepatocytes, clearance of BPAF (half-life 6-15min) and BPS (half-life 29-97min) was similar. In conclusion, BPAF and BPS were well absorbed following oral exposure, underwent extensive first-pass metabolism and recirculation and have low bioavailability similar to that reported previously for BPA.

3205 2,4,6-Tribromophenol (TBP) Disposition and Kinetics in Female Sprague-Dawley Rats after Single and Repeated Dosing


2,4,6-tribromophenol (TBP, CAS No. 118-79-6) is widely used as a brominated flame retardant and wood fungitidal agent. TBP is frequently detected in environmental matrices, biota, and humans. In female SD rats, systemically available TBP (10 µmol/kg, IV) was rapidly excreted primarily via urine, with ~61% of the dose recovered after 4h, and 89-94% in 24h. 5% of the dose was recovered in feces and 1-2% in blood/tissues. TBP administered to female SD rats (0.1-1000 µmol/kg) by gavage was well absorbed, with ~25% eliminated via urine after 4h and ~88% after 24h. Fecal recoveries varied only slightly by dose and route. Approx. 11% of a single oral dose was recovered in bile. Toxicokinetic analyses of TBP in blood found IV time-concentration profiles fit a two-compartment model indicating a rapid distribution to tissues followed by a slower excretion phase. After oral dosing, concentrations rapidly rose to C<sub>max</sub> followed by a single elimination phase. Oral bioavailability was 31-37%. Urine was found to contain a mixture of TBP, TBP-glucuronide, and TBP-sulfate. Blood collected after IV dosing contained a consistent 85%-15% ratio of TBP to TBP-glucuronide at all timepoints investigated, but blood collected after oral dosing contained a 50%-50% ratio. Fecal extracts contained only parent TBP while bile contained only TBP-glucuronide. TBP did not appear to bioaccumulate or alter its own metabolism after repeated administration. TBP was readily absorbed at all doses and routes tested, and is likely to be bioavailable when ingested by humans. Supported by the Intramural Research Program at NCI/NIEHS.
Understanding the processes of absorption, distribution, metabolism and excretion (ADME) is important for evaluating potential inter- and intra-species differences in response to chemical exposure, which is in turn important for extrapolating health effects and effect levels observed in laboratory animals to humans, for purposes of establishing regulatory criteria. We conducted a comprehensive analysis of key ADME parameters across different species for five perfluorinated chemicals (PFCs), including perfluorobutanoic acid (PFBA), perfluorobutane sulfonate (PFBS), perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA), and perfluorooctanoate (PFOA). We identified parameters from approximately 60 studies, which we identified from PubMed, and from health effects review documents prepared by US EPA and ATSDR. Our analysis yielded the following observations: 1) Across species, liver partitioning decreases in the order of PFOS > PFOA > PFBA = PFHxS, with PFOS and PFOA distributing preferentially to the liver and PFBA and PFHxS distributing preferentially to the serum. 2) Compared to primates, PFOS distributes preferentially to the liver in rats. 3) Half-lives increase in the order of PFOS < PFBS < PFBA < PFHxS < PFOA, and in the order of rodents < non-human primate < human. 4) Half lives of PFBA, PFHxS, and PFOA are shorter in female vs. male rats. 5) Although placental and lactational transfer of PFOA are comparable in rats and humans, offspring/maternal serum ratios are lower in humans compared to rats. 6) Although placental and lactational transfer of PFOS are greater in rats than humans, offspring/maternal serum ratios are comparable. Based on these observations, we conclude the following: 1) Large differences in half-lives among species highlights the importance of extrapolating effects across species based on pharmacokinetic characterization of physical-chemical properties. 2) Tissue distribution of these PFCs is consistent with the liver being the target organ for toxicity, with highest liver/serum ratios observed for rats exposed to PFOS. 3) Based on serum concentrations, primates are expected to be less susceptible to than rats to the liver effects of PFOS, and the human fetus is expected to be less susceptible than the rat fetus to the developmental effects of PFOS. Considering these conclusions, our analysis will facilitate interpreting human relevance of findings observed in rodents exposed to PFCs.
The gut microbiota contributes to diverse mammalian processes including defense against pathogens, immunity, and energy production through metabolic processing. More recently the gut microbiome has been shown to play a role in the metabolism of xenobiotics including antibiotics and drugs targeted against host physiology. Shifts in the composition of GI microbiota, whether induced by dietary changes, antibiotic treatment or inflammatory processes can disturb the balance of organisms in the gut and alter the metabolic networks of the host. Changes in the microbiome can also influence the overt toxicity and metabolism of therapeutic drugs administered orally. To determine how changes in the gut microbiome can alter drug metabolism and affect drug-drug interactions, the pharmacokinetics (PK) and biodistribution and metabolism of the model compound acetylaminocephalin was assessed in C57B/6 mice after treatment with the antibiotics amoxicillin (amox) or a cocktail of ampicillin/ neomycin (amp.neo). Altered tissue concentration, plasma PK, and metabolism of acetylaminocephalin was observed over 2 h, in animals exposed to antibiotic treatments. Acetylaminocephalin plasma area under the curve profiles were significantly decreased in antibiotic treated animals compared to control animals suggesting decreased bioavailability. Levels of acetylaminocephalin in the kidneys of animals exposed to amox were higher when compared to control and amp.neo treated animals at the early time points (0.25 h, 0.5 h, 4 h). Urinary metabolite files revealed decreases in acetylaminocephalin-sulfate metabolite levels in both the amox and amp.neo treated animals compared to control animals. Analysis of gut microbe composition using the LLNL Microbial Detection Array system revealed changes in microbiota content in antibiotic treated animals compared to untreated animals. These results suggest that exposure to amox or amp.neo can alter the biodistribution of acetylaminocephalin and that these alterations could be directly due to changes in gut microbiome composition. This work was performed under the auspices of the US DOE by LLNL under Contract DE-AC52-07NA27344 and supported by the National Institute of General Medical Sciences (P41GM103483-14) and LLNL-PLS-LDRD: 16-ERD-007.

Synergistic interaction between florfenicol (FF) and thiamphenicol (TAP) have been reported for several pathogenic bacteria of animal origins. Survival rates were demonstrated to be significantly increased against Pasteurella multocida and Streptococcus suis in rat and chicken models. Given that the efficacy of FF+TAP at the reduced dose of both drugs was uncompromised, lower drug residues in plasma and tissues were expected and warrant further investigation. Therefore, the plasma pharmacokinetics and tissue residues of the synergistic FF+TAP and its acute toxicity in broiler chickens was evaluated. To determine the pharmacokinetic profile, the mixture of 5 mg/kg FF and 10 mg/kg TAP was intramuscularly administered to 6 broiler chickens. Another 3 chickens were used for tissue residues study at 24 h after drug administration. Plasma and tissues (kidney, liver, skin with fat and breast muscle) concentrations of the two antimicrobials were analyzed by HPLC-UV. For acute toxicity study, the chickens were treated at 48 h after drug administration and examined for any gross and histopathological abnormalities. Significantly lower peak concentrations (0.04 and 2.63 µg/mL for FF and TAP, respectively) and area under the curves (0.25 and 13.99 h·µg/mL for FF and TAP, respectively) of both antibiotics were recorded when compared to those of the recommendation dose (30 mg/kg for each drug). The 1-day tissue residual levels of FF were approximately one order of magnitude lower than the results reported by other studies and are much lower than the maximum residue level (MRL). Consequently, the drug withdrawal period could potentially be shortened. In addition, the observed histopathological lesions were found following FF+TAP administration at this dosage level. Our results clearly demonstrated the benefits of using synergistic FF+TAP combination over the monotherapy in the aspects of consumers safety as the chicken plasma and tissue drug residues could be significantly reduced without compromising the antimicrobial activity.

Azithromycin is a widely-used macrolide antibiotic that is continually deposited into natural waterways by sewage effluent. Though recognized as an emerging contaminant of concern, little to nothing is known about its effect on non-target aquatic organisms. With less than 1% degradation after 150 days in simulated watershed conditions, azithromycin is an enduring contaminant in aquatic systems that has been shown to accumulate in aquatic invertebrates. Known to inhibit p-glycoprotein pumps, azithromycin may be extremely deleterious to aquatic systems as multixenobiotic resistance in aquatic organisms is largely mediated by p-glycoprotein. Crayfish (Procambarus clarkii) were used as a model non-target aquatic organism exposed to azithromycin through diet. Preliminary kinetic studies showed gastrointestinal adsorption of azithromycin to be as high as 75.73 ± 0.008%. Elimination was slow; after 14 days less than 10% of the parent compound was excreted with no potential non-target or target metabolites (deschlorido azithromycin, N'-desmethyl azithromycin, and 9a-N'-desmethyl azithromycin) observed, indicating that azithromycin is likely to biocumulate in the host magnifying potential toxic effects. Toxico-kinetic studies currently in progression are expected to further elucidate the fate of azithromycin in P. leniusculus, as well as identify potential azithromycin effects on multixenobiotic resistance in these crayfish.
Simultaneous Hepatocellular Disposition Profiling of Parent Compound and Its Major Metabolites in Sandwich-Cultured PXB-Cells by D-PREX

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This study aimed to examine the total disposition profiling of parent and its major metabolites by a single systematic procedure called D-PREX (Disposition Profile Exploration), using sandwich-cultured PXB-cells (SCPC). In this D-PREX system, drug uptake and metabolism is performed in intact SCPC, followed by simultaneous evaluation of biliary and basolateral efflux by measuring supernatants, then cell lysis to understand the intracellular residues. SCPC were exposed to the standard Hakus' balanced salt solution (Std HBSS) including 10 μM parent compound at 37°C for over time (10-120 min), which were terminated by rinsing with ice-cold Std HBSS. Subsequently, the cells were incubated 10 min with Std HBSS at 37°C for stabilization of the following efflux evaluation (conditioning efflux step). Then the cells were separated into three groups: intact and disrupted bile canalicular structures with and without Ca²⁺ ion conditions at 37°C, and that of intact with Ca²⁺ at 4°C. Then, each supernatant was collected after 10 min incubation, followed by the cell lysate samplings. The amount of compounds in the collected samples were quantified by LC-MS/MS analyses, which were used to calculate the respective disposition endpoints. In terms of acetonaphthphen (APAP), the metabolism seemed to follow the uptake of APAP, which saturated after 60 min incubation. The proportion of the major metabolites of glucuronide (APAP-Gluc) and sulfate (APAP-Sulf) corresponded to the known human metabolism properties. In addition, these compounds mostly transported to basolateral efflux than biliary excretion, which represented the tendency of urinary elimination. Further, in terms of basolateral efflux, APAP showed relatively a higher passive diffusion portion than that of transporter-mediated, whereas APAP-Gluc and -Sulf represented opposite trend, suggested the hydrophilic property of the conjugates mediated by the transporters. D-PREX can be used for the simultaneous evaluation of total disposition profiles not only the parent compounds but also their metabolites, resulted from the complex interplay of the hepatic metabolism and transport. This methodology may contribute to pre-estimate the toxic potential caused by active-metabolites due to an enterohepatic circulation such as SN-38, an active-metabolite of CPT-11. 

Interaction of Oatp1b2 Gene Dose and Nonalcoholic Steatohepatitis on Plasma Clearance of Pravastatin

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It has been reported that nonalcoholic steatohepatitis (NASH) downregulates hepatic uptake transporters, including the organic anion transporting poly peptide (OATP) transporters. It is known that the combination of NASH and genetic loss of Oatp1b2 causes a synergistic decrease in the plasma clearance of pravastatin (PRAV). The current study aimed to examine the effects of comorbidity NASH and genetic heterozygosity of Oatp1b2 on the plasma clearance of PRAV to model the overlap between the 24% of the human population who are heterozygous for OATP1B1 and the ~15% with NASH, potentially placing these people at higher risk of statin-induced myopathy. Therefore, male C57Bl/6 wild-type (WT), Oatp1b2+/− (HET), and Oatp1b2−/− (Null) mice were fed either a methionine and choline sufficient or methionine and choline-deficient (MCD) diet. After 6 weeks of control and MCD diet, pravastatin was administered via the carotid artery, and blood samples were collected at 2, 7, 15, 30, 50 and 90 minutes after pravastatin administration. Concentration of PRAV in plasma samples was determined by liquid chromatography-tandem mass spectrometry. MCD diet did not alter the plasma AUC values of PRAV in WT or HET mice. However, the Null-MCD group increased plasma AUC by 4.4-fold. 

Impact of Organic Cation Transporter 3 (SLC22A3) Knockout on [3H]Quinine and [3H]Mefloquine Uptake and Social Behavior in Aged Mice


Quinine is an old antimalarial drug that is still in use worldwide, however, it is better known as a bitterant of tonic water that makes it glow under blacklight. Quinine has affinity for and is known to block serotonin (5-HT) reuptake by serotonin transporters (SERT). However quinine is also a substrate of organic cation transporters (OCTs). OCTs are low-affinity, high capacity, polyspecific carriers that help regulate extraneuronal concentration of OCTs. There are three subfamilies of OCTs: OCT1, OCT2, and OCT3 expressed in the brain of both humans and mice. Currently, there is a great need for OCT3-specific inhibitors to differentiate its activities and to test its properties for the treatment of psychiatric disorder. The goal of our study is to determine the relative share of OCT3 to [3H] quinine and [3H] mefloquine uptake in aged mice. Adult male C57Bl/6 mice and OCT3 knockout mice were euthanized on day of experiment and their cerebellum harvested for uptake assay. Stocks, 10X uptake buffer, and ligand dilutions were prepared for synaptosomes. [3H] Quinine and [3H] mefloquine were used as substrates and decyinium-22, lopinavir, and sertraline were used as blockers. Cerebellum was harvested from 2 year old mice, stored and homogenized in sucrose then centrifuged. Synaptosomal preparations were incubated once pipetted into labeled ligand dilutions. Synaptosomes were harvested using a Brandel Cell Harvester for vacuum filtration and then collected into scintillation tubes and analyzed using a scintillation counter for 24 hours. Lastly, a Bradford protein assay was used to identify protein concentration. Surprisingly we found that uptake of [3H] quinine was greater in OCT3 knockout mice than in wildtype mice, and that sertraline (1 μM) was a more potent blocker than D2-2 or lopinavir. There was no statistically significant difference between the wildtype and OCT3 knockout and wildtype mice. Furthermore, like the pseudoisocyanine decyinium-22 that blocks all ancillary transporters of 5-HT, we find that OCT3 knockout in mice and to a lesser extent substrates such as mefloquine or quinine can enhance social behaviors in adult male mice strain-dependently. 

Hepregen Hepatoc: A Novel In Vitro Transporter Tool to Support DILI Risk Assessment

Using an empirically derived cutoff based on inhibition of BSEP and the compound’s estimated unbound concentration at the inlet of the human liver (fu*Iin,max), we were able to establish a screening assay to identify instances where inhibition of BSEP may be predictive of human DILI. Interestingly, inhibition of MRPs did not significantly enhance DILI predictivity compared to BSEP inhibition alone. To further understand the ability of inhibitors to inhibit bile salt transport in vivo, laboratory animals were then tested as an inhibitor of taurocholate (TCA) transport in a long term micropatterned human hepatocyte based system (Hepatopac®). In contrast to BSEP containing membrane vesicles, Hepatopac allows for a more holistic assessment of bile salt transport inhibition as multiple transport pathways, protein binding, intracellular sequestration, and compound metabolism are taken into account. The resulting IC50 for TCA in vitro biliary clearance (Clbiliary) and biliary excretion index (BEI) in Hepatopac were compared to the compound’s estimated fu*Iin,max to assess potential risk for bile salt transport perturbation. The data showed that the specificity of the assay was high (89%) with only 1 false positive being observed. Furthermore, 11 out of 16 compounds which showed an IC50 in the TCA vesicular assay of < 5 μM did not result in an IC50 for BEI that was within 10 fold of the fu*Iin,max suggesting that these compounds are less likely to cause inhibition of BSEP in vivo. These data suggest that Hepatopac may be a more effective transport tool to identify compounds which inhibit biliary efflux of bile salts than the TCA vesicular inhibition assay.

**3219 The Role of Human MRP2 in the Cellular Efflux of Microcystin Congeners**

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Water contamination by cyanobacterial blooms is a worldwide health hazard to humans as well as livestock. Exposure to Microcystins (MCs), toxins produced by various cyanobacterial or blue green algae found in poorly treated drinking water or contaminated seafood such as fish, prawns etc., are associated with hepatotoxicity, nephropathy and neurotoxicity and in extreme cases, death in humans. At present more than 120 MC congeners are described. These congeners differ dramatically in their uptake kinetics, i.e. their uptake via organic anion transporting polypeptides (OATP), in OATP overexpressing human HEK293 cells and primary human hepatocytes. It is thus likely that MC congeners will also differ with respect to the cellular efflux of the parent and conjugated congeners, e.g. via Mrp’s, Bcrp or Bsep. Consequently, the role and kinetics of different human efflux transporters - Mrp, Bcrp and Bsep in MC efflux was studied using inside-out membrane vesicles. Of the efflux transporters investigated, hMrp2 suggested MC transport. In addition, a MC congener-specific efflux was also observed, whereby Mrp-c was transported faster than MC-LF. In conjunction with the previously reported faster cellular uptake of MC-LF and the slower MC-LF efflux (reported here) results in a higher cellular MC-LF concentration per unit time and thus would explain the higher cytotoxicity observed for MC-LF in human primary hepatocytes and OATP overexpressing human HEK293, when compared to MC-LR. In future, this approach will allow for the development of a toxicokinetic computational model that supports improved human risk assessment of the plethora of single MC congener and/or their mixtures in contaminated food and water.

**3220 Tissue Distribution of Organophosphate Flame Retardants in Mice: Analysis of Dose-Dependence, Chemical Species, and Sex Differences in a Subchronic Study**

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The industrial phase-out of polybrominated diphenyl ether flame retardants due to their persistence in the environment, bioaccumulation, and toxicity created a demand for alternatives such as organophosphate flame retardants (OPFRs). OPFRs are chemicals of emerging toxicological concern due to reports of endocrine disruption, neurotoxicity, and reproductive and developmental toxicity. Studies on the tissue distribution and accumulation of OPFRs in vivo are limited. In the present study, the tissue distribution of OPFRs was quantified in adult male and female C57BL/6 mice orally administered 1 or 10 mg/kg/day of each OPFR—tricresyl phosphate (TCP), triphenyl phosphate (TPP), and tri(1,3-dichloro-2-propyl) phosphate (TDCPP) in equal amounts for four weeks using gas chromatography-mass spectrometry. Circulating concentrations of TDCPP in male mice ranged from 1.4 to 3.8 ng/ml and were similar for 1 and 10 mg/kg/day dosing. TCP and TPP exhibited dose-dependent differences with 10- and 1.75-fold higher concentrations observed after 10 mg/kg/day dosing (TCP: 5.6 ng/ml; TPP: 2.8 ng/ml) compared to 1 mg/kg/day dosing (TCP: 0.56 ng/ml; TPP: 1.6 ng/ml), respectively. In male serum, concentrations of TDCPP (3.2 ng/ml) were higher than TPP (1.6 ng/ml) and TCP (0.56 ng/ml) after 1 mg/kg/day dosing. By comparison, serum concentrations of TPP (0.94 ng/ml) and TCP (0.16 ng/ml) were significantly lower in female mice, with no difference in TDCPP (2.5 ng/ml). Similar to circulating serum concentrations, increased tissue concentrations of TDCPP (M: 7.5 pg/mg; F: 9.2 pg/mg) relative to TPP (M: 1.4 pg/mg, F: 0.35 pg/mg) and TCP (M: 0.92 pg/mg, F: 0.33 pg/mg, F: 0.33 pg/mg) with no statistical differences between the sexes. Tissue distribution of OPFRs was also quantified in other tissues including liver, brain, adipose, ovaries, and testes. Comparative OPFR tissue distribution after subchronic exposure aids in the identification of target organs for toxicity and facilitates modeling to extrapolate tissue exposures in humans. Funded by NIH ES020721, ES027119, DK083457-51, ES021800, and ES005022.

**3221 Is a Common Mechanism of Action Essential to Conduct a Cumulative Risk Assessment or Just Nice to Have?**

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Current risk assessments of chemicals for regulatory purposes do not generally take into account the “real-life” exposure to multiple substances, but rely instead on the assessment of individual substances in individual commodities. Humans however are routinely exposed simultaneously to numerous chemicals via multiples routes of exposure. These mixtures can be variable, constantly changing and essentially undefinable. One major question is whether a common mechanism of action is critical element for conducting a cumulative risk assessment. The chemical toxicity of the chemical mixtures and the diversity of the routes of exposure may call for the development of both mechanism-based and non-mechanism-based quantitative frameworks for risk assessment to estimate the impact on health, thereby increasing the efficiency and effectiveness of these evaluations. Consideration of the recent advances in vitro as well as in vivo models, the exposome, adverse outcome pathways, and computational models and how this can help inform our discussion on chemical mixtures will be included in the roundtable talks. This roundtable will conclude with a debate on the different approaches to assess the potential health impacts of exposure to chemical mixtures.

**3222 Unlocking the Toxicity Archive: Enabling Toxicogenomic/Proteomic Investigation from Archival Samples**

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Formalin fixation and paraffin embedding (FFPE) is a cross-industry gold standard for preparing nonclinical and clinical samples for histopathological assessment which preserves tissue architecture and enables storage of tissue in archival banks. These archival banks are an untapped resource and vast repository of tissue from regulatory toxicology studies, novel animal bioassays, clinical trials, or epidemiologic studies with tremendous potential for assessing the toxicity profile of individual commodities. Humans however are routinely exposed to mixtures of chemicals through indirect routes such as via food and drinking water, thus use of FFPE archival tissues can inform our discussion on chemical mixtures will be included in the roundtable talks. This roundtable will conclude with a debate on the different approaches to assess the potential health impacts of exposure to chemical mixtures.
tions working in isolation and with limited resources, the ILSI Health Environmental and Sciences Institute (HESI) Genomics Committee FPFE Working Group has been developing methods to “demodify” FPFE RNA, enabling subsequent, more robust RNA-Seq analysis. Novel methods developed to improve RNA yield and sequencing results from limiting quantities and quality RNA FPFE samples will be highlighted, followed by discussion of frozen and fixed archival nonclonal and clinical FFPE tissue to interrogate mechanisms of toxicity, aid in biomarker development, enable creation of a critical translational bridge between emerging in vitro and in vivo data, and reproduce fully-induced adverse health effects established in prior toxicologic and epidemiologic studies, as well as enabling data gaps to be closed in adverse outcome pathways (AOPs).

3223  Good Cell and In Vitro Method Practices against the “Reproducibility Crisis”

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There is a strong belief that in vitro methods are fast becoming the key tool for a new way of doing toxicology. However, their potential will not be fully realized if they are not developed and applied in a way in which scientific integrity and quality are assured—the data they produce will not be trusted by decision makers. A reviving paper (Nature 2016, 533:452-454) showed that 70% of researchers have tried and failed to reproduce another scientist’s experiment, and more than half of the researchers failed to reproduce their own experiments. These dramatic data call for incentives for better practice. With the development of new high-throughput technologies, stem cells, and new culture technologies (organotypical cell cultures, organ-on-a-chip technologies), new challenges are presented for reproducibility of such advanced test systems. This session will summarize the challenges of working with in vitro cell and tissue-based culture methods and will describe the different initiatives that have taken place in recent years to give guidance on good cell and good in vitro method practices as measures against the reproducibility crisis in science. The session also will elaborate on the use of human-induced pluripotent stem cell-based systems for regulatory purposes and how to validate the new generation of high-throughput in vitro methods and microphysiological systems.

3224  Career Opportunities in Regulatory Toxicology

A. Lynch. Colorado State University and William H. Farland Consulting, LLC, Rockport, ME.

This session will serve as an introduction to current issues and opportunities in regulatory toxicology for graduate students and early-career scientists, as well as to motivate them to seek training experiences that will increase their knowledge of the approaches to and challenges of bringing modern-day toxicology into a risk or safety assessment process. The SOT Graduate Education Subcommittee, via its Awareness of Regulatory Toxicology (ART) Working Group, has assembled experts in regulatory toxicology to discuss opportunities available for toxicology trainees and others interested in pursuing careers in regulatory toxicology. Resources developed by ART for trainees interested in these careers also will be presented to attendees. Regulatory bodies within the United States and other countries use scientific data from various sources to assess the safety of chemicals and drug candidates in order to inform regulatory policy and determine approvals for use. In turn, industry uses scientific data to meet regulatory requirements and to achieve product stewardship and sustainability goals. Training in and application of modern, laboratory-based science must be married to the legislative and regulatory processes in order to inform decisions that are understandable and benefit the public. Many toxicology and postdoctoral training programs do not cover regulatory toxicology or regulatory processes to a significant degree. In addition, scientists who engage in the regulatory process through a government or public contact with specific issues may not be fully aware of the regulatory process and/or impacts of regulatory decisions. Although toxicologists often gain experience while on the job, accessing training or internships in regulatory toxicology early in a scientist’s career benefits trainees by increasing knowledge of how scientific data can be used in the public domain, as well as by increasing awareness of available job opportunities. In addition, knowledge of the regulatory use of toxicology information also benefits toxicologists considering service in an advisory capacity to government or industry.

3225  Atherosclerosis as a Model to Understand the Combined Effects of Environmental Chemical and Non-Chemical Stressors

D. Carlin. NIEHS, Research Triangle Park, NC.

Atherosclerosis can lead to cardiac infarction and stroke and is a foremost candidate for identifying health effects associated with chemical and non-chemical stressors since much is known about the morbidity and mortality of this multifactorial disease. However, the evaluation of cumulative human health effects from multiple environmental exposures (i.e., chemical and non-chemical) represents a special research challenge due to the inherent complexity of the topic and requires careful examination of the potential interaction of these exposures. For example, further exploration is required of the biological mechanisms/pathways by which exposure to both environmental chemicals (e.g., air pollution, polycyclic aromatic hydrocarbons, metals, polychlorinated biphenyls, pesticides, and endocrine disrupting chemicals) and non-chemical stressors (e.g., psychosocial, lifestyle, quality-of-life, poor nutrition, and physical stressors) over time leads to health effects and the roles the stressors may play in the development of diseases known to be associated with them (e.g., cancer, cardiac, metabolic, neurological, etc.). For the purposes of this session, combined exposures pertain to any set of environmental chemicals and non-chemical stressors that may contribute, individually or jointly, to damage in the human health vector of whether people are exposed to the chemical(s)/non-chemical stressors at the same/different times or through similar/distinct sources or routes. Some of the other areas requiring further research on this complex topic include a better understanding of both the composition of real-world exposure to chemical and non-chemical stressors; the potential biological interactions between chemical and non-chemical stressors; and the development and validation of predictive models of combined exposure toxicity to characterize the hazard associated with these combined exposures. This session will bring together experts to discuss the state-of-the-science pertaining to underlying complex biological mechanisms/pathways associated with, when combined, chemical and non-chemical stressors in relation to atherosclerosis. Specifically, presentations will include a general overview of the etiology of atherosclerosis from chemical and non-chemical stressors, the biological mechanisms being evaluated by the extramural community, atherogenic mechanisms of environmentally-relevant chemicals, how diet and physical activity may modify atherosclerotic events, and how conceptual models can be created to evaluate complex causal pathways in this disease. In addition, this symposium also may be able to be used as a model for other diseases known to be associated with both chemical and non-chemical stressors and the roles these stressors may play in cumulative risk.

3226  General Overview of the Public Health Burden of Atherosclerosis

W. Cascio. US EPA, Research Triangle Park, NC.

Cardiovascular disease is the most common cause of death globally and atherothrombosis is underlining pathological process that accounts for the vast majority of its morbidity and mortality. Atherosclerosis is a chronic non-communicable disease process with well-established risk factors that include age, sex, family history of premature myocardial infarction, smoking, high blood pressure, and cholesterol. Yet, these traditional risk factors do not account for all cases of cardiovascular disease implicating that other factors are acting independently or in concert with traditional factors to cause atherosclerosis and occlusive vascular disease. Exposure to environmental chemicals and non-chemical stressors are likely to be important determinants of atherothrombosis. In addition to providing a general review of the global burden of cardiovascular disease, this presentation will primarily focus on the environmental chemicals associated with atherosclerosis, their mechanisms of toxicity, and proposed links between chemical and non-chemical stressors in the development and progression of atherosclerosis. This presentation will also focus on the active hypotheses being explored and will introduce how the subsequent talks will address this interplay of chemical/ non-chemical stressors on atherosclerosis.
Atherosclerosis is a lipid-driven inflammatory disease characterized by macrophage accumulation in the vessel wall and is responsible for myocardial infarction and stroke. Global morbidity and mortality from this disease continue to rise, and undoubtedly atherosclerosis and its consequences are among the greatest health challenges faced in the US and around the world. Atherosclerosis is a chronic inflammatory process that begins several decades before its symptoms become clinically evident. The National Heart, Lung, and Blood Institute (NHLBI) supports a broad program of investigations evaluating the associations between non-chemical stressors and the progression of atherosclerosis and coronary artery disease, including population-based public health studies, clinical trials, and basic laboratory research. These include, but are not limited to, studies investigating 1) how chronic and acute psychosocial stress contributes to the initiation and progression of cardiovascular disease by interfering with macrophage dynamics, 2) how lifestyle (high fat diet, lack of exercise, chronic stress, sleep fragmentation, and depression) affects hematopoiesis and cellular and molecular macrophage function during atherosclerosis; 3) how social/emotional factors, diet, and stress affect atherosclerosis and the integrity of the blood vessels; 4) the study of neural, hormonal, and immunological mechanisms that may link biobehavioral variables to cardiovascular pathology. This presentation will focus on the potential mechanisms of non-chemical stressors linked to atherosclerosis and coronary heart disease and how they may possibly interact with known chemical stressors, which will further our understanding of the pathophysiology and prevention of this disease.

Atherosclerosis, the underlying cause of most CVD, is an inflammatory disease that develops slowly under the influence of genetic and environmental factors. Insulin resistance, endothelial activation/injury, and recruitment of leukocytes in the sub-endothelial space play a pivotal role in the etiology of atherosclerotic plaque development. Some toxic Superfund chemicals can injure endothelial cells or cause endothelial activation. This presentation focuses on our studies that show that exposure to toxicants such as volatile organic compounds (VOCs; i.e., acrolein and benzene) significantly decrease circulating endothelial progenitor cells (EPCs; Flik+/Sca+ cells) and augment insulin resistance in mice. Moreover, chronic exposure to acrolein or arsenic (another Superfund-relevant chemical) exacerbates atherosclerosis in apoE-KO mice. In vitro, both acrolein and arsenic augment the surface expression of adhesion molecules, promote monocyte adhesion and trans-endothelial migration and enhance cytokine formation. Mechanistic studies show that both acrolein and arsenic cause endothelial activation by triggering endoplasmic reticulum stress (ER-stress) and inducing unfolded protein response (UPR). Genetic ablation of UPR-associated inflammatory signaling or enhancing the protein-folding capacity by chemical chaperones of protein-folding attenuates acrolein or arsenic induced ER-stress and endothelial activation in vitro; and feeding with chemical chaperones of protein folding prevents arsenic-induced ER-stress, endothelial activation, and atherosclerotic plaque formation in apoE-KO mice. Acrolein also induces micro RNA-21 in macrophages, potentially as an adaptive response to inflammation; and myeloid cell specific deficiency of micro RNA-21 exacerbates atherosclerosis and lesion inflammation. Collectively, ER-stress induced endothelial activation and differential regulation of microRNAs associated with inflammatory signaling are plausible mechanisms by which toxic Superfund chemicals exacerbate atherosclerosis.

Atherosclerosis is a chronic inflammatory disease characterized by macrophage accumulation in the vessel wall and is responsible for myocardial infarction and stroke. Global morbidity and mortality from this disease continue to rise, and undoubtedly atherosclerosis and its consequences are among the greatest health challenges faced in the US and around the world. Atherosclerosis is a chronic inflammatory process that begins several decades before its symptoms become clinically evident. The National Heart, Lung, and Blood Institute (NHLBI) supports a broad program of investigations evaluating the associations between non-chemical stressors and the progression of atherosclerosis and coronary artery disease, including population-based public health studies, clinical trials, and basic laboratory research. These include, but are not limited to, studies investigating 1) how chronic and acute psychosocial stress contributes to the initiation and progression of cardiovascular disease by interfering with macrophage dynamics, 2) how lifestyle (high fat diet, lack of exercise, chronic stress, sleep fragmentation, and depression) affects hematopoiesis and cellular and molecular macrophage function during atherosclerosis; 3) how social/emotional factors, diet, and stress affect atherosclerosis and the integrity of the blood vessels; 4) the study of neural, hormonal, and immunological mechanisms that may link biobehavioral variables to cardiovascular pathology. This presentation will focus on the potential mechanisms of non-chemical stressors linked to atherosclerosis and coronary heart disease and how they may possibly interact with known chemical stressors, which will further our understanding of the pathophysiology and prevention of this disease.

Many human and environmental risk scenarios involve complex processes occurring across varying space and time scales, potentially following multiple causal pathways to the same endorgan, for example, the causal relationships between atherosclerosis and contributing environmental exposures and lifestyle choices, in the context of genetics and medical factors, is a challenging task. Complexity stems from differences in timing, magnitude, and mode of action of different types of stressors as well as their interaction with each other and with factors such as age and gender. One useful way to conceptualize this type of complex causal question is to visually map the problem space and the network of pathways from cause to effect. The resulting conceptual model, or series of models at different scales, organizes and enables cumulative risk assessment by identifying important risk components and mechanisms as well as multiple stressor junctures and multiple pathways to the same effect. For example, a variety of factors in sequence or separately can affect lipid status, such as dietary risk factors exacerbating diabetes or obesity or both. Having this understanding of the interrelationships between stressors is crucial in the search for data to quantify associations between causes and ensure the quality of data collected is appropriate to the causal question being addressed. If different stressors are measured with different levels of precision, or if they are not included as part of a joint pathway, then risk calculations are subject to error. As sub-pathways are better understood and quantified, conceptual models can and should be refined as part of the iterative process of real-world causal analysis. This leads to a more realistic, causal web-based, rather than linear chain-based, framework for cumulative toxicological inference. This presentation will provide information on how this modeling has been successful in the case of atherosclerosis and may be applicable to other diseases associated with chemical and non-chemical stressors.

This session will be composed of graduate and postdoctoral trainees presenting primary research focused on autophagic pathways that go awry in neurodegenerative diseases and how relevant environmental toxicants affect these pathways. Autophagy is a tightly regulated catabolic process that enables a cell to conduct bulk degradation of protein aggregates or dysfunctional organelles in a specific or nonspecific manner. Autophagic pathways have been observed to be impaired under a variety of neurotoxic and neurodegenerative disease conditions. Furthermore, environmental toxicants, such as neurotoxic metals, that are associated with altered risk or modification of neurodegenerative disease pathogenesis may impact with genetic and environmental factors by disrupting autophagy. Investigation of these pathways is likely to provide key insight into early cellular events of these diseases.
as disruptions to autophagic pathways occur prior to neurodegeneration or behavioral phenotypes. The session will begin with brief introductions regarding the structure of this trainee-led session and the trainees speaking, as well as a brief primer on autophagy and its potential role in neurodegenerative disease. In each talk, the trainee speakers will relate how autophagic dysfunction in their models contributes to disease pathogenesis and how their chemical(s) of interest exacerbate or ameliorate dysfunction. The first trainee speaker will discuss the role of autophagy in the toxicity of arsenic in cortical astrocytes. This speaker will present her findings on how AMPK and mTOR signaling regulate ATG5-dependent autophagy and apoptosis in astrocytes. The second trainee speaker will discuss the effects of manganese (Mn) exposure on autophagy-lysosome pathway in primary astrocytes, the mechanism(s) underlying Mn-induced autophagic dysregulation, and the functional relation between compromised autophagy and mitochondrial dysfunction. The third trainee speaker will continue the discussion about Mn in Huntington’s disease (HD), focusing on the contribution of excess manganese to neurons in HD and how the drug KB-R7943 can normalize Mn uptake. The fourth trainee speaker will change the topic to Parkinson’s disease (PD) and will delve into how optineurin, a protein previously not considered in PD, contributes to pathogenesis through autophagic dysfunction. The fifth trainee speaker will examine early mechanisms of PD and how they contribute to disease progression. The fifth trainee speaker will discuss the contribution of endosulfan to PD pathogenesis, as well as the relationship between autophagy and apoptosis in PD. The final trainee speaker will discuss the effects of low-dose chemical exposures on autophagic mechanisms in PD and further examine how these effects are altered even after chemical exposure is removed. Given that autophagy dysfunction is increasingly recognized as a common, early event in sporadic neurodegenerative diseases, it is imperative to understand how environmental chemicals disrupt normal autophagy, and 2) evaluate Mn-induced defects in autophagy in mouse primary astrocytes, the molecular mechanisms underlying this autophagic dysfunction, and its role in Mn toxicity. This presentation will show that Mn exposure disrupts autophagy in mouse astrocytes, partially by blocking the nuclear translocation of transcription factor EB (TFEB), a master regulator of autophagy and lysosome-related genes. Mn induces activation of ERK1/2, which phosphorylates TFEB to maintain it in an inactive cytosolic state. The causal relation between autophagy failure and mitochondrial dysfunction in order to determine whether impairment of autophagy directly contributes to the Mn-related cytotoxicity is currently being explored. This study highlights the mechanisms and functional role of impaired autophagy in Mn toxicity. These findings are critical in advancing Mn neurotoxicity studies as well as developing of therapeutic strategies for neurodegenerative diseases associated with autophagic dysfunction.

### 3232 Potential for Autophagy as a Primary Mechanism of Environmentally Induced Neurodegeneration

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

Autophagy is a broad term for the catabolic process of cellular degradation of cytosolic components, and is often referred to as a “double-edged sword” for the health of living cells. Disruptions in autophagy have been extensively examined in neurodegenerative disease models. However, most examinations, including those from neurotoxicological insults, have focused on autophagic disruptions as a pathological endpoint, instead of a primary mediator of toxicity or disease pathogenesis. This presentation will provide a brief overview on current knowledge in the role of autophagy in neurodegenerative diseases and how the work to be presented addresses key gaps of knowledge in the field. This brief introduction will set the stage for participants to understand the underlying scientific challenges being addressed by each speaker.

### 3233 Autophagy: Friend or Foe in Arsenic-Induced Toxicity?

**C. Garza-Lombo, University of Nebraska Lincoln, Lincoln, NE.**

Arsenic is a naturally occurring element found in groundwater as a byproduct of soil and rock erosion. Industrial and agricultural processes are also important sources for arsenic contamination. Chronic exposure to arsenic during development and adulthood induces cognitive dysfunctions, and is considered a risk factor for neurodegenerative disorders, but the mechanisms involved are still unclear. Astrocytes play an essential role in brain function as they regulate both neuronal excitability and homeostasis. They are also the first line of defense against xenobiotics crossing the blood brain barrier into the brain, buffering arsenic. It has been previously reported that autophagy protects neural cells against environmental xenobiotics. In contrast, in this work, the role of autophagy in the toxicity of trivalent inorganic arsenic species (AsIII) in glial cells will be reported. Exposure of primary cortical astrocytes to AsIII (arsenic trioxide [As2O3] or sodium arsenite [NaAsO2]) induced an increase in autophagy flux as evidenced by the dose-dependent accumulation of monomeric and aggregated p62, which was paralleled by the accumulation of ubiquitinated proteins. Impairment of autophagy by inhibition of class III phosphatidylinositol-3-kinases (PI3K) with wortmannin or by the overexpression of a dominant-negative (dn) form of the autophagy-related protein 5 (Atg5), significantly reduced the accumulation of p62 and ubiquitin-bound proteins. Interestingly, inhibition of autophagy, but not the proteasome (MG132), reduced caspase 3 cleavage/activation and cell death induced by AsIII. Similarly, trehalose protected astrocytes from AsIII, but this effect was shown to be independent from autophagy. Inhibition of the mammalian target of rapamycin (mTOR) with either rapamycin (mTOR complex I, mTORC1) or torin (mTORC1 and 2) stimulated AsIII toxicity. In addition, impairment of AMP-activated protein kinase (AMPK)–signaling by the overexpression of dnAMPK-ε1 and 2 reduced interest in autophagy against AsIII. Conversely, activation of AMPK with 5-aminimidazole-4-carboxamide ribonucleotide (AICAR) stimulated cell death in arsenic treated cells. The results demonstrate for the first time that AMPK/mTOR signaling and PI3K/Atg5-dependent autophagy regulate iAsIII-induced apoptosis in cortical astrocytes.

### 3234 Role of Autophagy in Manganese-Induced Neurotoxicity

**Z. Zhang, Albert Einstein College of Medicine, Bronx, NY.**

Metal exposure has long been recognized as an environmental factor that contributes to the development of neurodegenerative diseases. Exposure to excessive manganese (Mn) leads to an extrapyramidal disorder referred to as manganese, which shares a number of neurobehavioral deficits with Parkinson’s Disease. The mechanisms underlying Mn-induced neurotoxicity remain uncertain. The studies have characterized alterations in autophagy-lysosome pathway following 100 μM and 500 μM Mn exposures in mouse primary astrocytes, the molecular mechanisms underlying this autophagic dysfunction, and its role in Mn neurotoxicity. This presentation will show that Mn exposure disrupts autophagy in mouse astrocytes, partially by blocking the nuclear translocation of transcription factor EB (TFEB), a master regulator of autophagy and lysosome-related genes. Mn induces activation of ERK1/2, which phosphorylates TFEB to maintain it in an inactive cytosolic state. The causal relation between autophagy failure and mitochondrial dysfunction in order to determine whether impairment of autophagy directly contributes to the Mn-related cytotoxicity is currently being explored. This study highlights the mechanisms and functional role of impaired autophagy in Mn toxicity. These findings are critical in advancing Mn neurotoxicity studies as well as developing of therapeutic strategies for neurodegenerative diseases associated with autophagic dysfunction.

### 3235 Manganese Modifies the AKT/mTOR Pathway and Autophagy: Implications for Huntington’s Disease Pathology

**M. Bryan, Vanderbilt University Medical Center, Nashville, TN.**

Manganese (Mn) is both a neurotoxicant and essential in biological systems. This presentation will primarily discuss the speaker’s doctoral thesis project to 1) elucidate mechanisms by which Mn alters autophagy, and 2) evaluate the role of autophagy in Huntington’s Disease (HD). The STHdh model of HD exhibits decreased Mn uptake and reduced Mn-induced cell signaling (i.e. p53, AKT, and mTOR). A set of small molecules which impinge on Mn uptake have been identified that also impinge on p53 and AKT/mTOR pathways and downstream processes including autophagy. This suggests that Mn may be homoeostatically interwoven with autophagy. The effects of Mn exposure and Mn-increasing SMLs on neuronal and non-neuronal cell types were tested and found that Mn exposures (as low as 25μM) were capable of increasing LC3ii/I and p62 expression in the presence of chloroquine, a potent lysosomal autophagy inhibitor. These data indicate increased induction of autophagy by Mn. Furthermore, the effects of Mn and Mn-increasing small molecules was blunted in HD cells. This suggests that the HD mutation, which is known to impinge on autophagy, perturbs Mn-induced effects on autophagy as well. A role for Mn regulation in Huntington’s Disease is currently being explored. This study considers the role of optineurin (OPTN) in PD. OPTN, which is genetically linked to Parkinson’s Disease, is expressed in astrocytes. The OPTN protein is a key component of the autophagy machinery and is required for autophagic clearance of mitochondria (i.e. mitophagy) with many...
other biological roles that may be additionally implicated in PD. Here, we focused on the role of OPTN in mitophagy to evaluate the interaction between autophagic dysfunction and mitochondria dysfunction. The studies used the rotenone model of PD in vivo (wild-type Lewis rats exposed to rotenone at 3.0 mg/kg i.p.) and in vitro (SH-SYSY cells and primary embryonic midbrain cultures). The effects of OPTN overexpression on neurodegeneration and autophagy were investigated to evaluate its essential role in PD. Furthermore, we probed for OPTN immuno-staining in human brain tissues from PD patients and age-matched controls. The data suggest OPTN is a critical player in early cellular and molecular dysfunctions that contribute to PD pathogenesis.

3237 Environmental Neurotoxic Pesticide Endosulfan Induces Autophagy in Dopaminergic Neuronal Cells: Relevance to Etiopathogenesis of Parkinson’s Disease

A. Charli. Iowa State University, Ames, IA.

Chronic environmental exposure to pesticides, in particular organochlorine compounds, has been implicated in the etiopathogenesis of Parkinson’s Disease (PD). Although the extensive use of organochlorine insecticide endosulfan has been banned recently due to human health concerns, the compound is highly persistent in the environment. Previously, we showed that dieldrin, another potent organochlorine pesticide, induced dopaminergic neurotoxicity by activating a cascade of apoptotic signaling pathways in experimental models of PD. This study systematically investigated the effect of endosulfan on interplay between apoptosis and autophagy in dopaminergic neuronal cell culture model of PD. Exposure of endosulfan to the N27 dopaminergic neuronal cells rapidly induced autophagy as observed by increased number of autophagosomes and the accumulation of LC3-II. While the prolonged endosulfan exposure (>9 h), triggered apoptotic signaling including caspases activation (caspase-2 and -3), protein kinase C delta (PKCd) proteolytic activation, as well as cell death, demonstrated that autophagy preceded apoptosis during endosulfan neurotoxicity. Furthermore, inhibition of autophagy by wortmannin (phosphoinositide 3-kinase inhibitor) potentiated endosulfan-induced apoptosis, suggesting that autophagy is an early protective response against neurotoxic insult caused by the pesticide. Additionally, the studies found that Beclin-1, a major regulator of autophagy, was cleaved during the initiation of apoptotic cell death, and the cleavage was predominately mediated by caspase-2. Interestingly, CRISPR/Cas9 knockdown of prosapotic kinase PKCd significantly attenuated endosulfan-induced Beclin-1 cleavage and LC3-II accumulation. Also, caspase-2 and caspase-3 inhibitors effectively blocked endosulfan-induced apoptotic cell death. Additional studies in primary mesencephalic neuronal cultures confirmed the effect of endosulfan on autophagy and neuronal degeneration. Collectively, these results demonstrate that functional interplay between apoptosis and autophagy controls cell survival and death during pesticide-induced neuronal degenerative processes in dopaminergic neuronal cells. This study provides mechanistic insight into cell death mechanisms in environmentally linked neurodegenerative diseases. NIH grant ES10586, NS38644 and NS45133; Eugene and Linda Loyal endowment.

3238 Dopaminergic Cell Recovery and Resilience In Vitro: The Role of Autophagy

G. Harris. Johns Hopkins Center for Alternatives to Animal Testing, Baltimore, MD.

Currently, in vitro studies focus on short-term, high-dose exposures. Our work is aimed at understanding recovery or long-term effects after low-dose exposures and compound removal using rotenone as a known Parkinson’s-inducing compound and a 3D in vitro dopaminergic cell model. We have studied the short-term (24h) effects of non-cytotoxic concentrations and then compared these cellular responses to those which occur 7 days later (after compound removal). Acute mitochondrial impairment was observed by a decrease in cellular ATP after 100 nM, 24h treatment. One week after compound wash-out, ATP levels returned to control levels. Genes involved in autophagy (LC3, Optineurin, mTOR) were up-regulated short and long-term, indicating that this cellular process may play a role in rotenone-induced dopaminergic cell degeneration even after compound removal. Acutely, an increase in mitochondrial diameter was observed, suggesting swelling due to impaired fission. After 7 days, mitochondria were able to recover to the same size of controls. This presentation will discuss how rotenone-induced mitochondrial impairment can lead to long-term effects on autophagic clearance. The results demonstrate how in vitro low-dose, wash-out studies can be used to identify which mechanisms are relevant to long-term neurodegeneration and not only short term (reversible) neurotoxicity.

3239 The Role of the Epigenome in the Etiology of Metal-Induced Disease

R. Fry. University of North Carolina at Chapel Hill, Chapel Hill, NC.

Toxic metals, such as arsenic, cadmium, lead, and mercury, represent a major public health threat for populations worldwide. Exposures to these metals can be through various sources, including but not limited to, contaminated food and water. In terms of associated health effects, chronic exposure to toxic metals has been linked to cancers of numerous organs, including the lung, liver, and bladder. Toxic metals also are associated with non-cancer endpoints, such as adverse neurodevelopmental outcomes and cardiometabolic disease, including diabetes mellitus and cardiovascular disease in adults. Of particular concern are prenatal and early-life exposures to toxic metals that are associated with increased risk of low birth weight, preterm birth, susceptibility to infection, and later-life cancers. Among the many proposed cellular mechanisms that underlie toxic metals-associated disease are epigenetic modifications, including DNA methylation, histone modifications, and micro-RNA (miRNA) dysregulation. This symposium will highlight the complex role of the epigenome in the etiology of toxic metals-induced disease. The session speakers will present results from epigenomic studies spanning in vitro cell culture models, rodent models, and human populations exposed to toxic metals. The focus of this session will include diabetes, developmental effects in children, and metabolic disorders related to exposures to arsenic, cadmium, lead, and mercury. The presentations will highlight the role of toxic metals as epigenetic modifiers of DNA methylation and miRNA expression and mediators of a panoply of diseases. The use of epigenetic data for risk assessment and disease risk prediction also will be discussed.

3240 Prenatal Arsenic Exposure and the Epigenome: Informing Disease Mechanisms and the Risk Assessment Process

R. C. Fry. University of North Carolina at Chapel Hill, Chapel Hill, NC.

Inorganic arsenic is highly prevalent in the environment. Exposure to arsenic during pregnancy has been associated with detrimental health outcomes in infants as well as later in life. Among the early life outcomes related to prenatal arsenic exposure is a lower birthweight phenotype in infants. A molecular mechanism underlying the arsenic-associated birthweight effect is expected to include epigenetic modifications including CpG methylation and altered miRNAs expression. In a pregnancy cohort in Gomez Palacio, Mexico, arsenic-associated changes in CpG methylation were investigated using a genome-wide gene-specific assessment. Functional consequences of the DNA methylation at the transcript level were evaluated. Prenatal exposure to arsenic was associated with altered CpG methylation, subsequent gene expression and birth outcomes. Among the significant genes identified was potassium voltage-gated channel subfamily Q member 1, KCNQ1. This imprinted gene plays a role in infant birthweight/fetal size at birth as well as later life disease such as diabetes. Using a benchmark dose modeling approach, the arsenic concentration at which these epigenetic effects are observed was determined. This information, including a mechanism for the site-specific arsenic-associated DNA methylation, is useful for a more comprehensive understanding of the mechanisms underlying arsenic-associated disease. Furthermore, the data are critical for informing the risk assessment process.

3241 The Role of microRNAs in the Etiology of Diabetes Associated with Chronic Exposure to Arsenic

P. Sethupathy. Cornell University, Ithaca, NY. Sponsor: R. Fry

Inorganic arsenic (iAs) is a pervasive environmental diabetogen. Laboratory studies have shown that iAs may act as an inhibitor of insulin secretion or signaling, or may alter glucose metabolism in the liver or extrahepatic tissues. However, the molecular mechanisms underlying these effects are poorly understood. Over the last decade, microRNAs (miRNAs) have emerged as markers of environmental exposure and as potential regulators of pathways maintaining glucose homeostasis, including glucose stimulated insulin secretion (GSIS) by pancreatic beta
cells as well as the insulin-activated signal transduction pathway that regulates glucose utilization in the liver and peripheral tissues. Notably, several miRNAs that are linked to defects in the glucose regulating pathways have also been reported as responsive to iAs exposure in cultured cells or laboratory animals. Studies in our laboratory have found that miR-34a, which has been shown to regulate hepatic insulin sensitivity, is significantly upregulated in liver of mice exposed to iAs. Moreover, treatment with iAs increased glucose uptake into liver cells in vitro, and that miR-146a, which has been implicated in the control of GSIS, is significantly upregulated in pancreatic islets after iAs exposure. We have also shown that inhibition of GSIS in beta cells exposed in vitro to iAs is associated with altered expression of miR-146a, as well as at least one other miRNA, miR-20b and miR-217. While, like miR-29b, the miR-29b has been previously linked to the control of GSIS, it has not been well-studied in the context of beta cell function. Our current studies use samples and data from arsenic exposure cohorts in Mexico to characterize associations between the levels of these (and other) miRNAs and iAs exposure and metabolism, and clinical indicators of diabetes. The ultimate goal of this research is to determine if miRNAs are mediators of diabetes associated with iAs exposure and/or if they can be used as biomarkers of this disease.

C. Marsit, Emory University, Atlanta, GA. Sponsor: R. Fry

Cadmium is a ubiquitous environmental toxicant and exposures during pregnancy can impact fetal development leading to reduced fetal growth and neurobehavioral impairments. Unlike other toxic metals, the majority of cadmium does not pass from the maternal circulation to that of the fetus, but instead is sequestered within the placenta. This would suggest that the effects of cadmium on fetal and child health outcomes may be related to effects on placental function. We examined cadmium’s epigenetic effects by profiling genome-wide DNA methylation and transcription of all known imprinted genes in birth cohort studies from the northeastern United States, the New Hampshire Birth Cohort Study and the Rhode Island Child Health studies, using placental cadmium levels as the exposure of interest. We identified 17 Cd-associated differentially methylated CpG sites with meta-analysis p-values < 1e-05. Interestingly, methylation levels at 9 of the 17 loci were associated with increased expression of 6 genes (5% FDR); and higher placental expression of TNAIP2 and ACOT7, and lower expression of RORA, were associated with lower birth weight z-scores (p-values < 0.05). Placental cadmium concentrations were also associated with the expression of a panel of growth-related imprinted genes, such as H19 and IGF2, and the specific expression of these genes was associated with childhood growth at age 2, suggesting that these placental effects can lead to long-term programming of the offspring. Taken together, these results suggest that cadmium may elicit its reproductive effects by driving epigenetic changes within the placenta, and ultimately, this data may be used to improve to improve prevention or intervention efforts as well as to define biomarkers useful for identifying infants at-risk in order to assure early intervention efforts can be employed.

A. Barchowsky, University of Pittsburgh, Pittsburgh, PA.

Arsenic exposure is associated with increased metabolic disease risk and an inability to control metabolic syndromes. As skeletal muscle is the largest metabolic organ in the body, disruption of normal muscle metabolism, maintenance, and regeneration poses a high risk of all cause and cardiovascular mortality. Epidemiological studies indicate that muscle weakness and wasting is a primary pathologic sequelae caused by arsenic in at least 10% of exposed individuals, essentially affecting tens of millions of individuals worldwide. We find that exposure of mice to low to moderate levels of arsenic in drinking water targets the mitochondria in skeletal muscle fibers, interstitial cells, and stem (satellite) cells, producing a maladaptive shift in metabolism that impairs regeneration and promotes adipose infiltration (myosteatosis). The aberrant mitochondrial behavior of interstitial and satellite cells is retained and imprinted in the memory of their progeny long after arsenic is removed, and these phenotypes are reinforced by epigenetic changes, which includes DNA methylation, and histone modifications. The muscle progenitor cells are driven towards proliferation and stemness rather than being able to differentiate and regenerate muscle at the same time that interstitial cells are driven to elaborate a matrix and niche that directs the muscle stem cells towards fibrogenic differentiation. Intervention after arsenic exposure with a mitochondrially targeted radical scavenger, XJB-5-131, reverts the cell phenotypes allowing proper mitochondrial dynamics and function, as well as stem cell behavior. This reversion of mitochondrial homeostasis restores normal patterns of DNA methylation and histone marks on epigenetic regulatory genes. These studies identify mitochondrial-nuclear communications as potential target for prevention or reversion of pathogenic, epigenetic regulation of arsenic-induced metabolic disease and muscle morbidity.

A. Baccarelli. Columbia University, New York, NY. Sponsor: R. Fry

Prenatal exposure to metals, even at low levels, has been associated with a range of adverse health outcomes that may manifest themselves later in life. Epigenetic programming of life from conception to birth has been widely indicated as a biological process that may mediate the effects of metals on postnatal health outcomes. The wide availability of laboratory methods for epigenome-wide association studies (EWAS) now permits to conduct agnostic screens of the influences of environmental exposures on the human epigenome. In particular, DNA methylation-wide analysis are particularly cost effective and have been largely used in human cohorts. However, human data correlating prenatal exposure to metals with differences in DNA methylation in the child are still sparse. In this presentation, we will report novel findings from two EWAS data analyses conducted in the longitudinal pre-birth Project Viva cohort. We generated epigenome-wide methylation data using the Illumina Infinium 450K Methylation BeadChip in cord blood as well as during early childhood (range: 2.9 to 4.9 years) and mid-childhood (range: 6.7 to 10.5 years). We conducted the first EWAS analysis to identify methylation sites associated with prenatal levels of mercury exposures. A second EWAS analysis searched for methylation sites associated with lead exposures. Our EWAS analyses identified multiple methylation sites. We conducted in-silico analysis to determine the association of methylation at these sites with gene expression, as well as the correlation of blood methylation with target tissues, such as for instance brain methylation. We examine neurocognitive outcomes and their association with metal-related methylation sites. These studies provide an example of the use of EWAS analysis in human studies of metals and open the way to mechanistic studies to identify the specific role of DNA methylation in metal toxicity.

B. van Rensenwaay. BASF, Ludwigshafen am Rhein, Germany.

The microbiome has emerged as a key regulator of development and disease. A disruption in host-associated microbial communities is correlated with obesity, immune, and cardiovascular diseases and, increasingly, with adverse developmental outcomes. Current research is starting to unravel microbiome-host interactions that may explain these effects by examining microbiota as a target or mediator of chemical toxicity. One mechanism by which microbiota may modify toxicity is via chemical-dependent changes in the production of small molecules that disrupt normal biological processes in the host. Humans share 99.9% of their genome, yet the vastly larger microbiota-genomic space is much less conserved and, therefore, may partially explain inter-individual susceptibility to chemical exposures. Due to high inter- and intra-species variability within microbiota and its responsiveness to external factors, it is essential to determine the effects of chemical exposures on gut microbiota composition, function, community organization, and resulting host-microbiota interactions. There also is a need to identify molecules produced by gut microbiota and their effects on the host, as well as understanding how xenobiotic compounds are biotransformed by resident microbiota. This mechanistically-focused session highlights emerging interactions between gut microbes and the host that contribute to altering exposure and susceptibility to drugs and chemicals. The first talk will provide an overview of the metabolic capacity of the gut microbiome and, using a combination of metabolomics, metabolonomics, synthetic biology, and cutting-edge experimental host-microbiota model systems, reveal the metabolic characterization of individual microbial species and their roles in human biology and disease. The second talk will focus on how gut microbiota modify the biochemical impact of chemical transformations of food carcinogens and how these reactions are mediated by microbial communities. The third presenter will describe an innovative zebrafish model to test whether host-associated microbiota modify the developmental neurotoxicity of environmental chemicals. The fourth presenter will discuss microbiota in the context of cancer therapeutics. The fifth talk will provide a framework for the assessment of risks associated with microbiome changes via the investigation of the microbiome's functionality, defined as the production of
metabolites absorbed by the host. Hazard identification and risk assessment
do not currently consider host-microbiota interactions that are
disrupted by xenobiotic exposure. This session will bring together aca-
demic, government, and industry scientists who are using novel exper-
imentsal systems to determine the composition and organization of gut
microbiota. Relevant to the field of toxicology, the effects of chemical
exposures on host-microbiota interactions and the mechanisms by
which microbiota perform biotransformations of drugs and environ-
mental chemicals will be discussed in this session.

**3248 Environmental Chemicals Disrupt the Microbiota-Gut-Brain Axis during Zebrafish Development**

T. Tal, US EPA, Research Triangle Park, NC.

Growing evidence indicates that host-associated microbiota modify
the toxicokinetics and/or toxicodynamics of environmental chemicals;
however, current toxicological assessments do not consider interactions
between microbiota and chemical toxicity. Relative to conventionally
colonized zebrafish larvae, findings have previously reported that axenic
(microbe-free) or colonized zebrafish exposed to antibiotics are hyper-
active. Studies therefore hypothesized that neurobehavioral toxicity
elicited by exposure to environmental chemicals may be mediated by
altered microbial colonization during development. Differences were
explored in swimming behavior, microbial community structure, and
chemical metabolism in axenic and conventionally colonized zebrafish
larvae that were semi-statically exposed to the antimicrobial triclosan
(0.1-0.3 µM) or vehicle (0.1% DMSO) on 1, 6, 7, 8, and 9 days post fer-
tilization (dpf). Triclosan exposure had no effect on locomotor activity
in axenic larvae at 10 dpf. In comparison, locomotor hypoactivity was
observed in conventionally colonized larvae exposed to 0.3 µM triclosan.
Also at 10 dpf, triclosan exposure triggered concentration-dependent
shifts in microbial community structure. To understand the temporal
dynamics of triclosan-induced hypoactivity, conventionally colonized
larvae were exposed to 0.3 µM triclosan on 1 dpf or on 1, 6, 7, 8, and 9
dpf. Media changes were performed on days 6, 7, 8, and 9, regardless
of group. Triclosan caused hypoactivity at 10 dpf in larvae exposed on
1, 6, 7, 8, and 9 dpf, but not in larvae exposed on 1 dpf. High resolution
mass spectrometry will be used for non-targeted chemical analysis; pre-
liminary data suggests elevated concentrations of triclosan (ng/larva) in
larvae exposed at 1, 6, 7, 8, and 9 dpf as compared to larvae exposed at
1 dpf. Taken together, these data suggest that host-associated micro-
biota modulate the ability to bioactivate triclosan and trigger subsequent
locomotor hypoactivity. This abstract does not necessarily represent US
EPA policy.

**3249 Discovering and Controlling the Gut Microbiome’s Impact on Xenobiotic Metabolism**

M. Redinbo. University of North Carolina at Chapel Hill, Chapel Hill, NC. Sponsor: T. Tal

The diverse and complex communities of GI microbiota play astonish-
ingly important roles in human health via many mechanisms, including
tuning the immune system, impacting neurological function, and
enhancing digestion and energy utilization. Our studies sought to probe
the roles specific pathways play in overall mammalian homeostasis
within the GI. Thus, the studies focused on systems where the bacteria
catalyze a reaction that exerts a profoundly negative impact on intes-
ninal tissues. The anticancer drug irinotecan, a member of the campto-
thecin family of topoisomerase I poisons, is widely used in the treat-
ment of the FOLFIRI and FOLFIRINOX regimens to treat colon and pancreatic cancers,
respectively. Like many toxic compounds, irinotecan is eliminated as an
inactivated drug-glucuronide by the Phase II drug-metabolism systems
in mammalian protective tissues. Irinotecan’s dose-limiting toxicity is
severely delayed diarrhea hypothesized to arise from the reactivation of
the inactive irinotecan metabolite in the intestinal mucosa catalyzed by a
microbial gene product expressed by the symbiotic GI bacteria. Studies
pinpointed the molecular basis of this reactivation to the beta-glucuro-
nidase sugar scavenging enzymes present in a wide variety of GI resi-
dent bacteria. Potent, selective, and non-lethal inhibitors to bacterial
beta-glucuronidase (GUS) enzymes were developed and demonstrated
that they prevented GI injury in a mouse model of irinotecan-induced
intestinal damage. Findings resolved the crystal structures of numerous
GI microbial GUS enzymes both alone and in complexes with novel
inhibitors, and elucidating the basis for the 10,000-fold selectivity the
compounds show toward bacterial GUS relative to the mammalian
enzyme ortholog, which is essential. Finally, the work was extended to
NSAIDs, which are also glucuronidated and cause GI damage by pro-
ducing small intestinal ulcers. Findings show that bacterial GUS inhib-
itors prevent the formation of these sites of intestinal damage in mice
treated with the NSAIDs diclofenac, indomethacin, and ketoprofen.
Together, the data suggests that enzyme targets in the GI microbiota
can be inhibited to improve human health. Furthermore, the results
shed significant light on one aspect of the mammalian-microbial axes of
chemical communication on-going between the two domains of life in
the “higher-order” human superorganism.
The intestinal microbiome contributes to the metabolism of its host. With the help of an artificial shift of the microbiome with antibiotics, microbiome-derived metabolites have been identified that are absorbed by the host and thus can be found in the blood, amongst which hippuric acid and indole acid are of major importance. Effects of antibiotics on the “functionality of the microbiome” were studied, defined as the production of metabolites absorbed by the host, and determined the gut microbiome’s composition of Wistar rats via 16S rRNA sequencing. A further aim of this study was to prove if the same metabolites analyzed in plasma could be found in feces, cecum content and gut tissue. The studies applied broad-spectrum antibiotics from different classes which were administered 28 days orally to rats for metabolic profiling in plasma and in different matrices (feces, cecum content, gut tissue). For the community, analysis via a 16S rRNA sequencing the gDNA of the feces was isolated. Treatment-related effects could be observed in a principal component analysis for feces and cecum content, but not for gut tissue. For each class of antibiotics specific metabolome patterns from plasma could be established in the MetaMap Tox data base, which contains metabolome data for more than 550 reference compounds. The results indicate that many biomarker metabolites in plasma could be derived from the microbiome because they were found in the immediate surrounding tissue of the bacteria. To determine the functionality of the microbiome for its host, methods still need to be developed. The results of this present work suggest that blood based metabolic profiling could be a suitable tool for this purpose.
Rusyn metal mixtures have the potential to predict mammalian response. Those exposed to 8μg/mL and higher. These concordant findings indicate C. elegans larvae exposed to 2-4μg/mL mercury developmental delay in humans and rats. Conversely, in some studies low levels of mercury in ment reduces offspring and induces a strong oxidative stress response have shown that sodium arsenite exposure during spermatid development as a mechanism of arsenic toxicity on mammalian sperm. We for responses to inorganic arsenic and mercury.

Surveillance of chemical exposure requires analytical platforms offering rapid measurements, high sensitivity, efficient separations, wide dynamic ranges, and applicability to a broad chemical space. We have developed a platform and pipeline that meets these needs by combining solid phase extractions with ion mobility spectrometry and mass spectrometry (SPE-IMS-MS). This platform is capable of performing both targeted and global measurements of endogenous and exogenous small molecules in human biofluids and environmental samples with high reproducibility, sensitivity, and throughput. This exposomics approach overcomes many challenges for large scale assessment methods and is a viable way of screening environmental conditions and patient cohorts for insight into human exposure and disease mechanisms.

Population-Based Studies before, during, and after Emergencies: Building Resilience in Coastal Communities

G. Sansom. Texas A&M University, College Station, TX. Sponsor: L. Rusyn

Much of our current understanding of the population health impacts of disasters is based on disaster-specific case studies with relatively small sample sizes. Typically, reliable baseline data is not available for comparison. Post-disaster research can be expensive, time consuming, and may place burdens on individuals or systems during response and recovery. Because of their focus on a single event, this type of research limits capacity to enhance the resilience of individuals or the communities in which they live to future disasters of a different type, scale, or location. New approaches to environmental health research using open, citizen science, and interdisciplinary data are needed to address these gaps and engage the communities in solving environmental and man-made disasters.

Utility of Caenorhabditis elegans for Predictive Heavy Metal Toxicity Assessment


The toxicity effects of exposure to individual heavy metals have been well studied. Information on the effects of exposure to heavy metal mixtures is urgently required, however. Alternative toxicity models have the potential to provide relevant data for human safety assessment of chemicals with rapid turnaround times and at greatly reduced cost relative to traditional models. Caenorhabditis elegans is a tiny nematode that is inexpensive to maintain, and most assays can be completed in a week or less by a single researcher. However, before data obtained from an alternative model can be used for resource allocation and/or regulatory decisions, that model must first be evaluated for the capacity to predict human toxicity. We assessed the concordance between mammals and C. elegans for responses to inorganic arsenic and mercury. Inorganic arsenic exposure reduces sperm count and quality in rodents, and co-exposure to antioxidants reverses these effects, indicating oxidative stress as a mechanism of arsenic toxicity on mammalian sperm. We have shown that sodium arsenite exposure during spermatid development reduces offspring and induces a strong oxidative stress response in C. elegans, even at low concentrations that do not alter larval growth or motility. Mercury exposure is associated with reduced total movement, reduced speed of movement, and reduced coordination in both humans and rats. Conversely, in some studies low levels of mercury in blood are associated hyperactivity in children. Using a novel developmental neurotoxicity assay, we have identified hyperkinesia and slight developmental delay in C. elegans larvae exposed to 2-4μg/mL mercury acetate, and hypokinesis plus more extreme developmental delay for those exposed to 8μg/mL and higher. These concordant findings indicate that further studies in C. elegans assessing the effects of heavy metal mixtures have the potential to predict mammalian response.

Nuclear Receptor Disruption Alters Triphenyl Phosphate-Induced Cardiotoxicity in Zebrafish Embryos

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Triphenyl phosphate (TPHP) is an unsubstituted aryl phosphate ester used as a high-production volume flame retardant and plasticizer within the United States. Using zebrafish as a model, the objectives of this study were to 1) rely on mRNA-sequencing to uncover biological processes disrupted following embryonic TPHP exposure and 2) rely on high-content screening to identify nuclear receptor ligands that enhance or mitigate TPHP-induced cardiotoxicity. For mRNA-sequencing, embryos were exposed from 24 to 72 h post fertilization (hpf) under static conditions to vehicle (0.2% DMSO) or TPHP (5, 10, or 20 μM). Relative to vehicle controls, TPHP exposure resulted in a concentration-dependent increase in the number of transcripts significantly affected at 72 hpf (873, 1520, and 4256 transcripts following exposure to 5, 10, and 20 μM TPHP, respectively). While stress response-related effects represented the majority of impacted processes within embryos exposed to 10 and 20 μM TPHP, a cardiac-specific category represented the only process affected within embryos exposed to 5 μM TPHP - a concentration that did not result in detectable effects on larval development. For the ligand library screen, embryos were exposed from 24 to 72 hpf under static conditions to vehicle (0.2% DMSO) or 20 μM TPHP in the presence or absence of 74 unique nuclear receptor ligands. Hatched and alive 72-hpf embryos were analyzed for cardiac looping defects - a readily measurable biomarker for cardiac loop defects. Based on this screen and follow-up experiments, two compounds - ciglitazone (a peroxisome proliferator-activated receptor gamma, or PPARy, agonist) and fenofibrate (a pan-retinoic acid receptor, or RAR, agonist) - reliably mitigated TPHP-induced cardiotoxicity. These data suggested that TPHP may be activating retinoid X receptor (RXR, a heterodimer for both RARs and PPARy), co-exposed embryos to TX531 - a pan-RXR antagonist - from 24 to 72 hpf, and found that co-exposure to TX531 significantly enhanced TPHP-induced cardiotoxicity. However, using a luciferase reporter assay, we found that TPHP did not activate nor inhibit chimeric human RXR, RXRβ, or RRY within CHO cells, suggesting that TPHP does not bind to nor interact with RXRs. Overall, our data suggest that TPHP may directly or indirectly interfere with retinoid acid signaling pathways involved in cardiac development.
**3261 Expression of Genes Inolved in Xenobiotic Metabolism in the Chicken Egg Alternative Genotoxicity Model**

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The Chicken Egg Genotoxicity Assay (CEGA) has demonstrated an intrinsic ability to bioactivate various DNA-reactive chemicals. While the activities of major phase I and II metabolic enzymes in avian fetal livers has been described, data on gene expression profiles can significantly contribute to the understanding of metabolic capabilities of avian fetuses. For this purpose, fertilized chicken eggs were dosed under the CEGA protocol with vehicle (deionized water (DW)), a carcinogen that requires metabolic bioactivation, diethylnitrosamine (DEN), and its structural non-carcinogenic comparator, N-nitrosodietanolamine (NDELA). The gene expression data obtained from chicken fetal liver samples using microarray technology was analyzed. Recently in CEGA, DEN produced DNA damage, while NDELA did not. Expression in fetal livers of 463 genes that are known to be involved in the xenobiotic metabolism were detected on the array. DW did not affect gene expression profile of the selected genes, deregulating less than 1% of genes. In contrast, both chemicals, DEN and NDELA, at 4 mg/egg each produced significant changes in gene expression pattern, up-regulating over 33% and down-regulating over 43% of genes. When gene expression profiles of DEN and NDELA dosed eggs were compared, the majority of the genes were expressed in a similar manner, both compounds shared the same up-regulated and down-regulated genes. Among up-regulated genes were CYP 450 genes that are responsible for DEN biotransformation (hydroxylation) to the reactive moiety. Among down-regulated genes were those that regulate detoxication enzymes such as glutathione S-transferase, sulfotransferase and UDP glucuronosyltransferase. Thus, the analysis of gene expression data in fetal chicken livers exposed to dialkylnitrosamines confirmed the hypothesis that avian species possess complex metabolic activity, which underlies their capability to biotransform a variety of chemicals. In the pair of genotoxic and non-genotoxic compounds studied here, genes were deregulated in a similar fashion, indicating similarity in the metabolism of DEN and NDELA.

**3262 Valproate Analogue Developmental Toxicity Potency in a Human Pluripotent Stem Cell-Based Assay Is Concordant with In Vivo Potency**

J. Palmer1, A. Smith1, R. Burrier1, E. Donley1, F. Kirchner1, D. Kroese2, R. Stober3, and N. Kleinstreuer4. 1National Toxicology Program (NTP) in support of Tox21. In this study, the devTOX assay was consistent with observed developmental toxicity potency of chemicals based on changes in hPS cell metabolism. The assay has been used by multiple industries and, of note, by the United States Environmental Protection Agency (EPA) and the National Toxicology Program (NTP) in support of Tox21. In this study, the devTOX assay included phthalates, developmental vascular toxicants, and neuroactive GPCRs (dopamine, serotonin, endothelins), estrogen signaling, and RAR antagonists. The devTOX assay measures the responses of a pluripotent h9 stem cell response. To address this question, a logistic regression model was built via machine-learning to mine the strongest positive and negative correlates to 331 enzymatic and receptor signaling assays in the ToxCast NovaScreen dataset (NVS). An aggregate model using the lower dose NVS model defined 47 positive and 38 negative correlations. Top sensitive pathways in the STM-NVS model were kinase signaling, some neuroactive GPCRs, and corticotrophs. In contrast, top negative correlations were observed with other neuroactive GPCRs (dopamine, serotonin, endothelins), estrogen signaling, and RAR antagonists. These findings indicate that the scrT assay includes distinct cellular processes that potentially account for the sensitivity of the STM model and guide a battery of fit-for-purpose ToxCast assays covering potential in vitro outcomes. This abstract may not reflect US EPA policy.

**3263 Profiling the ToxCast Library with a Pluripotent Human (H9) Embryonic Stem Cell Assay**

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The Stemina devTOX quickPredict platform (STM) is a human pluripotent H9 stem-cell based assay that predicts developmental toxicants. Using the STM model, we screened 1065 ToxCast chemicals and entered the data into the ToxCast data analysis pipeline. Model performance was 83.3% accuracy (sensitivity 0.78, specificity 0.92) based on 30 common reference compounds in the dataset. Of the 1065 screened chemicals, the STM model predicted 181 (17% tested) as putative developmental toxicants. STM predictivity versus animal studies was examined by a concordance model using prenatal rat and rabbit developmental toxicity studies for 146 chemicals testing positive (dLEL ≤ 125 mg/kg/day) or negative (no dLEL ≥ 1000 mg/kg/day) in ToxRefDB. The cell-based STM model had 76.7% accuracy (sensitivity 0.34, specificity 0.84) when compared with the in vivo results. Compound classes detected by the assay included phthalates, developmental vascular toxicants, and developmental neurotoxicants; misses included glycol ethers, perfluorinated compounds, and endothelin antagonists. The lower sensitivity of the STM model suggests certain pathways/targets may fall outside the responses of a pluripotent H9 stem cell response. To address this question, a logistic regression model was built via machine-learning to mine the strongest positive and negative correlates to 331 enzymatic and receptor signaling assays in the ToxCast NovaScreen dataset (NVS). An aggregate model using the lower dose NVS model defined 47 positive and 38 negative correlations. Top sensitive pathways in the STM-NVS model were kinase signaling, some neuroactive GPCRs, and corticotrophs. In contrast, top negative correlations were observed with other neuroactive GPCRs (dopamine, serotonin, endothelins), estrogen signaling, and RAR antagonists. These findings indicate that the scrT assay includes distinct cellular processes that potentially account for the sensitivity of the STM model and guide a battery of fit-for-purpose ToxCast assays covering potential in vitro outcomes. This abstract may not reflect US EPA policy.

**3264 Medium-Throughput Procedures for Species-Specific, Functional Analyses of Developing Human, Rat, and Mouse Primary Neurospheres: From In Vitro to In Silico**

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Testing for developmental neurotoxicity (DNT) of compounds for regulatory purposes, e.g. for screening and prioritization, by using an in vitro testing battery is currently under discussion at the OECD level. Within such a battery a variety of neurodevelopmental processes at different developmental time-points needs consideration. We have been establishing a DNT high content assay based on time-matched fetal human and postnatal rodent neural progenitor cells (NPCs), which proliferate in culture and - under differentiating conditions - migrate and differentiate into neurons and glia cells. We tested more than thirty compounds with this so-called ‘Neurosphere Assay’ for their ability to interfere with human, mouse and rat neurodevelopmental processes. Manual and automatic endpoint evaluations are implemented in the Omnibus software, which exports analysis endpoints as complete concentration-response information into a relational database. Maintaining a structured database of concentration-response information provides a resource from which IC50 values for compound effects on neurodevelopmental endpoints and corresponding viability tests can be computed and compared. Specific heat maps visualize potency of DNT compounds on individual endpoints, across endpoints and in a species comparative manner. More generally, a database resource will facilitate evaluation of a set of preclinical compounds across multiple neurodevelopmental endpoints for DNT in vitro evaluation. As data from more substances will accumulate in such database, a structured data repository will allow to flexibly apply novel and advanced data analysis approaches in the future.
Embryotoxicity is an essential toxicological endpoint in the registration process of chemicals and drugs. Alternative methods to animal testing are being developed for developmental toxicity and applied already for screening purposes. Nevertheless, without transfer of an active substance via the placenta, potential direct effects on the embryo can be neglected. Therefore, considering placental transfer is necessary. The in vitro placental transfer model can be used to determine the placental transfer rate by determining the apparent permeability coefficient (Papp value) of a substance. Using a trophoblastic cell line (BeWo b30) on a transwell system, a cell barrier is formed separating the apical (representing maternal side) from the basolateral (representing fetal side) compartment. However, varied protocols exist and often insufficient characterization is performed. Therefore, in the present study the main methodology is extensively characterized and optimized to enable screening processes. Characterization parameters included are trans-epithelial electrical resistance (TEER), fluorescein transfer (paracellular lumen control), histology, immunohistochemistry of cell tight junctions and transfer of permeability controls amoxicillin (low) and antipyrine (high). The optimization alters the frequency of medium change. Stable cell layer integrity was observed on day 6 based on the characterization parameters, which also showed high reproducibility. The TEER increased from day 3 to day 6 reaching a value of 46.8 Ω·cm². This increase was followed by a decreased of 80% on the Fluorescein transfer, confirming the barrier integrity. The Papp values of permeability controls amoxicillin and antipyrine were in the literature range for both methodology. Moreover, several substances, difenoconazole, flusilazole and triadimenol, were tested for its placental transfer rate, resulting in Papp values from 7.8 to 41.8 x10⁻⁶ cm/s. This highlight that even compounds (high). The optimization alters the frequency of medium change. Stable cell layer integrity was observed on day 6 based on the characterization parameters, which also showed high reproducibility. The TEER increased from day 3 to day 6 reaching a value of 46.8 Ω·cm². This increase was followed by a decreased of 80% on the Fluorescein transfer, confirming the barrier integrity. The Papp values of permeability controls amoxicillin and antipyrine were in the literature range for both methodology. Moreover, several substances, difenoconazole, flusilazole and triadimenol, were tested for its placental transfer rate, resulting in Papp values from 7.8 to 41.8 x10⁻⁶ cm/s. This highlight that even compounds...
Mechanistic Evaluation of Improved Testes Structure and Function in Fathead Minnows Exposed to Insensitve Munitions

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Previous toxicological investigations of the insensitive munition, 3-nitro-1,2,4-triazol-5-one (NTO), demonstrated histopathological and physiological impacts in mammalian testes. Here, we investigated the effects of 21 days NTO exposure and IMX-101 (composed of NTO, 2,4-dinitroanisole (DNAN), and nitroguanidine) exposure in adult male fathead minnows to assess if impacts on testes were conserved in fish. NTO exposures included 0 (control), 94, 192, 383, and 720 mg/L (measured concentrations) where no significant effects on survivorship were observed. Conversely, the IMX-101 exposures at 6.3, 12.5, 25 and 50 mg/L (nominal, NTO + DNAN + NQ) significantly decreased survivorship at the two highest concentrations, driven by DNAN. NTO exposure significantly impaired spermatogenesis at the 383 mg/L and 720 mg/L exposures where development of stage 3 epithelium was impaired resulting in disproportionately high spermatocytes and necrosis in secondary spermatocytes. The highest NTO exposure also caused testicular degeneration. Microarray-based transcriptomics identified steroid biosynthesis as the most significantly enriched pathway in response to NTO exposure while the structure/function of the axoneme was the most significantly enriched annotation cluster. These results suggest potential impacts on steroidogenesis in testes in addition to the impacts of flagellar molecular motor structure critical in spermatogenesis. The 12.5 mg/L IMX-101 exposure caused a significant increase in sperm necrosis, interstitial fibrosis, and Sertoli-like cell hyperplasia. The most enriched pathways and annotations observed in the IMX-101 exposure indicated changes in genetic signals including RNA transport, zinc-finger PHD motifs, and helicase activity, perhaps indicative of systemic toxicity when approaching the lethal-threshold for IMX-101. The results indicate that NTO can elicit histopathological impacts on fathead minnow testes impairing spermatogenesis likely via impaired steroidogenesis, however these component effects of NTO occur at concentrations far exceeding the lethal concentration of DNAN in the IMX-101 mixture.

Assessing Endocrine-Disrupting Activity of Bisphenols across Multiple Species

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Bisphenol A (BPA) is an endocrine disrupting chemical, widely used in plastics, food packaging, and other products. Increasing concern over BPA has prompted the search for safer replacements. As adverse effects of BPA can vary significantly among humans and other species, we evaluated the endocrine disrupting activity of a panel of bisphenols, including BPA, bisphenol B (BPP), bisphenol F (BPF) and bisphenol AF (BPAF) on multiple nuclear receptors (NRs) of major vertebrate classes. Specifically, we evaluated responses of estrogen (ER), androgen, thyroid and PPARG receptors of fish, amphibians, reptiles, birds, and mammals. To do that, we used our harmonized multiplexed NR reporter assay (FACTORIAL-MS), which enables profiling the impact of a chemical on the activity of multiple NRs of multiple species in a single well of cells. The bisphenols were compared by the maximal NR activation (Emax) and the half-maximal response concentration (EC50). The predominant effects of bisphenols were ER activation, which varied significantly among species. According to the EC50 and Emax values, we ranked the overall ER potency across multiple species as BPA>BPF>BPF>BPF. The strongest BPA effects were at human ERβ (Emax=102% of estradiol, EC50=380nm) and zebrafish ER1 (Emax=106% of estradiol, EC50=2.1nm). Among species, the most susceptible NR to three bisphenols (BPA, BPP, BPF) was zebrafish ERβ (Emax of 62% of estradiol). Interestingly, we also found specific activation of human and mouse PPARG by BPF (Emax~11% of rosiglitazone). These results provide assessments of species-specific effects of bisphenols and demonstrate the utility of FACTORIAL-MS assay for high-throughput evaluation of endocrine disruptors and other drug candidates across multiple species.

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Comparison of Mammalian and Amphibian Tests for Detection of Thyroid Active Chemicals

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Test guidelines for detecting thyroid activity of a chemical include amphibian and mammalian tests. Only some of mammalian tests are included in standard information requirements of the EU legal frameworks for pesticides, biocides and industrial chemicals. The amphibian assays are not included. Data interpretation across taxa is needed for the identification of endocrine disruptors in these EU frameworks. To answer the question whether information derived from mammalian assays can be used for indicating chemicals with thyroid activity in amphibians or vise versa, publically available data of 50 chemicals tested in both amphibians and mammals were reviewed. Based on the changes in thyroid responsive endpoints including hind limb length (HLL), development of thyroid and thyroid histology in amphibians, as well as endpoints of thyroid hormones (T3, T4, TSH), thyroid weight, and thyroid histology in mammals, 15 chemicals are concluded to have the potential to interact with the hypothalamic-pituitary-thyroid (HPT) axis; with 6 chemicals responsive in both amphibians and mammals; 7 chemicals responsive in mammals but not in amphibians; and 2 chemicals responsive in amphibians but not in mammals. These results suggest that not all thyroid active chemicals showed the potential to interfere with the HPT axis across different vertebrate taxa, although there is a high degree of the thyroid system conservation. When interpreting data across taxa, some cautious should be paid due to the differences in exposure routes and in sensitivity of amphibian and mammalian assays. Recommendations for a testing strategy for the thyroid pathway are currently in development.

Trace Concentrations of Emerging Contaminants Dysregulates Nitric Oxide Levels and Superoxide Dismutase Activity in Mice: Differential Roles of Sex and PPARs

P. K. Gonnabathaula, and M. A. Yakubu. Texas Southern University, Houston, TX. Sponsor: A. Adedapo

Contaminants of Emerging Concern (CEC) are chemicals detected in water/environments that were not detected previously or were found at trace levels. Possibility exist for interactions of these diverse chemicals at low levels, multiple interactions-interfacing with sensitive biochemical pathways to cause harm. We have investigated the effects of trace concentrations (ng/L) of multiple CEC on essential biomolecules -Nitric oxide (NO), superoxide dismutase (SOD) activities in wild type and PPARα knockout mice. Mice (male and female) were exposed to trace concentrations of CEC comprising: atrazine, dieldrin, endrin, endosulfan and atrazine -1(100ng/L) in drinking water for six weeks. Blood and organs were collected, homogenized, NO levels and SOD activity were determined by Griess/SOD assays. In the blood samples, low CEC reduced NO (20%) in the female with no changes in male. In wild type, NO levels was 90% higher compared to PPARα knockout female. NO levels increased in tissues from wild type: spleen (80%), heart (75%), liver (80%), kidney (85%) and brain (85%), while in PPARα knockout NO levels was reduced in spleen (45%) but increased in the kidney (55%), no change in the brain, liver, and heart. In the male, NO levels were reduced in the heart (66%) and liver (40%) with no changes in the kidney, spleen, brain, and testicles. Blood SOD activity is 90% lower in male than female; CEC increased SOD activity by 20% in male with no change in the female. In female SOD activity was significantly increased in the heart (30%), liver (25%) and spleen (25%) by CEC with no effect on the brain and the kidney. In the PPARα knockout mice, SOD activity was significantly reduced in the heart (50%) and liver (25%) but not in the kidney, spleen, and brain. Thus, trace concentrations of multiple pesticides caused selective dysregulation of NO/SOD systems in different organs of the body. The effects observed was sex dependent and may be influenced by genetic status as in PPARα knockout. These results indicate that nano-concentrations of series of organic contaminants can cause cellular and molecular dysregulations of biomolecules precipitating toxicity and pathology that can be a threat to human health. Further investigation into the molecular mechanism(s) and signaling pathway(s) implicated in these dysregulations is warranted and of interest.
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from their wild-type (WT; Ctr1 hand, SC-KO mice had indistinguishable testis weight and histology (~83% by PND 41) with almost complete depletion of GCs. On the other hand, SC-KO mice had 35% reduced GC death a single high dose of 5 mg/kg of cDDP or equivalent volume of saline for 48 hours. We found that SC-KO mice had 35% reduced GC death compared to its SC-WT littermates. Platinum levels in testis tissue and in the seminiferous tubules of treated mice showed no difference between both genotypes, however intracellular platinum levels were reduced by 30% in cDDP-treated primary SCs derived from both genotypes. Taken together, these observations reveal for the first time 1) the required role of CTR1 in GCs, but not in SCs, for functional spermatogenesis and 2) the participation of CTR1 by the SCs on mediating cDDP-induced GC loss. Future investigations will utilize SC-KO as a mouse model to study the contribution of the environmental niche provided by the SC on spermatogenesis followed by clinically relevant multi-dose cDDP treatment, and the GC-KO mice will be utilized to explore the importance of CTR1 and/or copper on spermatogenesis.

Cisplatin (cDDP) is a highly effective chemotherapeutic drug. However, treatment with cDDP contributes to many adverse side effects including prolonged azoospermia in male patients. Although it is known that cDDP disrupts spermatogenesis, its main cellular target and mechanism responsible for its last effects on fertility remain unknown. The high affinity membrane copper transporter 1 (CTR1; SLC31A1) has been shown to be involved in cDDP uptake in both in vivo and in vitro studies. Our preliminary evaluation on mice testes indicates that CTR1 is primarily expressed in primary spermatocyte germ cells (GCs) and Sertoli cells (SCs). To examine the role of CTR1 in the testis as well as to discern the relative contribution between CTR1 in SC and GC to the lasting cDDP-induced disruption in spermatogenesis, we have developed two independent mouse models, with the conditional knockout of Ctr1 in either SCs (SC-KO; Amh-Cre, Ctr1fl/Δ) or GCs (GC-KO; Ddx4-Cre, Ctr1fl/Δ). Interestingly, GC-KOs exhibit a severe reduction in testis weight (~83% by PND 41) with almost complete depletion of GCs. On the other hand, SC-KO mice had indistinguishable testis weight and histology from their wild-type (WT; Ctr1fl/Δ) littermates, with all stages of spermatogenesis present. The SC-KO mice were further challenged with an acute dose of cDDP, where the SC-KO and WT mice were either exposed to a single high dose of 5 mg/kg of cDDP or equivalent volume of saline for 48 hours. We found that SC-KO mice had 35% reduced GC death compared to its SC-WT littermates. Platinum levels in testis tissue and in the seminiferous tubules of treated mice showed no difference between both genotypes, however intracellular platinum levels were reduced by 30% in cDDP-treated primary SCs derived from both genotypes. Taken together, these observations reveal for the first time 1) the required role of CTR1 in GCs, but not in SCs, for functional spermatogenesis and 2) the participation of CTR1 by the SCs on mediating cDDP-induced GC loss. Future investigations will utilize SC-KO as a mouse model to study the contribution of the environmental niche provided by the SC on spermatogenesis followed by clinically relevant multi-dose cDDP treatment, and the GC-KO mice will be utilized to explore the importance of CTR1 and/or copper on spermatogenesis.

In utero phthalate exposure results in significant testicular pathology in mammalian testes, despite the lack of an anti-androgenic effect on mouse and human fetal testes. Retinoic acid signaling is critical for sex determination and gonadal sex-determination pathways in Ex Vivo Cultured Rat and Mouse Fetal Testes. Phthalates are peroxisome proliferators that bind to peroxisome proliferator-activated receptors and may engage in crosstalk with other nuclear receptors, including retinoic acid receptors. Therefore, we hypothesized that retinoic acid signaling is disrupted by phthalates, contributing to phthalate toxicity in the fetal testes. To test this hypothesis, rat and mouse fetal testes isolated on gestation day 15 and 14, respectively, were exposed in tissue culture to 10-6 M all-trans retinoic acid (ATRA) with or without 10-6 to 10-4 M mono-(2-ethylhexyl) phthalate (MEHP). In rat fetal testes, ATRA exposure caused a loss of seminiferous cord structure, accompanied by an increase in expression of a retinoic acid receptor target gene, Rbp1, and sex determination genes Nr0b1 and Wnt4. The testicular pathology was reversed in a concentration-dependent manner by addition of MEHP. The interaction between MEHP and ATRA also led to an additive increase in Rbp1 expression, but non-linear response in expression of Nr0b1 and Wnt4. In the mouse, ATRA-MEHP co-exposure resulted in similar testicular pathology. Given this evidence that MEHP interacts with ATRA to influence both retinoic acid signaling and sex determination in the fetal testes, we conclude that disruption of retinoic acid signaling is a mechanism of phthalate toxicity in the fetal testis of both rats and mice.

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Fetal exposure to diethylstilbestrol (DES), a synthetic estrogen, is associated with an increased risk of both male and female reproductive tract defects including vaginal cancer, cryptorchidism, and infertility in humans. We have exposed a panel of genetically diverse inbred mouse strains, including Collaborative Cross (CC) strains, to either vehicle or DES (2 µg) on postnatal days (PND) 1-5. The CC is a mouse genetic reference population ideal for toxicogenomics studies, and this study is among the first to use the CC with a developmental exposure paradigm. DES exposure reduces male reproductive organ weights, and epididymal sperm counts in adult mice. Importantly, the severity of DES-induced abnormality in male reproductive tracts varies among strains, demonstrating the substantial contribution of genetic diversity to DES susceptibility. At adulthood, non-susceptible mouse strains exhibit relatively normal testicular structure following DES exposure, while DES-treated susceptible mouse strains show marked testicular degeneration with reduced germ cell numbers, decreased tubule diameters and increased areas of interstitial tissue. We observe an impaired first wave of spermatogenesis from a DES-treated susceptible CC strain that persists into adulthood. This disruption is evidenced by the presence of immature Sertoli cells, pre-mature depletion of spermatogenic cells, and the absence of pachytene spermatocytes and/or spermatids. DES exposure increases Nr0b2 level and decreases expression in susceptible CC mouse testes on PND21, indicative of Leydig cell damage associated with estrogen receptor activation. These results demonstrate that the CC is an ideal platform to identify specific gene by environment interactions in mice.

Organochlorides are among the most abundant and persistent chemicals in the environment. Organochlorine pesticides, such as dichlorodiphenyltrichloroethane (DDT), have been documented to cause effects upon fecundity were observed, however a dose-related decrease in reproductive tissue weights, genital malformations (cleft phallic, cleft prepucce, hypospadias, vaginal pouch), retained nipples, epididymal agenesis, and undescended testes. Mixture modeling of AGD reduction and incidence of hypospadias or cryptorchidism in adult males generally occurred in the 25-100% dose groups including reduced reproductive tissue weights, genital malformations (cleft phallus, cleft prepuce, hypospadias, vaginal pouch), retained nipples, epididymal agenesis, and undescended testes. Mixture modeling of AGD reduction and incidence of hypospadias or cryptorchidism in adult males generally occurred in the 25-100% dose groups including reduced reproductive tissue weights, genital malformations (cleft phallus, cleft prepuce, hypospadias, vaginal pouch), retained nipples, epididymal agenesis, and undescended testes. Mixture modeling of AGD reduction and incidence of hypospadias or cryptorchidism in adult males generally occurred in the 25-100% dose groups including reduced reproductive tissue weights, genital malformations (cleft phallus, cleft prepuce, hypospadias, vaginal pouch), retained nipples, epididymal agenesis, and undescended testes. Mixture modeling of AGD reduction and incidence of hypospadias or cryptorchidism in adult males generally occurred in the 25-100% dose groups including reduced reproductive tissue weights, genital malformations (cleft phallus, cleft prepuce, hypospadias, vaginal pouch), retained nipples, epididymal agenesis, and undescended testes. Mixture modeling of AGD reduction and incidence of hypospadias or cryptorchidism in adult males generally occurred in the 25-100% dose groups including reduced reproductive tissue weights, genital malformations (cleft phallus, cleft prepuce, hypospadias, vaginal pouch), retained nipples, epididymal agenesis, and undescended testes. Mixture modeling of AGD reduction and incidence of hypospadias or cryptorchidism in adult males generally occurred in the 25-100% dose groups including reduced reproductive tissue weights, genital malformations (cleft phallus, cleft prepuce, hypospadias, vaginal pouch), retained nipples, epididymal agenesis, and undescended testes. Mixture modeling of AGD reduction and incidence of hypospadias or cryptorchidism in adult males generally occurred in the 25-100% dose groups including reduced reproductive tissue weights, genital malformations (cleft phallus, cleft prepuce, hypospadias, vaginal pouch), retained nipples, epididymal agenesis, and undescended testes. Mixture modeling of AGD reduction and incidence of hypospadias or cryptorchidism in adult males generally occurred in the 25-100% dose groups including reduced reproductive tissue weights, genital malformations (cleft phallus, cleft prepuce, hypospadias, vaginal pouch), retained nipples, epididymal agenesis, and undescended testes. Mixture modeling of AGD reduction and incidence of hypospadias or cryptorchidism in adult males generally occurred in the 25-100% dose groups including reduced reproductive tissue weights, genital malformations (cleft phallus, cleft prepuce, hypospadias, vaginal pouch), retained nipples, epididymal agenesis, and undescended testes. Mixture modeling of AGD reduction and incidence of hypospadias or cryptorchidism in adult males generally occurred in the 25-100% dose groups including reduced reproductive tissue weights, genital malformations (cleft phallus, cleft prepuce, hypospadias, vaginal pouch), retained nipples, epididymal agenesis, and undescended testes. Mixture modeling of AGD reduction and incidence of hypospadias or cryptorchidism in adult males generally occurred in the 25-100% dose groups including reduced reproductive tissue weights, genital malformations (cleft phallus, cleft prepuce, hypospadias, vaginal pouch), retained nipples, epididymal agenesis, and undescended testes. Mixture modeling of AGD reduction and incidence of hypospadias or cryptorchidism in adult males generally occurred in the 25-100% dose groups including reduced reproductive tissue weights, genital malformations (cleft phallus, cleft prepuce, hypospadias, vaginal pouch), retained nipples, epididymal agenesis, and undescended testes. Mixture modeling of AGD reduction and incidence of hypospadias or cryptorchidism in adult males generally occurred in the 25-100% dose groups including reduced reproductive tissue weights, genital malformations (cleft phallus, cleft prepuce, hypospadias, vaginal pouch), retained nipples, epididymal agenesis, and undescended testes. Mixture modeling of AGD reduction and incidence of hypospadias or cryptorchidism in adult male...
in the described AOP for cryptorchidism, but may be a key event for other cryptorchidism AOPs during this window of development. While extensive data were relied upon to underpin the proposed AOPs, the confidence in each of the AOPs varies and gaps remain, including the molecular initiating events for all AOPs.

**3281 Uncertainty and Variability in High-Throughput Toxicokinetics for Risk Prioritization**


1US EPA, Research Triangle Park, NC; 2Cytospect, Watertown, MA; 3SciToxation, Research Triangle Park, NC; and 4ToxStrategies, Austin, TX.

Streamlined approaches that use in vitro experimental data to predict chemical toxicokinetics (TK) are increasingly being used to perform risk-based prioritization based upon dosimetric adjustment of high-throughput screening (HTS) data across thousands of chemicals. However, assessments of the impact of uncertainty and variability on these TK values and subsequent predictions are needed to guide data interpretation and provide overall confidence in high-throughput TK (HTTK) approaches. In this study, Bayesian methods were developed to provide chemical-specific uncertainty estimates for two in vitro TK parameters: plasma protein binding (\(K_p\)) and intrinsic hepatic clearance (\(CL_{int}\)), using chemical-specific experimental measurements derived. Inclusion of experimental measures across three physiologic plasma protein concentrations reduced the uncertainty in the \(K_p\) estimates. Uncertainty estimation was additionally conducted for predictions of volume of distribution (\(V_d\)) and steady-state serum concentration (\(C_s\)). Monte Carlo simulation to propagate both measurement uncertainty and biologic variability into the predicted TK for most chemicals, variability contributed more than uncertainty to \(C_s\) estimations of the 95th percentile. Risk-based prioritization of chemicals based upon high throughput exposure estimates and dosimetric adjustment of ToxCast HTS data using Bayesian-derived \(C_s\) estimates incorporating uncertainty and/or variability demonstrated that prioritization would change for a few chemicals when uncertainty is included. Incorporation of these methods provides a timely risk-based prioritization strategy that considers the relationship between in vitro bioactivities and exposures, overlaid with a metric for TK prediction uncertainties. This abstract does not necessarily reflect US EPA policy.

**3282 Feedback Regulation through the Hypothalamic-Pituitary-Endocrine Loop as a Potential Mechanism for Nonmonotonic Dose Responses of Endocrine-Disrupting Chemicals**

Q. Zhang. Emory University, Atlanta, GA.

Endocrine-disrupting chemicals (EDCs) often exhibit nonmonotonic dose response (NMDR) behaviors. A number of mechanisms have been proposed for the nonmonotonic effects, including existence of multiple hormone receptor isoforms with opposite effects, receptor desensitization, and formation of mixed-ligand heterodimers. Besides these local mechanisms, NMDR of EDCs is also believed to be a systems-level behavior of the neuroendocrine feedback regulation, although the exact mechanism is unclear. In the present study we use dynamical modeling of the hypothalamic-pituitary-endocrine organ (HPE) loop to investigate whether and how an NMDR may occur as a result of feedback regulation. In the model, a high loop-gain proportional control or an integral control is implemented in the central site containing the hypothalamic and pituitary gland. Such control mechanism can achieve near perfect adaption to EDCs disrupting the synthesis, secretion or clearance of the hormones secreted by the terminal endocrine organs. However, if an EDC acts as an agonist and has access to the central feedback sites in the hypothalamus and pituitary to inhibit the secretion of hypothalamic releasing hormones and the pituitary hormones, the levels of the peripheral hormones will decrease accordingly, compensating against the agonistic effects of the EDC. Model simulations indicate that when the agonist’s affinity for the hormone receptors in the peripheral target tissue is less than that of the endogenous hormone and/or when the agonist’s affinity for the hormone receptors in the central site is greater than that of the endogenous hormone, a J-shaped dose response relationship can arise between the agonist EDC and the peripheral tissue effects. In such cases although the EDC functions as an agonist by itself, due to feedback regulation it results in an inhibitory effect at low doses. In target tissues where the agonist’s affinity for the hormone receptor is no less than that of the endogenous hormone, a stimulatory effect can be obtained for low doses of EDCs. Conversely, an inverted U-shaped dose response occur when the EDC functions as an antagonist. In summary, our modeling results suggest that for an EDC biochemically functioning as a hormone agonist or antagonist, the HPE feedback regulation plays a pivotal role in determining the direction of the EDC’s effects in peripheral target tissues.

**3283 A Biologically Based Dose-Response (BBDR) Model for the Effects of Perchlorate Exposure and Iodine Intake on Thyroid Hormone Levels in Women Prior to and During Early Gestation**

P. M. Schlosser1, D. F. Kapraun1, and T. L. Leavens2.

1US EPA, Washington, DC; and 2PK Consultant, Cary, NC.

Perchlorate (ClO₄⁻) has both natural and synthetic sources and is released to the environment as a salt; it is highly stable and has high water solubility and mobility in aqueous environments. Once ingested, ClO₄⁻ is readily absorbed and distributed in blood plasma, allowing it to competitively inhibit iodide transfer by the sodium-iodide symporter (NIS). NIS-mediated iodide uptake by the thyroid is necessary for production of thyroid hormones (T₃ and T₄), which are essential for development and growth of fetuses, infants, and young children, and to metabolism and energy regulation at all ages. Iodide insufficiency during pregnancy is known to cause severe neurodevelopmental effects, while reductions in maternal TH levels in early pregnancy have been linked to subsequent levels in children. Perchlorate exposure in pregnant women is common, and is often considered for the lactating mother, describing dietary iodine economy, thyroidal function, and TH production and distribution, along with ClO₄⁻ pharmacokinetics and NIS inhibition, was adapted to describe these processes in the woman prior to pregnancy and through gestation week (GW) 14. This model incorporates two key improvements over a previous late gestation model: 1) a majority of maternal ClO₄⁻ to iodide ratio, that for most chemicals, variability contributed more than uncertainty to \(C_s\) estimations of the 95th percentile. Risk-based prioritization of chemicals based upon high throughput exposure estimates and dosimetric adjustment of ToxCast HTS data using Bayesian-derived \(C_s\) estimates incorporating uncertainty and/or variability demonstrated that prioritization would change for a few chemicals when uncertainty is included. Incorporation of these methods provides a timely risk-based prioritization strategy that considers the relationship between in vitro bioactivities and exposures, overlaid with a metric for TK prediction uncertainties. This abstract does not necessarily reflect US EPA policy.

**3284 PBPK Modeling of Piperacillin and Tazobactam in Premature Neonates**


1US FDA/NCTR, Jefferson, AR; 2Procter & Gamble Company, Cincinnati, OH; 3Duke University School of Medicine, Durham, NC; and 4US FDA, Silver Spring, MD.

Piperacillin (PIP) and Tazobactam (TAZ) injection, a combination broad-spectrum antibiotic agent, is used in neonatal intensive care units to treat bacterial infections in premature neonates. To assess the effect of early birth on the pharmacokinetics of PIP and TAZ, a novel 7-compartment flow-limited physiologically based pharmacokinetic (PBPK) model was constructed to characterize the rapid developmental changes in physiological and biochemical processes in neonates for gestational birth ages ranging from 25 to 40 weeks. This provisional model describes growth and maturation processes for up to 2 years after birth. Tissue:plasma partition coefficients for PIP and TAZ were calculated using published mechanistic algorithms in PK-Sim software or from reported experimental tissue to plasma concentration ratios. These drugs are primarily excreted in urine by glomerular filtration and tubular secretion. Urinary clearance rates for PIP and TAZ were derived by extrapolation of clearance rates reported for older infants. Hepatic metabolism, a minor clearance pathway in older infants, was also evaluated for its possible role in describing the development of this elimination pathway in preterm infants. Initial simulations, without any adiabatic values, were performed using computer simulation software. Initial model predictions were evaluated by comparing with observed data from 9 premature neonates. Based on visual inspection of the plasma PIP and TAZ concentration-time data, model predictions were typically within 2- to 4-fold of the measured values for the 9 neonates that were evaluated.
While a very promising outcome, a better mechanistic understanding of key biological determinants in very early life is needed to increase model predictive performance. In addition, expansion of this provisional model to characterize age-dependent growth into childhood and adulthood is planned.

**3285 Evaluating the Influence of Physiological Changes during Pregnancy on Oseltamivir Dosimetry Using Physiologically-Based Pharmacokinetic (PBPK) Modeling**

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Oseltamivir (brand name, Tamiflu) is a prescription antiviral drug used to treat influenza (virus types A and B) in humans. Oseltamivir is approved for use in adults and children with the dosage varying with age and body weight. However, the effective dosing regimen in pregnancy conditions remains to be evaluated. Physiological changes that occur during the gestational period affect the pharmacokinetics of the drug leading to the potential concern of under-dosing of oseltamivir in pregnant women. The overall goal of this project is to quantify the effects of physiological changes on oseltamivir pharmacokinetics and to determine the appropriate dose-adjustments for pregnant women to maintain equivalent internal exposure as in non-pregnant adults. In the absence of pregnancy, the therapeutic model in model in pregnant women was first, second, and third trimesters. The changes in physiological parameters were captured in the model using descriptive mathematical equations as a function of gestational age. The model predicted an overall increase in drug plasma concentration over the course of pregnancy. Following which we implemented an iterative modeling routine incrementally adjusting the oral dosage for each trimester of pregnancy. The findings suggest that the current treatment dose of 75 mg twice a day should be increased by 16%, 38%, and 35% in the first, second, and third trimesters, respectively, to yield plasma concentrations of the active drug equivalent to that determined in non-pregnant adults. Similarly, the dose-adjustments necessary for the prophylaxis dose of 75 mg once a day in pregnant women was predicted to be 17%, 41% and 63% higher. These efforts could help guide regulatory decision making and provide clinical practitioners with data-driven recommendations for oseltamivir dosimetry during pregnancy. Future work will focus on the expansion of the current research analysis to other anti-viral drugs to predict safe and efficacious dosing regimens in pregnant women.

**3286 Physiological Simulation of Thermal and Chemical Stressors in a United States Air Force Population**

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Thermal stress research has focused on understanding the critical physiological need to maintain a constant internal body temperature of 37°C despite changes in the external environment and levels of exercise. Stable core body temperature is critical to provide optimum physiological conditions for favorable cellular metabolism within blood and tissues. The DoD operational environment often incurs incidents of concurrent thermal/heat stress and toxic chemical exposure. Our objective was to introduce a recently developed thermal and chemical dosimetry model in human military personnel. Subjects wore Zephyr BioHarness chest straps to sense skin concurrent thermal/heat stress and toxic chemical exposure. Our objective was to translate in vitro constitutive androstane receptor (CAR) agonist and antagonist assay data for organophosphate (OP) pesticides to in vivo toxic potency values for the human, using physiologically-based pharmacokinetic (PBPK) modeling-based reverse dosimetry. To this purpose, PBPK models were developed for Malathion (Mal) and Parathion (Par). Each PBPK model consists of five compartments and can be used for inhalation, oral, and dermal routes of exposure. The hepatic tissue was used as a surrogate to describe tissue concentration. Using the PBPK dosimetry approach, in vitro concentration-response curves of the CAR test were translated into in vivo tissue dose-response curves from which PODs were derived for the pesticide exposure, indi vidual or in combination. For the CAR exposure, the concentration was 50µM for Mal and Par. For the CAR agonist the change was +50% at 100 µM for Mal and approximately 35% for Par. This biologic modeling estimated an equivalent external dose corresponding to the concentration in hepatic tissue for Mal oral (495), inhalation (4) and dermal (9025) mg/kg bw exposures, and for Par oral (380) and dermal (13600) mg/kg bw exposures. These results suggest the importance of the route of exposure for an equivalent tissue concentration. A current limitation is that only the parent compound and not the metabolite assay data are available in Tox21, they should be integrated as data become available. In conclusion, this study shows the feasibility of using in vitro data to estimate in vivo levels of pesticides and their mixtures using PBPK dosimetry for non-animal-based risk assessment of pesticides. The findings and conclusions in this presentation have not been formally disseminated by [the Centers for Disease Control and Prevention/ the Agency for Toxic Substances and Disease Registry] and should not be construed to represent any agency determination or policy.

**3288 Understanding Implications of Metabolic Interactions between Benzo[a]pyrene (Ba[a]P) and Dibenzo[def,p]chrysene (DBC) in Rodents and Humans Using Physiologically-Based Pharmacokinetic (PBPK) Modeling**


Humans are rarely exposed to a single polycyclic aromatic hydrocarbon (PAH) and, instead, are routinely exposed to complex PAH mixtures. Since multiple PAHs can be substrates for various individual cytochrome P450 enzymes (e.g. CYP1A1, CYP1B1, etc.), it is hypothesized that exposure to PAH mixtures can cause metabolic competition, inhibiting PAH metabolism, and affecting PAH clearance, detoxification, bioactivation, and toxicity. To test this hypothesis, the ability of parent PAHs to compete for metabolism in binary mixtures was quantified using in vitro assays to measure the required chemical concentration to produce an effect, the point of departure (POD). Such in vitro PODs data can be extrapolated to in vivo species including humans, at the biological effective dose concentration if no specific mechanism appeared in the target organ. The aim of this study was to determine the potential of metabolic interactions between Ba[a]P and DBC. For the parent-parent interactions, it was observed that DBC was a more potent inhibitor than Ba[a]P for rats and humans. However for mice, Ba[a]P was a more potent inhibitor than DBC. Similar to the parent-metabolite interactions, DBC-diol was found to be a more potent inhibitor than Ba[a]P-diol for mice and rats, whereas, for humans, Ba[a]P-diol was the more potent inhibitor. These results suggest that care should be taken when utilizing measured core body temperature change and chemical exposure simultaneously in the active military occupational environment, providing a robust in silico human simulation that allows computer based mission planning and hazard assessment with limited individual real-time data collection.
animal models to predict human hazard of PAH mixtures. PBPK model simulations predict the extent of metabolic inhibition and implications to internal dosimetry at various exposure levels of these binary PAH mixtures. Ultimately this model can be used as a tool for understanding implications of PAH mixtures on human health.

**3289 Human Plasma and Urinary Metabolic Profiles of Trimethylamine and Its N-Oxide Extrapolated Using Humanized-Liver Mice and Simple Physiologically-Based Pharmacokinetic Models**

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Trimethylamine, a dietary- and medicinal carnitine-derived amine, is extensively metabolized by liver to non-malodorous trimethylamine N-oxide. Although trimethylamine and trimethylamine N-oxide under daily dietary consumption or carnitine treatment are generally regarded as nontoxic, they have been, and remain, toxicological and clinical interest because of their potential association with atherosclerosis. The aim of the current study was to model the pharmacokinetics of trimethylamine after oral administration of trimethylamine in humans 1) based on rat reported values in literature and 2) experimental pharmacokinetics after orally administration to the humanized-liver mice with deuterium-labeled trimethylamine. Trimethylamine N-oxide was extensively formed by humanized liver, but not by mouse livers or in rats as reported. Adjusted trimethylamine biomonitoring equivalents from rat studies were scaled to human equivalents using known species allometric scaling factors and in vitro metabolic clearance data obtained using rat and human liver microsomal preparations. In this approach, renal clearances in humanized-liver mice and trimethylamine N-oxide were calculated with a clearance concept approach using reported 24-h urinary excretion rates and assumed areas under plasma concentration curves. The experimental pharmacokinetic data of deuterium-labeled trimethylamine and its N-oxide in humanized-liver mice were also scaled up to humans. The resulting modeled plasma and urinary concentration curves by simple physiologically based both pharmacokinetic models (or semi-physiological pharmacokinetic models) were consistent with reported concentrations. This study provides important information to help simulate human plasma levels of trimethylamine and trimethylamine N-oxide in trimethylamine loading tests and during treatment with prescribed medicinal l-carnitine, showing the similar range as that resulting from daily dietary foodstuff consumption along with little toxicological impacts. The present models could estimate relationship between plasma and urine concentrations of trimethylamine or trimethylamine N-oxide and the daily oral doses by both forward and reverse dosimetry from viewpoint of human risk assessment.

**3290 The Kinetically Derived Maximum Dose (KMD), a New Dimension to the Maximum Tolerated Dose (MTD)**

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Evaluations of chemicals tested under dose-selection protocols using a maximum tolerated dose (MTD) design (e.g., decreased body weight and/or histological injury) are increasingly revealing that interpretation is confounded by the onset of non-dose-proportionate systemic dose (i.e., nonlinear toxicokinetics). This is due to saturated absorption, distribution, and metabolism and/or clearance mechanisms of parent chemical and/or metabolites. Recent toxicity testing guidance from the Organisation for Economic Co-operation and Development (OECD) and US Environmental Protection Agency (US EPA) and other evaluations from the National Academy of Sciences (NAS) and ILSI Health and Environmental Sciences Institute (HESI) have clearly indicated that toxicity observed only under nonlinear toxicokinetic conditions often has limited, if any, quantitative relevance to risks to human health if the onset of dose-non-proportionality is well separated from real-world human exposures. Detection of nonlinear toxicokinetics has been improved by analytical techniques/approaches that have made assessment of systemic/internal dose in test animals substantially more sensitive, lower cost, and higher throughput. In addition, determination of whether the onset of toxicokinetic nonlinearity is well separated from human exposure is increasingly possible given rapid improvements in modeling and/or analytical biomonitoring analyses supporting human environmental exposure assessments. Saturation of any biological process should be evaluated for its relevance to dose-response relationships relative to human exposure. Systemic dose non-proportionality should not be treated any differently from toxicity findings due to excessive stress indicated by the conventional MTD approach. Use of evidence of dose non-proportionality for selection of an acceptable top dose for animal toxicity testing has been termed the kinetically-derived maximum dose (KMD). Despite the convergence of these indisputable advances, however, current toxicity testing protocols and interpretation of existing toxicity test findings continue to be largely dominated by long-ingrained concepts of conventional MTD thinking. Current toxicity test dose selection often is conducted oblivious to a prior evaluation of toxicokinetic internal non-dose-proportionate systemic measures sensitive, costly, and animal-intensive post hoc mode-of-action analyses are frequently implemented to address high-dose specific toxicity findings that could (should) otherwise be ruled out as quantitatively non-human relevant on systemic dose alone. Thus, effective implementation of the fundamental toxicology principle of “the dose makes poison” must include an ever-expanding commitment to consideration of animal and human internal dose evaluations as a key part of chemical risk assessment. The rapid expansion of analytical technologies, exposure assessments, and reducing the use of animals demands nothing less. The objective of this session is to catalyze understanding and discussion surrounding the use of systemic dose kinetics in selection of improved human-relevant doses in animal toxicity testing and/or retroactive interpretation of high-dose specific toxicity findings; and 4) reg-ulatory perspectives on use of findings at non-dose-proportional doses in human risk assessment. The roundtable discussion between speakers and the audience will address the implications of KMD-based assessments for the practice of toxicology and risk assessment, such as: 1) the KMD as a replacement for the MTD; 2) research and policy constraints in the use KMD; 3) adequacy of existing regulatory toxicity testing guid-ances; 4) protocol design (e.g., toxicokinetics to support KMD evaluations); 4) offering an alternative to conducting conventional high-dose specific mode-of-action investigations; 5) assisting design and interpretation of adverse outcome pathway (AOP)/mode-of-action studies; and 6) informing life-stage-specific differences in toxicity.

**3291 The US Tox21 Collaboration: A Decade of Experience and a New Vision for the Future**

B. Meek, University of Ottawa, Ottawa, ON, Canada.

In 2007, Tox21 was launched as a multi-agency collaborative effort among the National Institutes of Health (NIH)’s National Institute of Environmental Health Sciences (NIEHS), National Toxicology Program (NTP), and the National Center for Advancing Translational Sciences (NCATS); the US Environmental Protection Agency (US EPA)’s National Center for Computational Toxicology (NCCT); and the US Food and Drug Administration (US FDA). The objective of this partnership was to shift the assessment of chemical hazards from traditional experimental animal toxicology studies to one based on target-specific, mecha-nism-based, in vitro assays, with the ultimate aim of improving human and environmental risk assessments. In this regard, the collaborative was highly successful in that thousands of chemicals have been screened in hundreds of bioassays. These data have been publicly released for consideration and use in a range of contexts, including regulatory deci-sions. However, complex challenges remain. The intent of this symposium is to review the 2017 strategic plan for Tox21 and gain feedback from the diverse stakeholders at SOT. The new strategic plan promotes: 1) addressing key limitations in in vitro assays; 2) continued development and use of alternative test systems that predict human toxicity; 3) management and curatIon of large chemical libraries; 4) curatIon of legacy in vivo toxicity studies; 5) performance-based validation of high-throughput in vitro, in silico, stem cell and microphysiological systems, and other emerging technologies; and 6) development and use of in vitro methods for characterizing pharmacokinetics. Each of the speakers will summarize lessons learned and outline their agency’s approaches aimed at solving complex challenges. The strategy embraces transpar-ency and technological change, aims to protect human health environ-ment, with an ultimate goal of fostering broader acceptance of alterna-tive data streams in regulatory decision.
3292 Research-Based Approaches to Improve Teaching Effectiveness in Toxicology Classrooms

B. Kaplan, Mississippi State University, Mississippi State, MS.

Teaching students effectively at all levels in science requires continuous learning on the part of the instructor. Not only is content knowledge increasing, so are the tools to teach it. At the heart of pedagogy, though, is research about how students learn to maximize educational outcomes and retention. While lecturing has been the predominant method of instruction since the founding of universities, there is evidence to suggest that this is not the most effective way to teach. Through national initiatives such as the National Science Foundation’s Vision and Change in Undergraduate Biology Education, research-based teaching methods are having a growing impact in higher education. This session will bring together educators to talk about best practices in student-centered teaching that are applicable to all educational levels. The topics to be discussed include: 1) an introduction to designing course methods, assignments, and assessments to optimize students’ opportunities to learn; 2) lessons learned from a flipped classroom; 3) the use of problem formulation and experimental design in the context of a graduate immunotoxicology classroom; 4) how community-engaged learning and research can improve comprehension, recruitment, and retention; and 5) case studies from industry to teach about risk communication. This session will conclude with time for questions, giving audience members an opportunity to further explore these and other related topics. This session will be of interest to current educators and those interested in teaching, such as graduate students and postdocs.

3295 In Vivo-In Vitro Comparison of Acute Respiratory Tract Toxicity Using Different 2D and 3D Models and a Successful Example Using Physico-Chemical Characteristics of the Test Substance

R. Lansiedl. BASF, Ludwigshafen am Rhein, Germany.

The usefulness of in vitro systems to predict acute inhalation toxicity was investigated. Results obtained in testing different materials in three-dimensional human airway epithelial models, EpiAirway and MucuAir, as well as and in A549 and 3T3 monolayer cell cultures, were compared to rat four-hour LC50 values classified according to US EPA and GHS hazard categories. Best results were achieved with a prediction model distinguishing toxic from non-toxic substances. Differentiation of toxic substances by their potency was mediocre but could be improved by excluding substances causing pulmonary edema and emphysema in vivo. None of the test systems was outstanding, and there was no evidence that tissue or monolayer systems using respiratory tract cells provide an added value. However, the test systems only reflected bronchial epithelia and alveolar cells and investigated cytotoxicity. Effects occurring in other cells by other mechanisms could not be recognized. Further work should optimize test protocols and expand the set of substances tested to define applicability domains. In vivo respiratory toxicity data for in vitro comparisons should distinguish different modes of action, and their relevance for human health effects should be ensured.

3293 In Vitro Test Methods to Model Local Respiratory Effects after Exposure to Pulmonary Toxicants: Not Just Smoke and Mirrors

H. Behrsing, Institute for In Vitro Sciences, Gaithersburg, MD.

The lungs are exposed to a wide range of toxicants, including environmental, pharmaceutical, and occupational. Evaluating this assortment of toxicants has necessitated the development of physiologically-relevant in vitro models. These in vitro models have proven to be sensitive, robust, reproducible, and capable of providing a diverse outcome of responses. These in vitro models focus on biomarkers related events by cytokine secretion, mucus production, goblet cell hyperplasia, and increased ciliary beat frequency. This session will explore recent findings in vitro toxicology that link a variety of pulmonary toxicants with their common inflammatory outcomes. The first speaker will focus on in vitro pulmonary models and their incorporation into a testing scheme. In the second presentation, a comparison of cell lines to 3D air-liquid interface (ALI) will be detailed. The third speaker will discuss in vivo-in vitro comparisons, as well as 3D ALI. The fourth speaker will demonstrate dosimetry modeling of in vitro e-cigarette exposure. Speaker five will reveal the fact that in vitro models have been taken into animal exposure modeling. Further comparisons between different in vitro exposure systems will be explained by speaker six. Finally, the last speaker will lay out the requirements to achieve in vitro testing regulations and the role each sector must play in achieving regulation. These speakers, who come from a mix of government and industry backgrounds, demonstrate how a range of pulmonary exposures can be evaluated using in vitro methods for detecting local respiratory effects and the steps necessary to establish regulations for these testing methods.

3294 Modern In Vitro Test Systems Provide Human-Relevant Toxicity Data to Support Product Development and Regulatory Decision Making

H. Behrsing, Institute for In Vitro Sciences, Gaithersburg, MD.

With the increase in chemicals and materials that may cause pulmonary toxicity through inhalation, a need for cost-effective toxicity testing of new chemicals continues to grow. The 2007 report, Toxicity Testing in the 21st Century—A Vision and a Strategy, The National Academies Press, Washington DC, describes a path forward for toxicology and envisages the use of more predictive human-relevant in vitro models for estimating human health risks. The ethical considerations, poor predictive value of animal testing, and vast numbers of chemicals/materials requiring evaluation, further drives this argument for the application of in vitro models to assess pulmonary toxicity. in vitro models utilizing human-derived cell lines and primary cells provide endpoints that reflect cytotoxic, genotoxic, and other relevant adverse events following exposure to toxicants. The emergence of multi-cellular three-dimensional (3D) tissue culture systems of the respiratory tract provide toxicologists with test platforms that accurately model the complex processes observed in native tissues. Human donor-tissue derived spheroids/organoids, reconstructed human airways, and precision-cut lung slices provide conventional toxicity endpoints as well as complex, relevant events following chemical exposure. The varied cell types, physiological structure, relevant toxicokinetics, and other properties of these models allow additional evaluation from chronic exposure to human-relevant (e.g. persistent inflammation, goblet cell hyperplasia) to functional outcomes (e.g. ciliary beating assays) that can reflect serious health complications that may lead to chronic obstructive pulmonary disease.

3296 The Challenge of Integrating Non-Animal Alternative Approaches to Assess the Risk to Human Health from Inhaled Materials

J. Hotchkiss, The Dow Chemical Company, Midland, MI.

Current guideline-compliant acute inhalation toxicity testing provides critical data for hazard identification, risk assessment, product stewardship, and development of short-term exposure guidelines. These tests have historically involved whole-body or nose-only exposure of rodents, however, significant research efforts across the chemical industry are focused on the development and application of human-relevant non-animal alternatives to assess the acute toxicity of inhaled materials. in vitro exposure of 3D human organotypic airway cultures grown at the air-liquid interface (ALI) may provide an immediate path to assess airway epithelial responses to deposited and adsorbed gases, vapors, and aerosols that are relevant for human risk assessment. ALI cultures reproduce many features of in vivo human respiratory epithelium, including 3D epithelial structure, functional cilia, mucus secretion, barrier properties, and metabolic activity. in vitro exposure systems permit the sort of translational studies that are essential to bridging the gap, from traditional in vivo inhalation exposures of laboratory species, to predicted acute pulmonary responses in humans based on dosimetrically-relevant concentrations of test materials in vitro. Until validated alternative testing methods are accepted by regulatory authorities, an integrated stepwise approach can be used that reduces animal use while developing relevant non-animal approaches. This includes, waiving studies when possible, use of the computational GHS additivity approach to predict toxicity of formulations based on their components, and the refinement and validation of in silico approaches to predict and rank acute toxicity hazard. in vitro exposure of human 3D airway cultures to pulmonary toxicants with recognized high and low toxicity in vivo are being used to benchmark the exposure/response of these human airway 3D cultures relative to the available in vivo data. While there is growing acceptance of predictive in silico and in vitro models for hazard assessment, much work remains to prove that these alternative approaches are robust, transparent, reproducible, and accurate.
spheres (50-1000 nm) were used as a surrogate for particulate matter vapors, and gases to the cells: Diffusion, sedimentation, thermophoretic movements. We compared various ALI in vitro exposure systems, a mass-balance approach can be easier to apply. This approach determines constituent levels generated and subtracts constituent levels that deposit in the in vitro exposure system. The remaining constituent levels represent an estimate of cellular exposure. For mainstream tobacco smoke, the mass-balance approach is not feasible because of the number of constituents, however, a selection of constituents have been used in in vitro studies to estimate doses. For E-vapor and other products which have significantly fewer constituents, this approach may be feasible. With the diversity of products in the market place, quantitative particulate and vapor phase in vitro dosimetry determinations are vital to interpreting and integrating results of in vitro experiments.

Operating Procedures to Improve Efficiencies of In Vitro Exposure Systems at the Air-Liquid Interface

M. Higuchi, US EPA, Research Triangle Park, NC.

The expanding use of in vitro exposure systems for toxicity assessments has created regulatory concerns. Many of these same concerns surround the proper conduct of in vivo inhalation toxicology studies which are addressed in Guidelines and Good Laboratory Practice (GLPs) regulations. It is acknowledged that this area (exposure techniques) is in rapid development, and there is limited experience now on best practices to describe new in vitro exposure methods. For example, OECD GLP No 14-The Application of the Principles of GLP to in vitro Studies states "routine requirements for apparatus used in a GLP compliant environment apply equally to apparatus used for in vitro studies." Unfortunately, the supplier/developer of in vitro exposure systems cannot know all potential applications of the specific exposure system by an end-user. Therefore, the in vitro method end-users must make the determination if the specific in vitro exposure system will meet the requirements of their experimental design. Various factors, such as air flow rate, relative humidity, and temperature can adversely affect performance and reliability. For example, increasing the air flow rate in diffusion (gases and vapors) exposure systems, when air flow is perpendicular to the cells, increases gas delivery to the cells via turbulence. To demonstrate this effect biologically, we used US EPA's cell culture exposure system (CCES) to expose BEAS-2B cells to air only (sham) controls. The experiments were aimed at "defeating the technology" of the CCES by determining the cells viability in a sham exposure. The controllable parameters of the exposures were total airflow rate, relative humidity, and temperature. Determining cytotoxicity via the CaliTitre Glo assay using the worst set of operating parameters (low air flow rate and relative humidity) indicated an average cell viability versus incubator controls of 62%. These results show that air flow rate and relative humidity are critical factors that influences the biological effects. Characterization of in vitro exposure systems is needed by the end-users to understand their advantages and limitations for regulatory assessments. This abstract does not necessarily reflect the views or policies of the US EPA.

Understanding Air-Liquid Interface Cell Exposure Systems: A Comprehensive Assessment of Various Systems under Identical Conditions

J. Zavaleta, US EPA, Research Triangle Park, NC.

Exposure of cells to atmospheric pollutants at the air-liquid interface (ALI) is a more realistic approach than exposures of attached cells submerged in liquid medium. However, there is still limited understanding of the ideal ALI design features to meet requirements. We compared various ALI in vitro exposure systems under identical conditions for their ability to expose cells to particles and gases. The systems tested used different mechanisms to deliver aerosols, vapors, and gases to the cells: Diffusion, sedimentation, thermostats (THP), and electrostatic precipitation (ESP). Fluorescent polystyrene spheres (50-1000 nm) were used as a surrogate for particulate matter to assess particle deposition. Deposition was determined by dissolving the spheres in ethyl acetate and measuring the fluorescence. Applying external forces, such as THP or ESP, enhanced deposition for all particle sizes. For example, deposition of a 50-nm particle was 1.6- to 4.8-fold higher with a THP compared to diffusion systems. Similarly, deposition for both 50-nm and 1000-nm particles were 3.4- to 10.3-fold higher with ESP compared to diffusion, depending on in vitro system with ESP. Results indicated that THP is an effective external force on nano-sized particles with decrease effectiveness as particle size increases, whereas ESP maintains a stable performance regardless of particle size. Also assessed was the ability of the systems to deliver gases to cells by using ozone levels as a test gas. The reaction of O3 with an indicator in the ALI surface showed that diffusion allowed gas-cell interaction. Results show a 13-fold difference in gas delivery performance between the best and poorest performing system. Additionally, increasing the flow rate in diffusion systems where airflow was perpendicular to the cells increased gas delivery. The study showed that in vitro systems with THP or ESP were the most effective at delivering aerosols to the cells, whereas flow rate was a critical parameter for the delivery of vapors and gases. This abstract does not necessarily represent the views or policies of the US EPA.

Novel Non-Animal Respiratory Test Methods Show Great Promise, So How Do We Get Them into Routine Use: Points to Consider for Industrial and Regulatory Acceptance

H. Raabe, Institute for In Vitro Sciences, Gaithersburg, MD.

The adoption of new non-animal test methods for regulatory purposes requires the evaluation of the relevance and the reliability of the proposed strategies for predicting toxicological outcomes. Such approaches have already been achieved in replacing animal tests for eye and skin irritation events by formally evaluating the relevance and the reliability of the replacement methods in multi-lab validations. These validations were borne from the numerous applications of these methods in regulatory settings. For evaluating respiratory effects, the results from select in vitro and ex vivo test systems exposed to environmental smoke and pollutants, smoke from cooking or tobacco use, industrial chemicals, or to agricultural products, show great promise for industrial and regulatory hazard classification assessments. To facilitate the adoption and use of these promising methods, it is imperative that stakeholders from industry, the regulatory community, and validation experts define the purposes for the test methods in the regulatory contexts, and design validations to achieve those goals. This presentation outlines the need for early collaborative engagement, the process for educating the scientific basis for the methods, and points to consider in the design, management and execution of the validation plan.

Deliberations in Regulatory and Safety Assessment of Food Substances in Early Life

W. A. Hayes, Harvard University, Cambridge, MA.

Neonates and younger children have a greater degree of vulnerability from consuming food than older children and adults. Much of this vulnerability is due to higher consumption per kilogram body weight. In comparison to adult dietary patterns, neonates and younger children's food behavior is largely atypical. It also is important to remember that neonates and younger children are in a rapidly developing phase of their life cycle when most organs are still developing and differentiating, leaving them in a potentially vulnerable state regarding ingredients and foods consumed. For the most part, regulatory guidelines for foods and food ingredients are in place; however, in the case of neonates and younger children, deliberation regarding the need for additional information to ensure the safety of food ingredients in early life is an area of ongoing discussion. The current paradigm for safety characterization of an ingredient for use in infant formula is, for the most part, limited to chemical characterization, anticipated exposure levels, and data from nonclinical and clinical studies. Additionally, acceptable daily intakes (ADI) currently do not apply to infants less than 12 weeks of age. The scientific community is pondering the need for a paradigm shift in the regulation of ingredients added to infant formula consumed in early stages of life. The objectives of this workshop are to address: 1) the need to derive a holistic understanding of exposure to ingredien...
how normal inter-individual and other sources of variability should be included in modeling predictions, and 4) if there are any differences and similarities in the safety assessment strategies that are in place for the safety of ingredients in early stages of life and how these uncertainties impact regulatory decision making.

**3302 Does Infant Exposure Matter in Safety Assessment?**

W. A. Hayes, Harvard University and Michigan State University, Cambridge, MA.

Harm to any biological system, including infants, can only occur following exposure to a harmful agent at sufficient concentrations to overcome that organism’s threshold for induction of an untoward effect. In order to complete a safety assessment, not only do we need to understand the hazard and dose necessary for that hazard to manifest itself, but we also need the details regarding whether or not exposure of the individual(s) has occurred. Three simple principles help us understand why individuals often respond differently to the same chemical exposure: 1) Dose matters including timing; 2) people differ; and 3) things change. Each principle will be discussed with examples. In order to appreciate exposure, an understanding of timing including amount, duration, frequency, and route need to be determined. In the end, even an extremely hazardous substance that has no exposure presents no risk even to infants. Both hazard and exposure are essential components of risk leaving us with the word RITE (Risk is equal to toxicity time exposure). Any safety assessment must include an understanding of hazard/toxicity and exposure.

**3303 Applying Concepts of Life Stage for Safety Assessment and Understanding the Needs for Children**

E. Faustman, University of Washington, Seattle, WA.

This presentation will begin with an introduction to the concepts of life stage analysis and illustrate how safety assessment frameworks can be applied across development. In order to do this, a discussion of factors of both food exposure as well as benefits and potential toxicity needs to be plotted over the developmental trajectory. This will include considerations of the changing landscape of food options, nutritional, and dietary needs. In parallel, pharmacokinetic and dynamic changes that affect ADME and response are needed. Three case examples will be provided to illustrate the temporal and toxicological importance of these factors for safety assessment of food substances. The talk will conclude with a list of current and future research initiatives that are affecting our conceptual application of these concepts for safety assessment.

**3304 Integrated Safety Assessments of Food Additives in Early Life**

A. Constable, Nestlé Research Centre, Lausanne, Switzerland. Sponsor: W. Hayes

Infant foods and their constituent ingredients are subject to rigorous risk analysis in the development of international standards, and are strictly regulated by many authorities. Implicit in this process is the need for robust assessments to ensure safety in use. Infants, having a smaller body weight and only limited food sources, have a relatively high exposure than do adults, and so a lower safety margin to any safe intake reference values. The significance of these lower margins depends on any particular developmental sensitivities and if the toxicological database is adequate to cover the early life period. With the aim of better defining (and harmonizing) assessment approaches and data requirements, we reviewed the suitability of existing safety databases on examples of additives with historical uses in infant nutrition products to support safe use in early life. We concluded that consideration of whether or not the chemical is identical to endogenous physiological metabolites, and/or if organs known to be immature in early life are targets for toxicity, are key elements to combine with an in-depth review of existing relevant toxicological and nutritional studies. A decision tree was developed to identify data gaps and guide additional data requirements, which could include targeted juvenile studies to investigate safety for the target population at the intended use levels, depending on the specific case.
ecules, therapeutic index continues to be a challenge for ADCs. Lack of understanding of ADC behavior presents unique challenges to drug development that encompasses the entire process from molecule discovery to patient selection strategies. This session will first focus on the most recent understanding of mechanisms of ADC-mediated toxicity, including current regulatory thinking on the translatability of preclinical data to the clinic. The session will then address novel approaches to ADC technologies (e.g. non-antibody scaffolds, bispecific molecules, and site-specific conjugation) that are being explored to generate more efficacious ADCs with improved tolerability. Lastly, the session will describe translational strategies designed to maximize clinical benefit of ADCs to patients.

**3309 Translational Value of Nonclinical Safety Studies**

N. Staag, Genentech, Inc., San Francisco, CA.

ADCs are a rapidly evolving class of biopharmaceuticals employing a range of conjugation technologies to stably link cytotoxic agents with different mechanism of action to monoclonal antibodies. The majority of ADCs in the clinic are conjugated to microtubule inhibitors (MTI) with primary clinical toxicities of bone marrow toxicity and peripheral neuropathy (PN). While bone marrow toxicity has been predicted in rat and monkey toxicology studies, PN has not. Recent progress in understanding the lack of translatability with PN and improvements in nonclinical models to better understand this toxicity will be discussed. In addition to MTI ADCs, there has recently been significant interest in developing ADCs conjugated to more potent DNA damaging agent payloads such as pyrrolobenzodiazepine (PBD) dimers, calicheamicins, duocarmycins, and indolobenzodiazepine dimers. While these ADCs have more severe nonclinical toxicity profiles than MTI ADCs, they also demonstrate impressive tumor activity at very low concentrations. The translatability of preclinical studies with these agents in the context of preliminary safety findings from the clinic will also be discussed in this talk.

**3310 Safety Perspectives Including Class Effects and Off-Target Toxicity**

H. Neff-LaFord, Seattle Genetics, Bothell, WA.

ADCs are a relatively new class of therapeutics developed under the premise that delivering a potent payload via a disease-specific target would minimize systemic toxicity. Two ADCs are currently on the market, with many more in development that are demonstrating promising activity in cancer patients. As experience grows with this class of compounds, we are gaining knowledge about the relative contribution of antigen-mediated (on-target) versus non-antigen-mediated (off-target) adverse events, both preclinically and clinically. Due to their complex nature, multiple components can be manipulated to change the properties of the ADC, and thus alter the toxicity profiles. Examples include changes in linker cleavage systems, linker stability, drug loading, released payload and antibody engineering. Some of these variations have been applied both preclinically and clinically, including site-specific conjugation (e.g. vadastuximab talirine), linker modifications (e.g. addition of a sulfo group to the SPDB linker for IMGN853), and released payloads (e.g. cantuzumab mertansine and cantuzumab ravtansine). These and additional examples for reducing non-antigen-mediated adverse events will be discussed.

**3311 A Regulatory Update on Nonclinical Expectations to Support the Safety of ADCs**

W. Helms, US FDA, Silver Spring, MD.

Antibody drug conjugates continue to be an active area of clinical development in the treatment of cancer. Improvements in stability and the utilization of more potent toxins are some of the factors driving innovation in the field. This presentation will discuss the current expectations for nonclinical packages submitted to support these products with a focus on the updates being discussed in the ICH S9 Q and A document currently under discussion. Case examples with regulatory outcomes from recent submissions will be included.

**3312 Translational Strategies to Maximize Therapeutic Index of ADCs**

M. Hinrichs, Medimmune, Gaithersburg, MD.

Successful development of ADCs requires strategic translational strategies to approach therapeutic index in patients. One potential mitigation strategy is to optimize dosing schedule to improve tolerability of ADCs with narrow therapeutic windows. Doing so requires a greater understanding of the exposure-response relationship to enable selection of a dosing regimen that decreases adverse events while maintaining efficacy. This presentation will address use of preclinical models to understand the pharmacokinetic drivers of safety and efficacy for ADCs in animals. Another important translational strategy being explored is to use biomarker selection strategies to understand which patient populations may benefit from treatment. For the majority of ADCs, the expression level of the target is likely to play key role in defining the appropriate patient population; however, the biology of target expression and relationship to efficacy can be complex. Therefore, this presentation will explore the relationship between target expression and anti-tumor activity using preclinical and clinical examples.

**3313 A Search for Biomarkers of Neurotoxicity: A Practical Approach**

D. Herr, US EPA, Research Triangle Park, NC.

Human exposures to drugs, chemicals, and chemical mixtures often are associated with symptoms suggestive of nervous system involvement (headache, fatigue, cognitive changes, etc.), yet broadly applicable screening methods to assess the neurotoxic condition in animal models are lacking. Thus, there is a need for more sensitive and specific biomarkers that can help diagnose and predict neurotoxicity that are relevant across animal models and translatable to the clinic. Fluid-based biomarkers, such as those found in serum, plasma, urine, and cerebrospinal fluid, have great potential due to the relative ease of sampling, but at present, data on their expression and translation are lacking or inconsistent. In order to identify such novel fluidic biomarkers associated with the development and expression of neurotoxicity and to evaluate their relative sensitivity with established phenotypic anchors of neurotoxicity, a pilot study was designed under the auspices of the ILSI Health and Environmental Sciences Institute (HESI) Technical Committee on Translational Biomarkers of Neurotoxicity—members include representatives from academia, industry, and government. Trimethyltin (TMT) was selected as a prototypic compound since relevant data are available on dose response, time course, and site of action. Neuropathology was confirmed by traditional histopathological assessments, behavioral changes, and alterations in neurochemical and neuroinflammatory biomarkers in brain areas targeted by a single dose of TMT (7.0 mg/kg body weight) at two, six, 10, and 14 days. Using state-of-the-art assessment techniques, the researchers identified specific biological chemicals or patterns in biological chemicals in serum, plasma, or cerebral spinal fluid that are associated with nerve cell damage/degeneration caused by TMT. Such changes include alterations in the status of the metabolome and the expression of microRNAs, as well as levels of interleukins and other related circulating antigen factors. Histopathological assessments of TMT effects in select non-brain tissues enabled improved interpretation of the brain specificity of these changes. Correlation of high-throughput endpoints with markers of neurotoxicity, especially glial fibrillary acid protein, neuropathological loss of neurons, and oxidative damage to neurons as presented here provide a guideline by which to establish fluidic biomarkers of neurotoxicity. The data from this pilot study also will help design follow-on studies utilizing other known neurotoxicants to determine the generalizability of the findings in an effort to develop and validate a set of biochemical markers of neurotoxicity that will be accessible clinically. Such clinical biomarkers should prove valuable to research ranging from preclinical studies to clinical trials and assist with the monitoring of the severity of and recovery from brain injury.
Overview of the Biomarker Initiative to Identify Biological Fluid-Based Indicators of Neurotoxicity

W. Slikker, US FDA/NCTR, Jefferson, AR.

In order to identify biomarkers associated with the development and expansion of neurotoxicity, the Health and Environmental Sciences Institute (HESI) Technical Committee on Translational Biomarkers of Neurotoxicity conducted a pilot study in which potential biomarkers were assessed in concert with confirmation of TMT-induced neuropathology by traditional histopathological assessments and behavioral changes. Tissue samples, behavioral observations, and MRI scans were obtained simultaneously at 2, 6, 10, or 14 days post-TMT treatment. Samples collected included plasma, serum, CSF, and urine with a goal towards identifying in bodily fluids key biomolecules associated with the expression of frank neurotoxicity. Behavioral and neuropathology data confirmed the effectiveness of TMT to induce functional and morphological derangements and will be key anchors in attempts to identify biomarkers that portend the onset, or confirm the presence of, neurotoxicity.

Glial Fibrillary Acidic Protein (GFAP) and Related Astroglial Proteins as Biomarkers of Neurotoxicity

J. O’Callaghan, NIOSH, Morgantown, WV.

The glial reaction to nervous system damage, often termed gliosis, represents a hallmark of all types of nervous system injury. As such, development and implementation of gliosis biomarkers represents a broadly applicable approach for neurotoxicity safety assessment. Using a panel of known neurotoxic agents, we have previously shown that the astroglial protein, GFAP, can serve as one such biomarker of neurotoxicity. Qualitative and quantitative analyses of GFAP have shown this biomarker to be a sensitive and specific indicator of the neurotoxic condition. Decades ago, assays and immunohistochemistry of GFAP were used to detect and quantify TMT-induced damage to its known target, hippocampus, and to identify novel CNS targets damaged by this compound. These studies revealed the sensitivity and target specificity of the GFAP/glial biomarker-based approach to neurotoxicity assessment. In the current study, time-dependent and significant increases in GFAP reactivity was observed in hippocampus and increased protein levels were detected in plasma and serum. The GFAP data from the HESI pilot study using TMT was used as a metric to which other potential markers of neurotoxicity can be compared for relative sensitivity and specificity. GFAP and related glial biomarkers may serve as the basis for further development of molecular signatures predictive of adverse effects on the nervous system.

Changes in the Metabolome May Serve as Peripheral Biomarkers of CNS Toxicity

D. Herr, US EPA, Research Triangle Park, NC.

Since our observation that an acute exposure to different classes of pesticides resulted in different changes in plasma metabolomics markers, a study of the metabolome has become of high interest for identifying markers of neurotoxicity. A Biocrates AbsoluteIDQp180 platform was used for targeted identification of metabolite changes in rat CSF, plasma, and urine. Metabolite classes included acylcarnitines, amino acids, biogenic amines, hexoses, phosphatidylcholines, lysophosphatidylcholines, and sphingomyelins. From among 186 metabolites, 31 were detected in all CSF samples, and 135 were detected in all plasma samples. A principal component analysis indicated that certain metabolites were differentially produced in TMT-treated groups compared to controls. This was especially true in the CSF. Analysis of metabolite fold changes in the CSF indicated increases in acylcarnitines and phosphatidylcholines at 2 and 6 days, with increases in amino acids observed as long as 14 days after treatment. These changes suggest alterations in energy metabolism and mitochondrial and membrane damage in the nervous system. In the plasma, there were increases in levels of acylcarnitines, phosphatidylyl- and lyso-phosphatidylcholines, amino acids, and sphingomyelins at 2 and 6 days. Additionally, the increased levels of acylcarnitines in urine at 2 and 6 days were most similar to the changes in metabolites noted in CSF and plasma. Our data provide evidence for significant changes in energy metabolism and membrane damage in CNS, which were reflected in a peripheral fluid. Should it be demonstrated that plasma and urine markers mirror CSF markers and track aspects of CNS damage as evidenced by frank neuropathology (cell death), they may serve as useful, readily accessible surrogates of neurotoxicity. This is an abstract of a proposed presentation, and does not represent US EPA policy.

Neurotoxicant Effects on Non-Brain Tissues: Understanding Biomarker Specificity

J. D. Pardo, Pfizer, Inc., Groton, CT.

Animal testing of a potential new drug often reveals multiple target organs, some of which may limit further drug development, and others that are manageable. A biomarker used to understand translation of target organ toxicity to human risk will need to have both high sensitivity and specificity for the target organ of concern. Thus, a peripheral biomarker of neurotoxicity must be very sensitive to nervous system injury while not being influenced by effects in non-nervous tissues. In the present study, the phenotypic anchor of frank CNS toxicity was quantified using Fluoro-jade stain of hippocampal cells. Histopathological evaluation of kidney, liver, thymus, adrenal glands, sciatic nerve, and lumbar cord was then performed. TMT-related microscopic findings consisted of minimal to mild degeneration of renal tubules within the distal nephron. These findings were accompanied by renal tubular epithelial necrosis and/or tubular dilation at 6 (at least n=3/10), 10 (3/6) and 14 (3/5) days. Minimal hepatocellular hypertrophy and minimal vacuolation (lipid) of portal hepatocytes were observed in one animal of one post-TMT group. Similarly, diffuse decreased cellularity (lymphoid cells) was observed in the thymus cortex in single animal on post-dosing days 6, 10, and 14. There were no TMT-related microscopic findings in the lumbar spinal cord, sciatic nerve, or adrenal glands. Thus, putative biomarkers described in other presentations in this session are likely reflective of TMT-induced brain toxicity and not derived from other organ systems. However, because urinary TGF-beta may be altered by renal toxicity this marker requires further study to determine its brain specificity.

Biochemical and Molecular End-Points as Biomarkers of Neurotoxicity and Their Correlation with Neuropathological Damage

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An effort to perform a comprehensive identification of biomarkers that circulate in bodily fluids and tissue that are associated with the expression of neurotoxicity was undertaken using a young adult male rat model. Here, preliminary observations are presented on biomolecules that have some promise for identification of neurotoxicity that was induced by a single intraperitoneal injection of the known neurotoxic agent, trimethyltin (TMT). A single dose of TMT led to significant alterations in total oxidative stress markers, changes in lipid homeostasis, circulating interleukins and related factors, and markers of neuroinflammation, thus, providing opportunities to explore their correlation with the traditional pathology that defines neurotoxicity. Finally, a comprehensive correlation of TMT-induced neuropathology with several observed biomarkers suggest specific pathways that can be assessed using peripheral fluids.
The widespread use of engineered nanomaterials (ENMs) in consumer products is a concern for potential unintended exposure to ENMs and the impact on health. The overarching goal of the National Institute of Environmental Health Sciences (NIEHS) Nano Environmental Health and Safety (Nano EHS) program is to gain fundamental understanding of the molecular and pathological pathways implicated in potential adverse health effects of ENMs. In the absence of an identified pathology associated with ENMs, the NIEHS took a proactive approach aimed at developing an ENMs-biological interactions knowledge base, based on physicochemical properties of ENMs. This comprehensive knowledge base is hoped to guide and develop *in silico* approaches for human health risk characterization and potential intervention or remedial measures, as well the design of benign nanomaterials. The NIEHS recognized the need to promote collaborative team science efforts to address the multidisciplinary nature of assessing ENMs on environmental health and safety. Towards this goal, based on the past two decades of promoted research through the Nano GO Consortium and NIEHS Centers for Nanotechnology Health Implications Research (NCNHIR) and the research outcomes from these efforts, the NIEHS Nanomaterials Health Implications Research (NHIR) Consortium was established in 2016. This consortium will expand the library of ENMs and physico-chemical properties focusing on specific materials with high production and use in consumer products, as well as recently emerging 2D and 3D ENMs containing new transitional metals and whose toxicology is unknown at the nanoscale. The comprehensive toxicological profile for these ENMs surveyed using a wide range of systems reflecting more physiologically relevant models will benefit the future goals of this program (i.e., to promote computational modeling efforts to predict association between ENM physicochemical properties and potential health effects).
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